REMEDIATION OF ACID SULFATE SOILS BY ORGANIC MATTER ADDITION

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Contents

Abstract

Acid sulfate soils (ASS) are soils containing iron sulfide minerals (predominantly pyrite) and/or their oxidation products. ASS have a large distribution in the world (10^7–10^8 ha coastal ASS) and in Australia (215,000 km^2). Sulfide in ASS is formed from sulfate in sea water or fresh water under anaerobic conditions by sulfate-reducing bacteria, which need organic matter (OM) as the energy source. Sulfide then reacts with dissolved Fe(II) to form pyrite. Sulfuric material (containing sulfuric acid) is formed during oxidation of ASS with sulfidic material (containing sulfide minerals), resulting in significant release of acid and dissolved metals that can have detrimental effects on soil and water quality and thus ecosystem services. Remediation of sulfuric material and prevention of oxidization of sulfidic material are therefore of great environmental concern. Conventional remedial strategies, such as liming sulfuric material and covering sulfidic material with water or non-ASS soil, can be costly or not practically feasible due to the large amount of lime, water or soil required.

Organic matter (OM) is the energy source for sulfate reducers, which play a critical role in the formation of sulfidic material and generate alkalinity during sulfate reduction. OM could influence the oxidation of pyrite through oxygen consumption by OM decomposers, complexation of ferric iron, and coating of pyrite. OM can also buffer acid generated from pyrite oxidation. However, the availability of native OM in ASS can be low due to binding to clay particles, occlusion in aggregates, or complexion by dissolved metals. Therefore application of OM may be an economical and environmentally friendly option to remediate ASS. But systematic studies are required on the effectiveness of OM application for the management of ASS.

Ten ASS with sulfuric material, with pH increased to 5.5 and OM (finely ground mature wheat straw) added at 2% (w/w) separately or combined, were incubated submerged for 36 weeks. Unamended soils served as controls. Only the combined treatment (pH increased and OM added) increased the concentration of reduced inorganic sulfur significantly compared to the control and had higher soil pore water pH than the treatment with only pH increased. But the stimulation of sulfate reduction compared to the control of the combined treatment differed among soils which could be attributed to the initial soil properties. Stimulation of sulfate reduction in the combined treatment was negatively correlated with soil clay content and initial nitrate concentration. Clay can limit the availability of the added OM by binding and nitrate is a competing electron acceptor for sulfur reduction. In a subsequent experiment, ASS with smaller increase in sulfate reduction compared with other soils in the previous study were incubated with up to 6% (w/w) OM added for 36 weeks. The concentration of reduced inorganic sulfur increased with OM addition rate, with the increase between 4% and 6% being smaller than that between 2% and 4%, suggesting that besides OM other factors influenced sulfate reduction. Further, the impact of nitrate (competing electron acceptor) at different OM addition rates on sulfate reduction was examined. Nitrate addition inhibited sulfate reduction but the extent was smaller with OM added at 4% compared to 2%, indicating that the inhibition by nitrate was overcome by higher OM addition.

In the two following experiments, the effect of OM addition on oxidation and acidification of sulfidic material was investigated by laboratory incubation under oxidizing conditions for 6 weeks. In the first experiment, OM (finely ground mature wheat straw) was added to a sulfidic material at 3% (w/w) as a layer on the soil surface or by mixing into soil. Soil pH decreased by 0.9 unit in the unamended control, increased by 0.2 unit in the treatment with OM as a layer, and increased by 0.8 unit in the treatment with OM mixed into the soil. In the second experiment, 1, 2, 3, and 4% (w/w) OM was mixed into the sulfidic material. The pH decrease was strongest in the unamended control and was smaller with 1% and 2% OM. Only 3% and 4% OM addition prevented acidification. However, the increase in soil sulfate concentration was similar in all the amended treatments. Prevention of acidification of sulfidic material by OM addition can be explained by consumption of oxygen by OM decomposers and pH buffer capacity of OM.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree.

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Acknowledgment

In this thesis, the first chapter was revised 5 times, the second chapter 10 times, the third chapter 7 times, the fourth chapter 3 times, and the fifth chapter 3 times. It was painful for me. Therefore, I can understand Petra's feeling and how much effort she put into helping me during my PhD candidature. And she has a group of students! For them and science, she spends all her time and energy, which stimulates us to do our best. From Petra I learned to design an experiment more systematically and write a paper more concisely.

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The flowers, the people and more make Adelaide a beautiful and wonderful place, where I had a lot of unforgettable experiences.

I will tell these experiences to my dear friends back in China. They are expecting me to go home.

I will also tell my parents and sister. They don't know English, they don't know what Adelaide looks like, and they don’t even know what I currently look like. They haven't seen me since I came here. They must miss me.

Chaolei Yuan

List of abbreviations

ANOVA	analysis of variance
ASS	acid sulfate soils
AVS	acid volatile sulfide
$\mathbf{DO_2}$	dissolved oxygen
ICP-AES	inductively coupled plasma atomic emission spectroscopy
MaOC	mineral-associated organic carbon
OM	organic matter
PCA	principal component analysis
pH(1:1)	pH measured in a 1:1 soil to water ratio
pH(1:5)	pH measured in a 1:5 soil to water ratio
POC	particulate organic carbon
RIS	reduced inorganic sulfur
Scr	chromium reducible sulfur
$\mathbf{S_{HCl}}$	4 M HCl extractable sulfur
$\mathbf{S_{KCl}}$	1 M KCl extractable sulfur

CHAPTER 1

Introduction and Literature Review

Introduction and literature review

1. Properties and distribution of acid sulfate soils

Acid sulfate soils (ASS) are soils that contain sulfide minerals or are affected by geochemical or biochemical transformations of iron sulfide minerals. These soils may either contain sulfuric materials ($pH<4$) (Isbell, 2002) or have the potential to form sulfuric acid in amounts that have an effect on the main soil characteristics (Dent, 1986; Dent and Pons, 1995; Fanning, 2013; Fitzpatrick et al., 2011; Fitzpatrick et al., 2009; Pons, 1973). The iron sulfides in ASS form under anaerobic conditions where sulfide reacts with dissolved ferrous iron. Oxidation of iron sulfides results in sulfuric material containing sulfuric acid, which can cause acidic groundwater/leachate (Mosley et al., 2014b). These processes will be discussed in more detail below.

ASS can be found in coastal and inland environments (Fitzpatrick et al., 2009). The global extent of coastal ASS is between 10^7 and 10^8 ha (Andriesse and van Mensvoort, 2007; Macdonald et al., 2011), but the world extent of inland ASS is unknown. In Australia, the estimated area of ASS is 215,000 km^2, of which coastal ASS occupy 58,000 km^2 and inland ASS 157,000 km^2 (Fitzpatrick et al., 2010). ASS can occur, for example, in farmlands, wetlands, mine spoil, or densely populated areas (Andriesse and van Mensvoort, 2007; Baldwin and Fraser, 2009; Brinkman, 1981; Fitzpatrick et al., 2009; Mosley et al., 2014b). Their significant environmental and social impact on ecosystems, agricultural and fishery production, and infrastructure in both Australia and the world has attracted attention by scientists, farmers and natural resource managers (Brinkman, 1981; Degens et al., 2008; Fanning, 2007; Fanning and Rabenhorst, 2007; Shamshuddin et al., 2014).

2. Sulfur in ASS

Sulfur in ASS can be present in reduced and oxidized forms. Generally, three soil materials are recognized in ASS (Fitzpatrick et al., 2009; Sullivan et al., 2010a): (i) sulfuric material (pH<4) containing sulfuric acid, (ii) sulfidic material containing mainly pyrite (FeS_2), and (iii) monosulfidic material containing monosulfide minerals (FeS). Sulfidic material can be further classified into two types: hypersulfidic material and hyposulfidic material. The former is capable of severe soil acidification following oxidation of sulfides; the latter is not (Sullivan et al., 2010a). Monosulfidic and sulfidic material can transform into sulfuric material after oxidation. Often more than one material can be found in the same soil profile with sulfidic material generally underlying sulfuric material (Ahern et al., 2004; Powell and Martens, 2005).

2.1. Formation of sulfidic material

Under undisturbed saturated conditions, sulfur in ASS occurs as iron sulfides, mainly pyrite (FeS_2). These iron sulfides are produced via sulfate-reducing microorganisms and their content is controlled by the duration of reducing conditions and the availability of organic matter, reactive iron, and dissolved sulfate (Berner, 1984; Fitzpatrick et al., 2009). Sulfate from ocean or fresh water can be reduced to H_2S by sulfate-reducing bacteria, most of which are heterotrophs and therefore require organic matter as an energy source (Berner, 1984; Muyzer and Stams, 2008):

$$2CH_2O+SO_4^{2-}\rightarrow 2HCO_3^{-}+H_2S \text{ (1).}$$

In the equation, CH_2O represents organic matter in which the oxidation state of carbon is zero.

Other factors influencing sulfate reduction include, for example, pH (most sulfate reducers are inactive at pH<5), presence of other competing electron acceptors, redox potential and temperature (Johnston et al., 2005; Neculita et al., 2007; Ponnamperuma, 1972).

H_2S produced reacts with aqueous FeS (FeS_{aq}) to form pyrite (the H_2S mechanism); another mechanism of pyrite formation is the reaction between polysulfide and FeS_{aq} (the polysulfide mechanism) (Luther III, 2005; Rickard, 2012; Rickard and Morse, 2005) (Fig.1). FeS_{aq} is formed by dissolution of solid FeS or reaction between H_2S/HS^- and iron species dissolved from other Fe minerals. Polysulfides are formed by several reactions, most commonly through the reaction between S^0 and S^{2-} (Luther III, 2005; Rickard, 2012; Rickard and Morse, 2005).

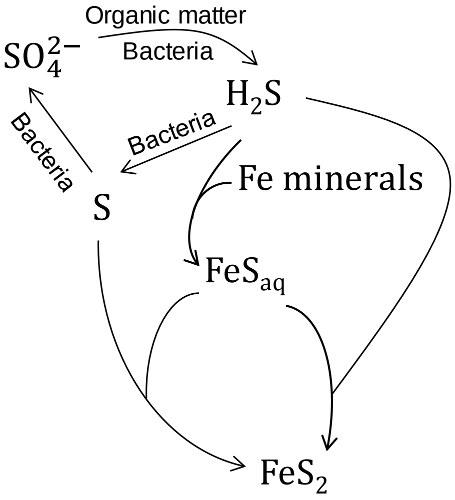

Fig.1 Formation of pyrite (according to Berner (1984) and Rickard (2012)).

2.2. Oxidation of sulfidic material

When ASS with sulfidic material is drained or no longer covered by water, iron sulfides can be oxidized with release of sulfuric acid as indicated in the following overall reaction (Johnson and Hallberg, 2005):

$$4FeS_2+15O_2+14H_2O\rightarrow 4Fe(OH)_3+8SO_4^{2-}+16H^+ \text{ (2).}$$

This is also a complex process with multiple steps and the involvement of microorganisms (Ahern et al., 2004; Chandra and Gerson, 2010; Fanning and Fanning, 1989; Fitzpatrick et al., 2009; Johnson and Hallberg, 2005; Neculita et al., 2007). Pyrite oxidation is initiated by exposure to oxygen:

$$FeS_2+7/2O_2+H_2O\rightarrow Fe^{2+}+2SO_4^{2-}+2H^+ \text{ (3).}$$

Ferrous iron is then oxidized to ferric iron:

$$Fe^{2+}+1/4O_2+H^+\rightarrow Fe^{3+}+1/2H_2O \text{ (4).}$$

Hydrolysis of Fe^{3+} results in precipitation of ferric hydroxide and liberation of acid:

$$Fe^{3+}+3H_2O\rightarrow 3Fe(OH)_3\downarrow+3H^+ \text{ (5).}$$

The produced ferric iron (Equation 4) can significantly accelerate pyrite oxidation and H^+ production (Johnson and Hallberg, 2005; Neculita et al., 2007):

$$FeS_2+14Fe^{3+}+8H_2O\rightarrow 15Fe^{2+}+2SO_4^{2-}+18H^+ \text{ (6).}$$

Oxidation of ferrous to ferric iron (Equation 4) is considered as the rate-limiting step in pyrite oxidation, and this step is mainly catalyzed by acidophilic iron-oxidizing bacteria (Johnson and Hallberg, 2005; Neculita et al., 2007). These bacteria are chemolithotrophic (Emerson et al., 2010), therefore organic carbon is not required as energy source.

The overall consequences of pyrite oxidation are the substantial release of acidity (Equation 2) and mobilization of metals, which have higher solubility at low pH (Mosley et al., 2014c; Neculita et al., 2007). Other products of iron sulfide oxidation such as jarosite $KFe_3(SO_4)_2(OH)_6$, natrojarosite $NaFe_3(SO_4)_2(OH)_6$, and schwertmannite $Fe_8O_8(OH)_6(SO_4)$

can also form in the soil. These secondary sulfate minerals retain acidity that can be slowly released on hydrolysis (Ahern et al., 2004; Breemen, 1973).

Factors influencing the rate of pyrite oxidation include pyrite surface area, oxygen concentration, temperature, pH, concentration of ferric iron, the presence and activity of bacteria, organic matter, and soil texture (Breemen, 1973; Ward et al., 2004).

2.3. Environmental impacts linked with ASS

When submerged, pyrite in ASS is stable and has limited environmental influence. Oxidation of sulfidic material can be induced by natural and/or human disturbance such as drought, changed direction of rivers and water ways, drainage or high usage of river water causing reduced flows into wetlands, and post-glacial rebound (Åström and Deng, 2003; Fitzpatrick et al., 2009; Mosley et al., 2014a; Mosley et al., 2014c; Österholm et al., 2010). Australia is expected to experience more droughts in the future (Hobday and McDonald, 2014; Stokes et al., 2008), which will increase the chance of oxidation of sulfidic material. Once oxidized, the acidity together with mobilized metals from ASS can have detrimental impacts on: (i) the soils themselves and surrounding soils, (ii) water quality, causing deterioration of aquatic or wetland ecosystems and loss of fisheries, (iii) agricultural production, and (iv) infrastructure (Bronswijk et al., 1993; Fitzpatrick et al., 2009). Fitzpatrick et al. (2012) studied the environmental impacts of ASS in the Lower Murray Reclaimed Irrigation Area (LMRIA), which is an important agricultural area in South Australia. Before 2007, hypersulfidic material accumulated in the saturated soil profiles due to stable water levels and sufficient supply of iron, sulfate and organic matter. Between 2008 and early 2010, a persistent drought resulted in exposure of these soils to the atmosphere which caused cracking of soil and oxidation of previously undisturbed hypersulfidic material to sulfuric material even at depth (>1.5 m) (Mosley et al., 2014b). After re-flooding and irrigation (since 2011), surface water acidification (pH 2.5–3.5) and iron-rich precipitates (mainly schwertmannite) have been observed in drains (Fitzpatrick et al., 2012).

ASS also have potential influence on global climate. Some studies have shown that processes in ASS can be linked to the emission of greenhouse gases including carbon dioxide, methane, nitrous oxide (Jugsujinda et al., 1996; Macdonald et al., 2011; Simek et al., 2011). However, most current studies of ASS are focusing on sulfur and metal dynamics, while little attention has been given to the role of organic matter in these soils.

3. Organic matter and ASS management

Soil organic matter is a vital component of the soil ecosystem and accounts for a significant pool of global terrestrial carbon (Baldock, 2007; Chapin et al., 2002; Lal, 2004). Organic matter has been proposed as an attractive remedial reagent for ASS via stimulating sulfate-reducing bacteria or retarding pyrite oxidation (Baldwin and Fraser, 2009; Dear et al., 2002; Fitzpatrick et al., 2009).

3.1. Organic matter and remediation of sulfuric material

To remediate ASS with sulfuric material, soil acidity needs to be neutralized, usually by application of chemical ameliorants such as hydrated lime ($CaOH_2$) or ground limestone (mainly $CaCO_3$) (Baldwin and Fraser, 2009; Dear et al., 2002; Fitzpatrick et al., 2009). However, liming may be costly and ineffective, particularly when sulfuric material occurs in large areas or deep soil layers, or has a high clay content (Dear et al., 2002). Limestone may also become coated in gypsum and Fe minerals, reducing its dissolution efficiency (Dear et al., 2002; Hammarstrom et al., 2003).

Adding organic matter to stimulate sulfate reduction has been suggested as a more economical and environmentally-friendly remedial option for sulfuric material than other approaches (Ahern et al., 2004; Baldwin and Fraser, 2009; Fitzpatrick et al., 2009). Sulfate reduction results in formation of pyrite but also generates alkalinity (bicarbonate, see Equation 1) that can neutralize acidity. However, little is known about the effectiveness of sulfate reduction as a remediation strategy in ASS.

Sullivan et al. (2010b) observed a positive relationship between organic carbon concentration and sulfate reduction rate in lake sediments containing sulfuric material during laboratory inundation. In field trials conducted by Fraser et al. (2012) in an Australian wetland with sulfuric sediments, vegetation combined with liming increased soil pH significantly. However, further systematic investigation is needed to better understand the influence of organic matter addition on sulfate reduction after re-flooding ASS with sulfuric material. In addition, the influence of soil properties on the effectiveness of organic matter addition to remediate sulfuric material needs to be examined for this strategy to be useful. Firstly, the availability of native and added organic matter can be affected by soil texture and minerals (Lützow et al., 2006; Roychand and Marschner, 2013; Shi and Marschner, 2013). Clay reduces the availability of organic matter because organic matter can be (i) bound via ligand exchange and cation (e.g. Ca^{2+}, Fe^{3+}, Al^{3+}) bridges, (ii) intercalated into clay layers, and (iii) occluded within aggregates (Lützow et al., 2006). Metal ions, such as Fe^{3+} and Al^{3+} which can be abundant in sulfuric material (Ahern et al., 2004; Fitzpatrick et al., 2013), can also bind organic matter (Bronswijk et al., 1993; Lützow et al., 2006). Secondly, sulfate-reducing bacteria are active only between pH 5 and 8 (Neculita et al., 2007). Therefore organic matter addition may only stimulate sulfate reduction when accompanied by pH increase to pH >5. Thirdly, the presence of competing electron acceptors, such as NO_3^-, Mn(IV), Fe(III), inhibits sulfate reduction (Ponnamperuma, 1972) by outcompeting sulfate for electron donors (organic matter) during reduction (Hubert and Voordouw, 2007; Lovley and Phillips, 1987). After flooding, soil organic matter is first decomposed by microbes using oxygen, NO_3^-, Mn(IV) and Fe(III) sequentially as electron acceptors (Ponnamperuma, 1972). If after the reduction of these electron acceptors there is still organic matter available, sulfate reduction can occur (Dugdale et al., 1977; Lovley and Phillips, 1987; Ontiveros-Valencia et al., 2012; Ponnamperuma, 1972). Also NO_3^-, Mn(IV) and Fe(III), if present, can oxidize sulfide (Canfield et al., 1993; Hubert and Voordouw, 2007; Myers and Nealson, 1988; Zhang et al., 2009). Nitrate inhibits sulfate reduction also because nitrite, the product of nitrate reduction, inhibits the dissimilatory sulfite reductase (Haveman et al., 2004; Kaster et al., 2007). Therefore, soil properties such as texture, pH, and concentrations of competing electron acceptors need to be taken into account in attempting remediation of sulfuric material using organic matter.

3.2. Organic matter and prevention of oxidation of sulfidic material

Another option to prevent detrimental impacts of ASS on the environment is the prevention of oxidation of sulfidic material, which can be achieved by maintaining the saturated state of ASS by covering with soil or water. But this method may be not feasible or costly (Baldwin and Fraser, 2009; Dear et al., 2002; Fitzpatrick et al., 2009).

Several previous studies suggest that organic matter can retard the oxidation of pyrite due to oxygen consumption by microbes during OM decomposition, complexation of ferric iron, and coating pyrite (Bronswijk et al., 1993; Bush and Sullivan, 1999; Pichtel et al., 1989; Rigby et al., 2006; Ward et al., 2004). OM can also buffer acid generated (Paul and Ulf, 2011). Rigby et al. (2006) observed mitigation of pyrite oxidation by organic matter in a reaction vessel with suspension of pyritic fines. Adding organic matter as a mulch on the soil surface is also a suggested method to prevent pyrite oxidation or acidification of sulfidic material by maintaining anoxia and buffering acid (Baldwin and Fraser, 2009; Cook et al., 2004; Fitzpatrick et al., 2009). However, there are no systematic studies on the effect of adding organic matter to prevent oxidation and acidification of sulfidic material. Further, the form of organic matter addition (e.g. as a mulch or by mixing) may influence its effectiveness.

4. Structure of the thesis

This thesis focuses on investigations involving remedial effects of organic matter in ASS management.

Chapter 1 is the introduction and provides a brief literature review.

Chapters 2 and 3 examine the effectiveness of organic matter in remediation of sulfuric material. Chapter 2 investigates the effects of pH increase to 5.5 and organic matter addition at 2% (w/w) separately or combined on sulfate reduction after the re-flooding of sulfuric material. Subsequently, in Chapter 3, ASS is incubated with varying amounts (0–6% w/w) of organic matter added. Furthermore, the impact of nitrate (competing electron acceptor) at different organic matter addition rates on sulfate reduction is tested by a nitrate addition experiment.

In Chapter 4 the effect of organic matter addition on acidification of sulfidic material is investigated by laboratory incubation experiments under oxidizing conditions. In the first experiment organic matter is added to sulfidic material at 3% (w/w) by mixing into soil or as a layer on the soil surface. The second experiment extends this work by investigating the effect of the rate of organic matter addition on acidification of sulfidic material. Sulfidic material is mixed with 0–4% (w/w) organic matter.

Finally, in Chapter 5 overall conclusions and further research directions are presented.

References

Ahern, C. R., McElnea, A. E., and Sullivan, L. A. (2004). "Acid Sulphate Soils: Laboratory Methods Guidelines," Department of Natural Resources, Mines and Energy, Indooroopilly, Queensland, Australia.

Andriesse, W., and van Mensvoort, M. (2007). Acid Sulfate Soils: Distribution and Extent. *In* "Encyclopedia of Soil Science, Second Edition", pp. 14-19. Taylor & Francis.

Åström, M., and Deng, H. (2003). Assessment of the mobility of trace elements in acidic soils using soil and stream geochemical data. *Geochemistry: Exploration, Environment, Analysis* **3**, 197-203.

Baldock, J. (2007). Composition and Cycling of Organic Carbon in Soil. *In* "Nutrient Cycling in Terrestrial Ecosystems" (P. Marschner and Z. Rengel, eds.), Vol. 10, pp. 1-35. Springer Berlin Heidelberg.

Baldwin, D. S., and Fraser, M. (2009). Rehabilitation options for inland waterways impacted by sulfidic sediments - A synthesis. *Journal of Environmental Management* **91**, 311-319.

Berner, R. A. (1984). Sedimentary pyrite formation: An update. *Geochimica et Cosmochimica Acta* **48**, 605-615.

Breemen, N. v. (1973). Soil forming processes in acid sulphate soils. *In* "Proceedings of the International Symposium on Acid Sulphate Soils, 13-20 August 1972, Wageningen, The Netherlands " (H. Dost, ed.), pp. 66-129. International Institute for Land Reclamation and Improvement, Wageningen.

Brinkman, R. (1981). Social and economic aspects of the reclamation of acid sulfate soil areas. *In* "Proceedings of Second International Symposium on Acid Sulphate Soils, Bangkok, Thailand, 18-24 January, 1981" (H. Dost and N. v. Breemen, eds.), Vol.

Publication 31, pp. 21-36. International Institute for Land Reclamation and Improvement/ILRI, Wageningen, The Netherlands.

Bronswijk, J. J. B., Nugroho, K., Aribawa, I. B., Groenenberg, J. E., and Ritsema, C. J. (1993). Modeling of Oxygen-Transport and Pyrite Oxidation in Acid Sulfate Soils. *Journal of Environmental Quality* **22**, 544-554.

Bush, R. T., and Sullivan, L. A. (1999). Pyrite micromorphology in three Australian Holocene sediments. *Soil Research* **37**, 637-654.

Canfield, D. E., Thamdrup, B., and Hansen, J. W. (1993). The anaerobic degradation of organic matter in Danish coastal sediments: Iron reduction, manganese reduction, and sulfate reduction. *Geochimica et Cosmochimica Acta* **57**, 3867-3883.

Chandra, A. P., and Gerson, A. R. (2010). The mechanisms of pyrite oxidation and leaching: A fundamental perspective. *Surface Science Reports* **65**, 293-315.

Chapin, F. S., Matson, P. A., and Mooney, H. A. (2002). "Principles of terrestrial ecosystem ecology," Springer Verlag, New York.

Cook, F. J., Dobos, S. K., Carlin, G. D., and Millar, G. E. (2004). Oxidation rate of pyrite in acid sulfate soils: in situ measurements and modelling. *Australian Journal of Soil Research* **42**, 499-507.

Dear, S.-E., Moore, N. G., Dobos, S. K., Watling, K. M., and Ahern, C. R. (2002). Soil Management Guidelines. *In* "Queensland Acid Sulfate Soil Technical Manual". Department of Natural Resources and Mines, Indooroopilly, Queensland, Australia.

Degens, B., Fitzpatrick, R., and Hicks, W. (2008). Avon Basin, WA Wheatbelt: Acidification and Formation of Inland ASS Materials in Salt Lakes by Acid Drainage and Regional Groundwater Discharge. *In* "Inland Acid Sulfate Soil Systems Across Australia" (R.

Fitzpatrick and P. Shand, eds.), pp. 176-188. CRC LEME Open File Report No. 249. (Thematic Volume) CRC LEME, Perth, Australia.

Dent, D. (1986). "Acid sulphate soils: a baseline for research and development," International Institute for Land Reclamation and Improvement/ILRI, Wageningen, Netherlands.

Dent, D. L., and Pons, L. J. (1995). A world perspective on acid sulphate soils. *Geoderma* **67**, 263-276.

Dugdale, R. C., Goering, J. J., Barber, R. T., Smith, R. L., and Packard, T. T. (1977). Denitrification and hydrogen sulfide in the Peru upwelling region during 1976. *Deep Sea Research* **24**, 601-608.

Emerson, D., Fleming, E. J., and McBeth, J. M. (2010). Iron-Oxidizing Bacteria: An Environmental and Genomic Perspective. *Annual Review of Microbiology* **64**, 561-583.

Fanning, D. S. (2007). Acid Sulfate Soils. *In* "Encyclopedia of Soil Science, Second Edition" (R. Lal, ed.), pp. 11-13. Taylor & Francis.

Fanning, D. S. (2013). Acid Sulfate Soils. *In* "Encyclopedia of Environmental Management", Vol. null, pp. 26-30. Taylor & Francis.

Fanning, D. S., and Fanning, M. C. B. (1989). "Soil: morphology, genesis, and classification," Wiley.

Fanning, D. S., and Rabenhorst, M. C. (2007). Acid Sulfate Soils: Problems. *In* "Encyclopedia of Soil Science, Second Edition" (R. Lal, ed.), pp. 29-32. Taylor & Francis.

Fitzpatrick, R., Marvanek, S., Powell, B., Grealish, G., and Gilkes, R. (2010). Atlas of Australian Acid Sulfate Soils: recent developments and future priorities. *In*

"Proceedings of the 19th World Congress of Soil Science: Soil solutions for a changing world. Brisbane, Australia, 1-6 August, 2010" (R. Gilkes and N. Prakongkep, eds.), pp. 24-27. Published on DVD; ISBN 987-0-646-53783-2; http//www.iuss.org; Symposium WG 3.1 Processes in acid sulfate soil materials.

Fitzpatrick, R., Raven, M., Self, P., Shand, P., Mosley, L., Simpson, S., and Baker, A. (2012). Occurrence, genesis and environmental significance of schwertmannite in re-flooded Acid Sulfate Soils in the Lower Murray Reclaimed Irrigation Area. *In* "Combined Australian Regolith Geoscientists Association and Australian Clay Minerals Conference ", Mildura, Australia.

Fitzpatrick, R., Shand, P., and Hicks, W. (2011). "Technical Guidelines for Assessment and Management of Inland Freshwater Areas impacted by Acid Sulfate Soils," CSIRO Land and Water Science Report, 05/11. 171 pp.

Fitzpatrick, R., Shand, P., Simpson, S., Grocke, S., Raven, M., Baker, A., Mosley, L., and Self, P. (2013). "Assessment of re-flooded acid sulfate soil environments at Long Flat, Jervois, Toora and Pompoota in the Lower Murray Reclaimed Irrigation Area (LMRIA)," CSIRO Land and Water Science Report 2013. 03/12. 250 pp. (In press).

Fitzpatrick, R. W., Shand, P., and Merry, R. H. (2009). Acid sulfate soils. *In* "Natural History of the Riverland and Murraylands" (J. T. Jennings, ed.), pp. 65-111. Royal Society of South Australia (Inc.), Adelaide, South Australia.

Fraser, M. A., Baldwin, D. S., Rees, G. N., Silvester, E. J., and Whitworth, K. L. (2012). Rehabilitation options for inland waterways impacted by sulfidic sediments--field trials in a south-eastern Australian wetland. *J Environ Manage* **102**, 71-8.

Hammarstrom, J. M., Sibrell, P. L., and Belkin, H. E. (2003). Characterization of limestone reacted with acid-mine drainage in a pulsed limestone bed treatment system at the Friendship Hill National Historical Site, Pennsylvania, USA. *Applied Geochemistry* **18**, 1705-1721.

Haveman, S. A., Greene, E. A., Stilwell, C. P., Voordouw, J. K., and Voordouw, G. (2004). Physiological and Gene Expression Analysis of Inhibition of Desulfovibrio vulgaris Hildenborough by Nitrite. *Journal of Bacteriology* **186**, 7944-7950.

Hobday, A. J., and McDonald, J. (2014). Environmental Issues in Australia. *Annual Review of Environment and Resources* **39**, 1-28.

Hubert, C., and Voordouw, G. (2007). Oil Field Souring Control by Nitrate-Reducing *Sulfurospirillum* spp. That Outcompete Sulfate-Reducing Bacteria for Organic Electron Donors. *Applied and Environmental Microbiology* **73**, 2644-2652.

Isbell, R. (2002). "The Australian soil classification," CSIRO publishing, Australia.

Johnson, D. B., and Hallberg, K. B. (2005). Acid mine drainage remediation options: a review. *Sci Total Environ* **338**, 3-14.

Johnston, S. G., Slavich, P. G., and Hirst, P. (2005). Changes in surface water quality after inundation of acid sulfate soils of different vegetation cover. *Australian Journal of Soil Research* **43**, 1-12.

Jugsujinda, A., Delaune, R. D., Lindau, C. W., Sulaeman, E., and Pezeshki, S. R. (1996). Factors controlling carbon dioxide and methane production in acid sulfate soils. *Water Air and Soil Pollution* **87**, 345-355.

Kaster, K., Grigoriyan, A., Jennneman, G., and Voordouw, G. (2007). Effect of nitrate and nitrite on sulfide production by two thermophilic, sulfate-reducing enrichments from an oil field in the North Sea. *Applied Microbiology and Biotechnology* **75**, 195-203.

Lal, R. (2004). Agricultural activities and the global carbon cycle. *Nutrient Cycling in Agroecosystems* **70**, 103-116.

Lovley, D. R., and Phillips, E. J. (1987). Competitive mechanisms for inhibition of sulfate reduction and methane production in the zone of ferric iron reduction in sediments. *Applied and Environmental Microbiology* **53**, 2636-2641.

Luther III, G. W. (2005). Acid volatile sulfide—a comment. *Marine Chemistry* **97**, 198-205.

Lützow, M. v., Kögel-Knabner, I., Ekschmitt, K., Matzner, E., Guggenberger, G., Marschner, B., and Flessa, H. (2006). Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions – a review. *European Journal of Soil Science* **57**, 426-445.

Macdonald, B. C. T., Denmead, O. T., White, I., and Byrant, G. (2011). Gaseous Nitrogen Losses from Coastal Acid Sulfate Soils: A Short-Term Study. *Pedosphere* **21**, 197-206.

Mosley, L. M., Fitzpatrick, R. W., Palmer, D., Leyden, E., and Shand, P. (2014a). Changes in acidity and metal geochemistry in soils, groundwater, drain and river water in the Lower Murray River after a severe drought. *Science of The Total Environment* **485–486**, 281-291.

Mosley, L. M., Palmer, D., Leyden, E., Cook, F., Zammit, B., Shand, P., Baker, A., and Fitzpatrick, R. W. (2014b). Acidification of floodplains due to river level decline during drought. *Journal of Contaminant Hydrology* **161**, 10-23.

Mosley, L. M., Zammit, B., Jolley, A. M., and Barnett, L. (2014c). Acidification of lake water due to drought. *Journal of Hydrology* **511**, 484-493.

Muyzer, G., and Stams, A. J. M. (2008). The ecology and biotechnology of sulphate-reducing bacteria. *Nature Reviews Microbiology* **6**, 441-454.

Myers, C. R., and Nealson, K. H. (1988). Microbial reduction of manganese oxides: interactions with iron and sulfur. *Geochimica et Cosmochimica Acta* **52**, 2727-2732.

Neculita, C.-M., Zagury, G. J., and Bussiere, B. (2007). Passive treatment of acid mine drainage in bioreactors using sulfate-reducing bacteria: Critical review and research needs. *Journal of Environmental Quality* **36**, 1-16.

Ontiveros-Valencia, A., Ziv-El, M., Zhao, H.-P., Feng, L., Rittmann, B. E., and Krajmalnik-Brown, R. (2012). Interactions between Nitrate-Reducing and Sulfate-Reducing Bacteria Coexisting in a Hydrogen-Fed Biofilm. *Environmental Science & Technology* **46**, 11289-11298.

Österholm, P., Edén, P., Martinkauppi, A., Beucher, A., Yli-Halla, M., and Auri, J. (2010). Mapping and characterization of Boreal acid sulfate soils. *In* "19th World Congress of Soil Science, Soil Solutions for a Changing World", pp. 82-85, Brisbane, Australia.

Paul, R. B., and Ulf, S. (2011). Soil pH and pH Buffering. *In* "Handbook of Soil Sciences" (P. M. Huang, Y. Li and M. E. Sumner, eds.). CRC Press.

Pichtel, J. R., Dick, W. A., and McCoy, E. L. (1989). Binding of iron from pyritic mine spoil by water-soluble organic materials extracted from sewage sludge. *Soil Science* **148**, 140-148.

Ponnamperuma, F. (1972). The chemistry of submerged soils. *Advances in Agronomy* **24**, 29.

Pons, L. J. (1973). Outline of the genesis, characteristics, classification and improvement of acid sulphate soil. *In* "Proceedings of the 1972 (Wageningen, Netherlands) International Acid Sulfate Soils Symposium, Volume 1" (H. Dost, ed.). International Land Reclamation Institute Publication 18, Wageningen, Netherlands.

Powell, B., and Martens, M. (2005). A review of acid sulfate soil impacts, actions and policies that impact on water quality in Great Barrier Reef catchments, including a case study on remediation at East Trinity. *Marine Pollution Bulletin* **51**, 149-164.

Rickard, D. (2012). Chapter 6 - Sedimentary Pyrite. *In* "Developments in Sedimentology" (D. Rickard, ed.), Vol. 65, pp. 233-285. Elsevier.

Rickard, D., and Morse, J. W. (2005). Acid volatile sulfide (AVS). *Marine Chemistry* **97**, 141-197.

Rigby, P. A., Dobos, S. K., Cook, F. J., and Goonetilleke, A. (2006). Role of organic matter in framboidal pyrite oxidation. *Science of The Total Environment* **367**, 847-854.

Roychand, P., and Marschner, P. (2013). Respiration in a sand amended with clay – Effect of residue type and rate. *European Journal of Soil Biology* **58**, 19-23.

Shamshuddin, J., Elisa Azura, A., Shazana, M. A. R. S., Fauziah, C. I., Panhwar, Q. A., and Naher, U. A. (2014). Chapter Three - Properties and Management of Acid Sulfate Soils in Southeast Asia for Sustainable Cultivation of Rice, Oil Palm, and Cocoa. *In* "Advances in Agronomy" (L. S. Donald, ed.), Vol. Volume 124, pp. 91-142. Academic Press.

Shi, A., and Marschner, P. (2013). Addition of a clay subsoil to a sandy top soil alters CO_2 release and the interactions in residue mixtures. *Science of The Total Environment* **465**, 248-254.

Simek, M., Virtanen, S., Kristufek, V., Simojoki, A., and Yli-Halla, M. (2011). Evidence of rich microbial communities in the subsoil of a boreal acid sulphate soil conducive to greenhouse gas emissions. *Agriculture Ecosystems & Environment* **140**, 113-122.

Stokes, C. J., Ash, A., and Howden, S. M. (2008). Climate Change Impacts on Australian Rangelands. *Rangelands* **30**, 40-45.

Sullivan, L., Fitzpatrick, R., Bush, R., Burton, E., Shand, P., and Ward, N. (2010a). "The classification of acid sulfate soil materials: further modifications," Southern Cross University, Lismore, NSW, Australia.

Sullivan, L. A., Bush, R. T., Ward, N. J., Fyfe, D. M., Johnston, M., Burton, E. D., Cheeseman, P., Bush, M., Maher, C., Cheetham, M., Watling, K. M., Wong, V. N. L., R., M., and Weber, E. (2010b). "Lower Lakes laboratory study of contaminant mobilisation under seawater and freshwater inundation," Prepared by Southern Cross GeoScience for the SA Department of Environment and Natural Resources, Adelaide. .

Ward, N. J., Sullivan, L. A., and Bush, R. T. (2004). Soil pH, oxygen availability, and the rate of sulfide oxidation in acid sulfate soil materials: implications for environmental hazard assessment. *Australian Journal of Soil Research* **42**, 509-514.

Zhang, L., Keller, J., and Yuan, Z. (2009). Inhibition of sulfate-reducing and methanogenic activities of anaerobic sewer biofilms by ferric iron dosing. *Water Research* **43**, 4123-4132.

CHAPTER 2

Sulfate reduction in sulfuric material after re-flooding: Effectiveness of organic carbon addition and pH increase depends on soil properties

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Sulfate reduction in sulfuric material after re-flooding: Effectiveness of organic carbon addition and pH increase depends on soil properties

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HIGHLIGHTS

- Sulfate reduction can increase pH of sulfuric acid sulfate soils upon reflooding.
- Four treatments were imposed control, pH 5.5 + organic C and pH 5.5 + organic C.
- Sulfate reduction only occurred in treatment pH 5.5 + organic C.
- Sulfate reduction was negatively correlated with soil clay and nitrate concentrations.

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ABSTRACT

Sulfuric material is formed upon oxidation of sulfidic material; it is extremely acidic, and therefore, an environmental hazard. One option for increasing pH of sulfuric material may be stimulation of bacterial sulfate reduction. We investigated the effects of organic carbon addition and pH increase on sulfate reduction after re-flooding in ten sulfuric materials with four treatments: control, pH increase to 5.5 (+pH), organic carbon addition with 2% w/w finely ground wheat straw (+C), and organic carbon addition and pH increase (+C + pH). After 36 weeks, in five of the ten soils, only treatment +C + pH significantly increased the concentration of reduced inorganic sulfur (RIS) compared to the control and increased the soil pore water pH compared to treatment + pH. In four other soils, pH increase or/and organic carbon addition had no significant effect on RIS concentration compared to the control. The RIS concentration in treatment +C + pH as percentage of the control was negatively correlated with soil clay content and initial nitrate concentration. The results suggest that organic carbon addition and pH increase can stimulate sulfate reduction after re-flooding, but the effectiveness of this treatment depends on soil properties.

1. Introduction

Acid sulfate soils (ASS) are soils containing iron sulfide minerals (sulfidic material) or materials affected by their transformations such as sulfuric and monosulfidic materials. Acid sulfate soils can be found in coastal, inland, mine spoil and wetland environments [1]. Globally, the extent of coastal ASS is between 10^5 and 10^6 km^2; the extent of inland ASS is unknown [2,3]. In Australia, the estimated area of ASS is 215,000 km^2, of which coastal ASS occupy 27% and inland ASS 73% [4]. Under flooded conditions, sulfur in ASS is present as iron sulfide minerals, mainly pyrite (FeS_2). Its content is influenced by the duration of saturation and availability of organic matter, reactive iron, and dissolved sulfate [5]. Sulfate from ocean or fresh water is first reduced to H_2S by sulfate-reducing bacteria. Polysulfide can be derived from H_2S by microbial oxidation or chemical transformation. H_2S or polysulfide react with dissolved Fe(II) to form pyrite [5–9]. Pyrite in sulfidic material is stable under anaerobic conditions. Oxidation of pyrite, which can be caused by human or natural disturbance such as drainage or drought, induces formation of sulfuric acid which may lead to sulfuric material ($pH < 4$) [1,10,11]. Sulfuric material can release acid and dissolved metals, which may cause, for example, poor water quality, deterioration of aquatic or wetland ecosystems, loss of fisheries and agricultural production, acidic corrosion of infrastructure [1].

To remediate sulfuric material, soil acidity can be neutralized by addition of chemical ameliorants such as lime. However, liming may be costly and ineffective, particularly when the sulfuric

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Table 1
Selected properties of soils used in the experiment.[a]

No.	Depth (cm)	Water content (g g–^{1})	Sand (%)	Clay (%)	pH (1:1)	TOC (%)	Nitrate-N (mg kg^{-1})	S_{HCl} (%)	S_{KCl} (%)
Profile: DSa01A[b], sulfuric clay soil[b], TypicSulfaquert or SulficSulfaquert[c]									
1	57–95	0.6	1.3	75.3	3.87	2.5	1.8	0.48	0.36
2	95–190	0.64	1.9	53.7	2.9	2.3	0.5	0.83	0.55
3	190–280	0.45	1.6	56.1	3.59	1.4	24	0.45	0.31
4	280–350	0.48	1.8	58.7	3.61	1.4	72.5	0.41	0.29
Profile: DSa01B[b], sulfuric clay soil[b], TypicSulfaquert or SulficSulfaquert[c]									
5	76–190	0.48	5.5	56.2	4	1.6	4.2	0.2	0.13
6	190–250	0.67	1.1	57.4	3.34	1.8	4.7	0.56	0.32
7	250–290	0.6	2.2	56.9	3.51	1.7	0.2	0.49	0.34
8	290–340	0.26	1.8	65.1	3.82	1.6	2.4	0.37	0.27
Profile: DSa01C[b], sulfuric clay soil[b], TypicSulfaquert or SulficSulfaquert[c]									
9	140–200	0.11	22.3	42.1	3.55	2.8	6	0.23	0.11
10	200–255	0.16	2.9	44.9	3.36	2.1	0	0.55	0.41

[a] Values are means of two or three replicates except for depth, water content, and texture where $n = 1$. pH(1:1), pH measured in a 1:1 soil to water ratio; TOC, total organic carbon; SHCl, 4 M HCl extractable sulfur; SKCl, 1 M KCl extractable sulfur.

[b] ASS Subtype classification; modified from [24].

[c] Soil taxonomy [27].

material is in deeper soil layers or has a high clay content [12]. Microbial sulfate reduction after re-saturation of sulfuric material could result in pH increase, and therefore, be considered as a more economical and environmentally-friendly amelioration strategy. However, microbial sulfate reduction is constrained by environmental factors such as redox conditions, availability of sulfate and organic carbon (as energy source for sulfate-reducing bacteria), and pH (most sulfate reducers are inactive at pH < 5) [1,5,9,13]. Sulfate reduction can also be inhibited by the presence of competing electron acceptors, such as Fe(III) and nitrate [9,13,14]. Due to these constraining factors, re-flooding or re-saturation of sulfuric material may not lead to sulfate reduction [14,15]. Additionally, the relative influence of the constraining factors may vary with ASS properties. A better understanding of factors influencing microbial sulfate reduction after re-flooding is needed to improve amelioration strategies for sulfuric material.

Organic matter has been used to ameliorate acid mine drainage [13,16,17], but little is known about the effectiveness of this treatment in sulfuric material. Sullivan et al. [15] monitored sulfate reduction after re-flooding of lake sediments containing sulfuric material and concluded that it was mainly limited by the availability of organic carbon based on the positive relationship between sulfate reduction rate and organic carbon concentration of the sediment. Field trials conducted by Fraser et al. [18] in an Australian wetland with sulfuric sediments showed that only liming or liming with revegetation increased soil pH significantly. They observed black monosulfidic material only in limed and planted plots, not in plots that were only limed. However, to our knowledge, there are no published studies systematically investigating the influence of organic matter addition and pH increase separately or in combination on sulfate reduction after re-flooding of sulfuric material. Further, the effects of these treatments may depend on soil properties such as texture and concentrations of competing electron acceptors. The aims of this study were to: (i) investigate the effects of pH increase to 5.5 and organic matter addition separately or combined on sulfate reduction after re-flooding of sulfuric material, and (ii) determine the influence of selected soil properties on the effects of these treatments. Previous studies and field observations in South Australia showed that sulfate reduction did not occur after re-flooding of sulfuric material even when they were limed [10,19]. Limitation of sulfate reduction by sulfate or pyrite formation by iron is unlikely because sulfate and iron concentrations in drainage water from these ASS are high [10,19]. This indicates that not only low pH but also the low availability of native organic matter may limit sulfate reduction. In soils, availability of native and added organic matter to microbes can be reduced by binding to clay [20–22]. Therefore, we hypothesized that after re-flooding of sulfuric material: (i) pH increase or organic matter addition alone will have little effect on sulfate reduction, (ii) only a combined treatment of organic matter addition and pH increase will induce a significant increase in sulfate reduction compared to the control, and (iii) the increase in sulfate reduction by this treatment will be related to soil properties such as texture and competing electron acceptors such as nitrate.

2. Materials and methods

2.1. Sulfuric material

Sulfuric materials were collected from the Lower Murray Reclaimed Irrigation Area (LMRIA), which is an important agricultural area in South Australia with a large distribution of ASS [10]. Before 2007, soils in the LMRIA were mainly sulfidic, but persistent drought and groundwater level declined between 2008 and early 2010 resulted in oxidation of sulfidic material to form sulfuric material even at depth [23–25]. Now after re-flooding and irrigation (since 2011), even limed soils still contain sulfuric material and sulfate reduction does not seem to occur [10,24]. The ten sulfuric materials used in this experiment were from three soil profiles along a transect in a pasture adjacent to the River Murray at Long Flat (35°7′28.05″S–139°17′55.17″E) near Murray Bridge, South Australia (Table 1) [10,24]. After taking the soil cores in 2011, soil layers and horizons were defined, classified and soil properties determined for each layer. The three profiles were classified as acid sulfate soil subtype sulfuric clay soils in accordance with the Atlas of Australian acid sulfate soils [4,24,26] or Typic or Sulfic Sulfaquerts in Soil Taxonomy [27] (Table 1). The soil cores were then stored in plastic boxes covered loosely at room temperature until they were used in this experiment (May 2013). The ten soils used for the present study were classified as sulfuric material [27,28] (i.e. pH (1:1) < 4.0; Table 1) and comprised a wide range of other properties such as soil texture and concentrations of soil nitrate and organic carbon (Table 1).

2.2. Experimental design

There were four treatments with three replicates for each soil: Ctrl (control), +pH (pH increase to 5.5 ± 0.1), +C (organic carbon (wheat straw) addition), and +C + pH (organic carbon addition and pH increase). Twenty grams of moist soil (corresponding to 12–18 g dry soil) was mixed with 20 ml reverse osmosis (RO) water in a 70 ml polypropylene container. Wheat straw (total organic carbon

31%, total nitrogen 0.7%; ground to <2 mm) was added at 2% (w/w dry weight basis). Soil pH was then increased to 5.5 ± 0.1 by adding 1 M NaOH and mixing it with soil until the pH of the soil paste was 5.5 ± 0.1 24 h after adjustment. Between 0.80 and 4.25 ml 1 M NaOH was required. Then 40 ml RO water was added, and lids of the containers were screwed on tightly to minimize oxygen diffusion into the soils. The soil was incubated for 36 weeks at room temperature (20–25 °C) in the dark. Small amounts of water were added if necessary to maintain the water level in the containers.

2.3. Measurements

Before the start of the experiment, selected soil properties were determined (Table 1). Soil total organic carbon (TOC) was measured by the Walkley–Black method [29]. Sulfur was extracted by shaking for 4 h with 1 M KCl (S_{KCl}) or for 16 h with 4 M HCl (S_{HCl}) according to Ahern et al. [30]. Sulfur in the extract was measured by ICP-AES. S_{KCl} represents mainly soluble and exchangeable sulfate, while S_{HCl} also recovers relatively insoluble sulfate in iron oxyhydroxy sulfate minerals such as jarosite and natrojarosite that are commonly found in sulfuric material [24,30]. Nitrate concentrations in the 1 M KCl extracts were determined using the cadmium reduction method [31]. Soil texture (sand and clay content) was analyzed according to Kettler et al. [32].

During incubation, pH of the overlaying water (pHow) and of the soil pore water (pHspw; by inserting the electrode into soil) were monitored with an electrode (Model IJ44C, Ionode, Australia) weekly from weeks 0 to 12 and every two weeks from weeks 12 to 36. After 36 weeks, dissolved oxygen (DO_2) in the overlaying water was measured with a DO_2 electrode (Model ED1, TPS, Australia). Redox potential of overlying water (Ehow) and soil pore water (Ehspw; by inserting the electrode into soil) were measured with an electrode (Model IJ64, Ionode, Australia) only in soil 10 due to the long stabilization time. Soil 10 was chosen because it was considered representative of most soils used in this study. After incubation, soil reduced inorganic sulfur (RIS) concentration was determined as the sum of acid volatile sulfide (AVS) and chromium reducible sulfur (Scr mainly pyrite). Acid volatile sulfide was measured using the method described by Simpson [33]. The remaining soil was dried at 80 °C for 48 h (which removes AVS [30]), ground and passed through a 0.25 mm sieve for pyrite measurement as described by Ahern et al. [30]. About 94% of RIS was pyrite (Scr); AVS accounted for only 6%.

2.4. Data analysis

Pearson's correlation and two-way ANOVA (soil and treatment as fixed factors) were carried out for properties of soil and overlying water at the end of the experiment (week 36) and significant differences among soils and treatments were determined by Fisher's protected least significant difference test using GenStat 16.2 (VSN International Ltd., UK). For the relationship between RIS concentration and initial soil properties, Pearson's correlations were calculated using Genstat, and principal component analysis (PCA) was carried out with PRIMER 6 (Primer-E Ltd Plymouth Marine Laboratory, UK). Measured Ehow and Ehspw data of soil 10 was superimposed in a pH–Eh diagram for the iron–sulfur system as described in Mosley et al. [10].

3. Results

3.1. Soil morphology

After one week under flooded conditions, yellow–brown films (likely Fe(III)-oxyhydroxides) began to form on the soil surface (Fig. S1a); later they were also found on the inner container wall and/or, for some soils, at the bottom of the soil. The yellow–brown films occurred only or to a larger extent in treatments +C and +C + pH (Fig. S1b).

After two weeks, black material (likely monosulfides (FeS)) became visible (Fig. S1). Substantial amounts of black material were observed in soils 1, 5, 8, 9 and 10 with the highest amounts in soils 5 and 9, but only in treatment +C + pH. There was no or only negligible black material in soils 2, 3, 4, 6 and 7. The amount of black material changed with time. In soils 1, 5 and 9 the amount of black material peaked before week 6 and then either decreased (soil 1) or remained stable (soils 5 and 9). The amount of black material in soils 8 and 10 increased until week 12, then remained stable before decreasing from around week 24 (see Fig. S1b for soil 10).

3.2. pH of overlying water and soil pore water

After about 24 weeks, the pH of overlying water and soil pore water became relatively stable (Fig. 1). For most soils, values of pHow and pHspw were in the order: Ctrl ≤ + C < + pH ≤ + C + pH. During incubation, for treatments Ctrl and +C, both pHow and pHspw remained stable or dropped only slightly (by <0.5). But for treatment +pH, pHow and pHspw decreased substantially from the adjusted value of 5.5 by 0.7–1.9 units in all soils except soil 5.

In treatment + C + pH, compared with week 0, pHow decreased rapidly in the first 4–8 weeks except in soils 5 and 9, after which it stabilized (soils 2, 3, 4, 6 and 7) or increased (soils 1, 8 and 10) (Fig. 1). In contrast, pHspw increased rapidly during the first four weeks in soils 1, 3, 6, 7, 8 and 10, and increased more gradually in soils 5 and 9. After this increase, pHspw decreased or stabilized in most soils. However, pHspw did not increase in soils 2 and 4. pHow and pHspw differed from each other more strongly in the first 12 weeks than towards the end of the experiment.

3.3. Properties of overlying water and soil after 36 weeks

Treatment, soil, and their interaction had significant effects on pHow pHspw, RIS, and DO_2 expressed as absolute values (Fig. 2) and as percentages in the amended treatments relative to the control (Fig. 3).

Treatment effects were similar for pHow and pHspw. Compared to the control, addition of organic carbon alone (treatment +C) did not influence pHow and pHspw significantly except in soil 2 where pHspw was higher in +C, but treatments +pH and +C + pH increased pHow and pHspw significantly in all soils by 12–92% (Figs. 2 and 3). In soils 3, 5, 8 and 9, the percentage increase in pHow and pHspw relative to the control was greater in +C + pH (35–92%) than in +pH (12–36%). In soil 10, the percentage increase in pHspw (but not pHow) was also greater in +C + pH (65%) than in +pH (35%). In the other soils (1, 2, 4, 6 and 7), treatments C + pH and +pH increased both pHow and pHspw by 21–44% relative to the control.

Compared to the control, the RIS concentration was significantly increased in treatment +C + pH in six of the ten soils, but not in soils 1, 4, 6 and 7 (Fig. 2). The increase in RIS concentration in +C + pH as percentage of the control was greatest in soils 5, 9 and 10 (10-fold–12-fold) (Fig. 3). It was smaller in soil 8 (around 5-fold) and smallest in soils 2 and 3 (around 3-fold). The RIS concentration in treatment +C was no significantly different from in the control. Treatment +pH significantly increased the RIS concentration compared to the control only in soil 2 where it was twice as high as in the control.

Compared to the control, the pH adjustment alone (treatment +pH) significantly reduced the concentration of dissolved oxygen in the overlying water (DO_2) only in soils 1 and 9. However, in eight of the ten soils (except soils 2 and 10), the DO_2 concentration was lower in treatments +C or +C + pH than in the control by 14–42% (Figs. 2 and 3).

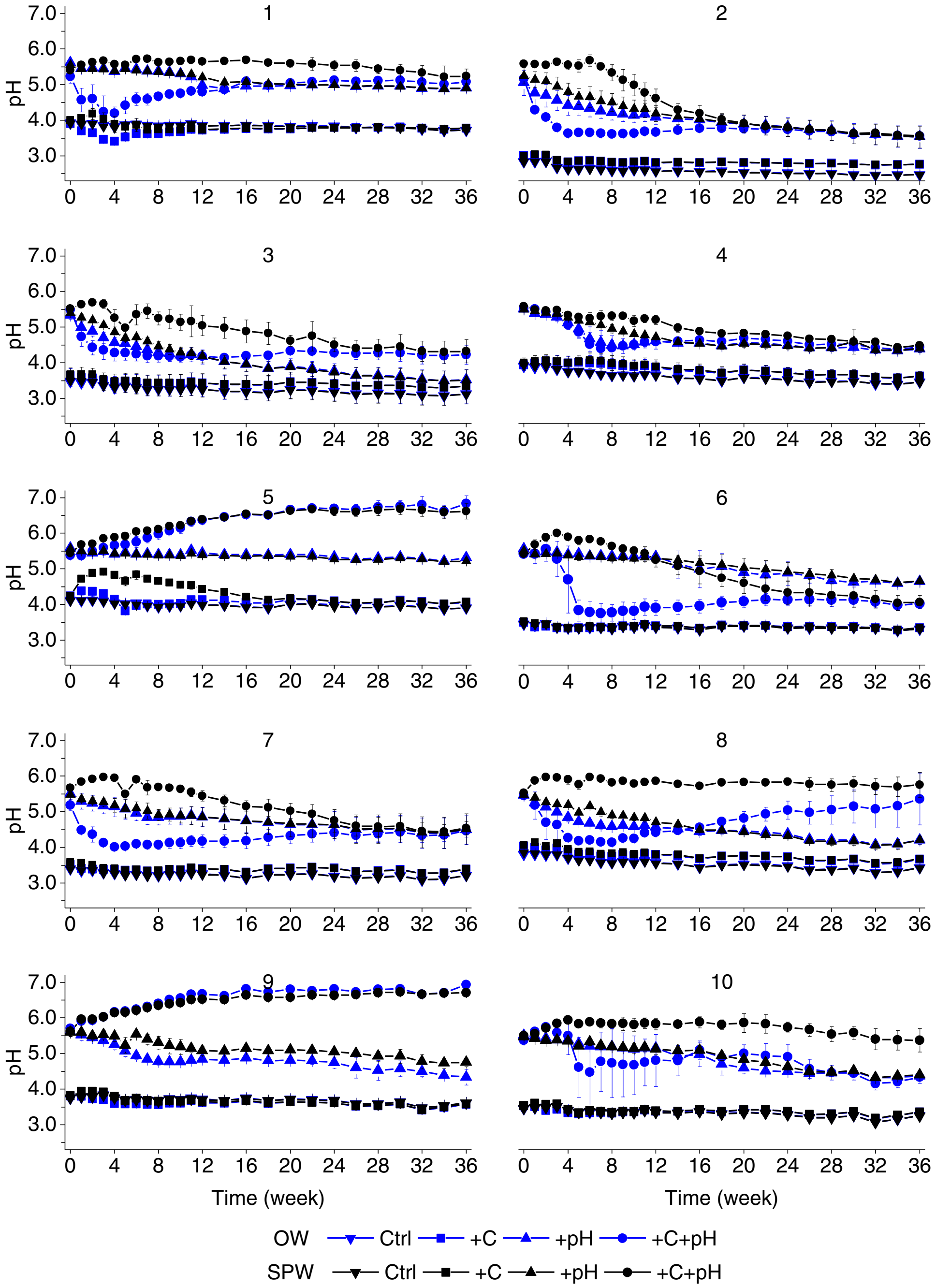

Fig. 1. pH of overlying water (OW, blue symbols) and soil pore water (SPW, black symbols) in ten soils over 36 weeks in treatments: control (Ctrl); organic carbon addition (+C); pH increase to 5.5 ± 0.1 (+pH); organic carbon addition and pH increase (+C + pH). Error bars show standard deviations ($n = 3$).

Expressing parameters in the amended treatments as percentage of the control, RIS concentration was positively correlated with pH of the overlying water and soil pore water ($r = 0.63$ and 0.70) and negatively correlated with oxygen concentration of the overlying water ($r = -0.34$) (Fig. 3).

3.4. Relationship between RIS concentration in treatment +C + pH and initial soil properties

To understand how initial soil properties influenced the effectiveness of treatment +C + pH in stimulating sulfate reduction, correlations between RIS concentration in this treatment in percentage of the control and initial soil properties were calculated (Table 2). RIS concentration was positively correlated with sand content, but negatively correlated with initial soil water content, clay content, and concentrations of nitrate and extractable sulfur. Soil water content was negatively correlated with sand content but positively correlated with clay content and extractable sulfur concentrations. TOC content was negatively correlated with soil depth and nitrate concentration but positively correlated with sand content. These relationships can also be visualized in a PCA plot (Fig. S2).

3.5. Redox potential in soil 10

The pH–Eh (redox potential) diagram for the iron–sulfur system suggested that in treatment +C + pH conditions in soil 10 were favorable for pyrite predominance in both weeks 18 and 36 (Fig. S3).

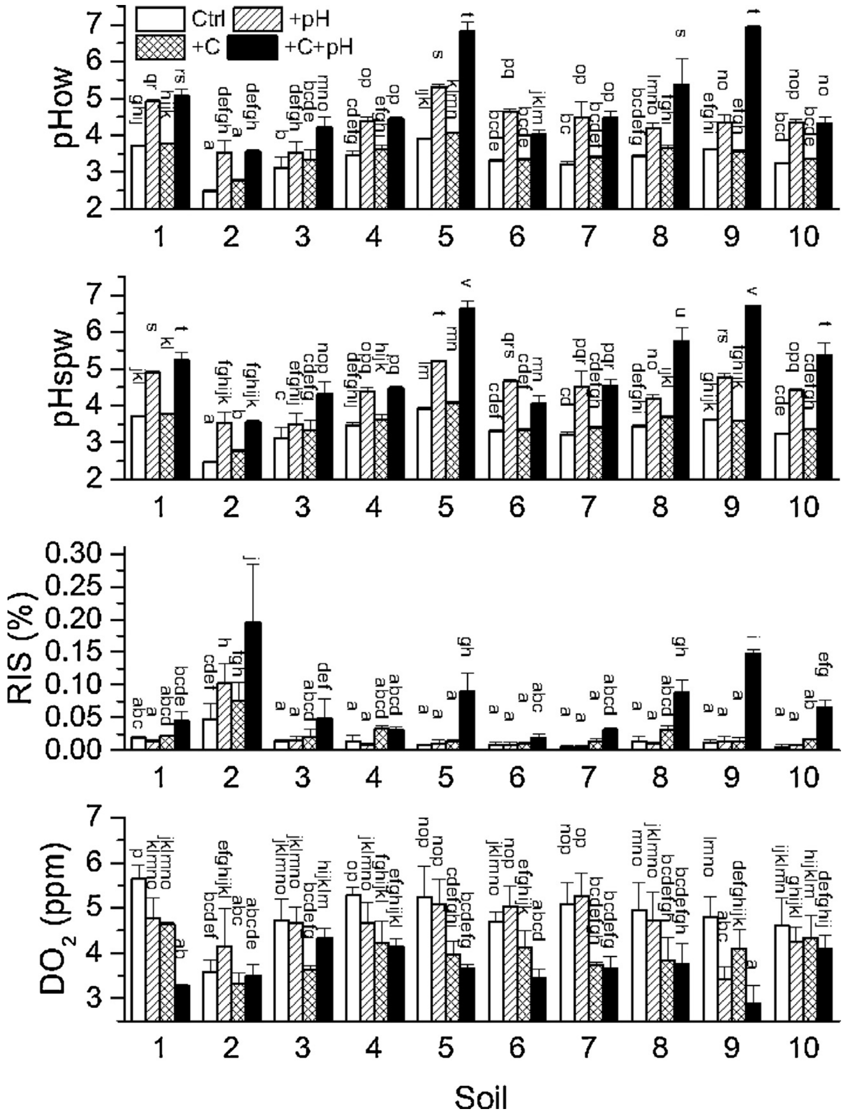

Fig. 2. pH of overlying water (pHow) and soil pore water (pHspw), reduced inorganic sulfur in the soil (RIS), and concentration of dissolved oxygen in overlying water (DO_2) in soils 1–10 after 36 weeks. Treatments: control (Ctrl); pH increase to 5.5 ± 0.1 (+ pH); organic carbon addition (+ C); organic carbon addition and pH increase (+C + pH). Error bars show standard deviations ($n = 3$). Values that do not share same letters are significantly different at $P \leq 0.05$ (soil×treatment interaction).

In contrast, conditions in the overlying water in this treatment were suitable for the formation of poorly crystalline Fe(III)-oxyhydroxy oxides such as ferrihydrite or [$Fe(OH)_3(a)$] in week 18 but not in week 36 when the pH was lower than in week 18 and ferrous iron (Fe^{2+}) was predicted to be dominant. In the other treatments, pH and Eh in both the overlying and soil pore water were similar in weeks 18 and 36 and the diagram predicted the dominance of ferrous iron.

4. Discussion

This study showed that only the combined treatment of increasing pH to 5.5 and adding organic carbon (+C + pH) induced significant sulfate reduction in the sulfuric materials (pH < 4) after re-flooding, which increased soil pH compared to treatment with pH increase alone (+pH). However, this was only the case in some soils, indicating that the effectiveness of treatment +C + pH to stimulate sulfate reduction is influenced by other soil properties. The results confirm our hypotheses, namely, after re-flooding of sulfuric material: (i) pH increase or organic matter addition alone has little effect on sulfate reduction, (ii) only the combined treatment (+C + pH) induces a significant increase in sulfate reduction compared to the control, and (iii) the increase in sulfate reduction by this treatment is related to soil properties such as soil texture and the presence of competing electron acceptors such as nitrate.

4.1. Factors limiting sulfate reduction

In the sulfuric materials used in this study, both low pH and low organic carbon availability limited microbial sulfate reduction because neither pH increase alone (treatment +pH) nor organic carbon addition alone (treatment +C) induced sulfate reduction compared to the control, while the combined treatment (+C + pH) resulted in a significant increase in sulfate reduction in some soils (Fig. 2).

Most sulfate reducing bacteria require a pH range from 5 to 8 for their growth [13,34]. Although microbial sulfate reduction has been reported at low pH in lakes and rivers, wetlands, mine tailings and bioreactors [34,35], to our knowledge, it has not been observed in soils with pH < 4. In the soils used, the absence of acidotolerant or acidophilic sulfate reducing bacteria may be because the soils were not acidic before acidification [25].

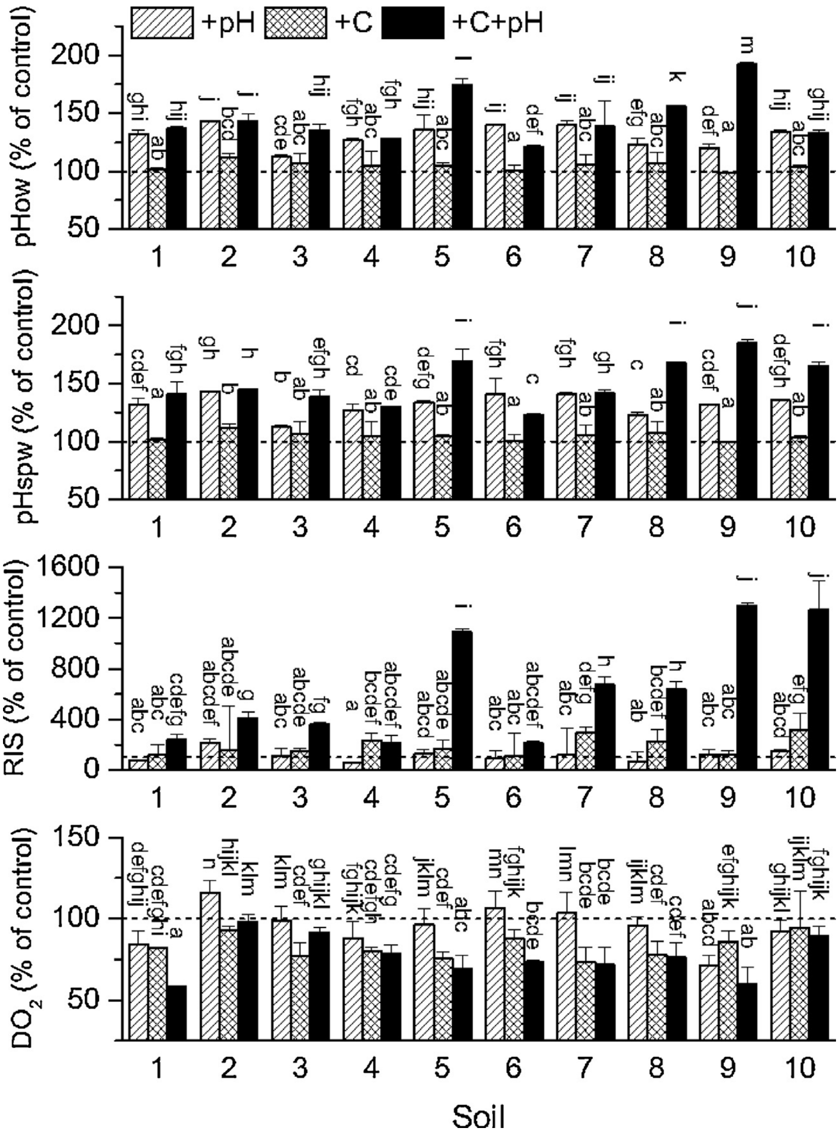

Fig. 3. Properties of overlying water and soil pore water in soils 1–10 after 36 weeks as percentage relative to the control. Error bars show standard deviations ($n = 3$). Values that do not share same letters are significantly different at $P \leq 0.05$ (soil × treatment interaction). The dashed line indicates 100% (control). For property and treatment abbreviations see Fig. 2.

Native organic matter was limiting sulfate reduction even though the native TOC content ranged between 1.4% and 2.8% (Table 1), which is relatively high compared to many Australian soils [36]. The low availability of organic carbon can be explained by the high clay content (mostly >50%) of these soils and soil acidification during the oxidation period prior to the collection of these soils. Accessibility of organic matter to decomposing microbes is reduced by (i) binding to soil particles via ligand exchange and cation (e.g. Ca^{2+}, Fe^{3+}, Al^{3+}) bridges, particularly to clay or precipitated minerals (e.g. iron minerals such as jarosite),

Table 2
Correlations between RIS concentration in treatment +C + pH in percentage of the control and initial soil properties.[a]

Variables	Depth	Water content	pH (1:1)	TOC	Nitrate	S_{HCl}	S_{KCl}	%Sand	%Clay	%RIS (+C + pH)
Depth	**1**									
Water content	−0.22	**1**								
pH(1:1)	−0.03	−0.19	**1**							
TOC	**−0.67**	−0.27	-0.22	**1**						
Nitrate-N	**0.47**	0.04	0.10	**−0.47**	**1**					
S_{HCl}	−0.07	**0.49**	**−0.83**	0.09	−0.15	1				
S_{KCl}	−0.04	**0.43**	**−0.71**	0.04	−0.12	0.97	1			
%Sand	−0.22	**-0.61**	0.07	**0.61**	−0.12	**-0.53**	**−0.63**	**1**		
%Clay	−0.12	**0.54**	**0.46**	−0.21	0.05	0.06	0.16	**−0.60**	**1**	
%RIS (+C + pH)	−0.11	**−0.72**	0.12	0.34	**−0.37**	**−0.44**	**−0.42**	**0.62**	**−0.65**	**1**

[a] Soil properties: depth, depth; water content, %sand, sand content; %clay, clay content; %RIS (+C + pH), reduced inorganic sulfur in the treatment +C + pH in percentage of the control. For other soil properties see Table 1. *r* values in bold are significant at $P \leq 0.05$.

(ii) intercalation in clay layers, and (iii) occlusion within aggregates [20]. The low pH in sulfuric material results in high concentrations of soluble Al and Fe, which may further reduce accessibility of soil organic carbon by the formation of stable complexes [20]. These mechanisms can reduce the availability of both native and added organic carbon to soil microbes [20–22].

Competing electron acceptors can also prevent or reduce sulfate reduction. After re-flooding, oxygen is first used for the decomposition of organic carbon. With the decrease of redox potential, nitrate, Mn(IV), Fe(III), and then sulfate are sequentially reduced [14]. Nitrate, which was abundant in soils 3 and 4 (Table 1), can inhibit sulfate reduction because it: (i) is a more energetically favorable electron acceptor competing with sulfate for electrons generated by decomposition of organic carbon [14,37], (ii) can oxidize reduced sulfur [37], and (iii) can indirectly inhibit a key enzyme involved in sulfate reduction [38]. Fe(III) is also a more energetically favorable electron acceptor, which can inhibit sulfate reduction [14] by competing for electrons from organic matter and by oxidizing reduced sulfur [39,40]. In the soils used here, Fe(III) could be in the form of jarosite [$KFe_3(SO_4)_2(OH)_6$] and natrojarosite [$NaFe_3(SO_4)_2(OH)_6$] as indicated by the difference between S_{HCl} and S_{KCl} (Table 1). Mosley et al. [10] and Fitzpatrick et al. [24] have reported these minerals in similar soils in the LMRIA. The yellow–brown films at the sediment–water interface (Fig. S1) also suggested the presence of Fe(III). After re-flooding, reductive dissolution of Fe(III) minerals releases dissolved Fe^{2+}, which can diffuse into the overlying water and be re-oxidized and precipitated as iron oxides if oxygen is present [10,41]. The reduction of Fe(III) is desirable because it produces alkalinity and dissolved Fe(II) which together with reduced sulfur can be used for RIS formation [42,43].

4.2. *Correlation of sulfate reduction with initial soil properties*

The RIS concentration expressed as percentage of the control allows better comparison of the effectiveness of treatments among soils than absolute RIS concentration. The correlation analysis (Table 2) showed that RIS concentration in treatment +C + pH as percentage of the control was positively correlated with sand content but negatively correlated with clay content, nitrate and extractable sulfur concentrations and soil water content. The positive correlation with sand content and negative correlation with clay content can be explained by the decrease in organic matter accessibility to microbes by clay [20]. Sand particles have a low potential for binding organic matter [20], therefore, most of the added organic carbon likely remained available. Clay particles on the other hand can bind organic matter; therefore, the proportion of added organic carbon available for sulfate reducers decreased with increasing clay content. Reduction of availability of freshly added organic matter to soil microbes by clay has been shown in previous studies [21,22]. The negative correlation with nitrate can be explained by the fact that nitrate is a competing electron acceptor, as mentioned above. In agreement with this, we recently showed that high nitrate addition rates have a negative effect on sulfate reduction [44]. Electrons from organic matter decomposition would be used for nitrate reduction first [14] and less would remain for sulfate reduction. The negative correlations with initial soil water content and extractable sulfur concentrations may be indirect. Soil water content was positively correlated with clay content. During storage of the soil cores prior to this experiment, clay-richer soils would have retained more water than lighter-textured soils. This higher water content may facilitate pyrite oxidation during storage [45], thus leading to higher extractable sulfur concentrations and lower pH in the moister soils. Pyrite oxidation/sulfate production leads to the generation of Fe(III) minerals including jarosite [1,13], which can inhibit sulfate reduction as competing electron acceptors and by binding organic carbon as mentioned above.

Treatment +C + pH significantly increased RIS concentration compared to the control in soils 2, 3, 5, 8, 9 and 10 (Fig. 2); the percentage increase in RIS was least in soils 2 and 3 and greatest in soils 5, 9 and 10 (Fig. 3). The strong stimulation of sulfate reduction in soils 5, 9, and 10 can be explained by their low concentration of clay, nitrate and extractable sulfur. In these soils, more of the added organic matter remained available for sulfate reducers compared to other soils where it was bound by clay or utilized for reducing other more energetically favorable electron acceptors (nitrate, Fe(III)). The percentage increase in RIS was smaller in soil 8 which had a higher clay content than soils 5, 9 and 10. Treatment +C + pH did not increase RIS concentration or induced only a small increase compared to the control in soils 1, 2, 3, 4, 6 and 7. The lower effectiveness of treatment +C + pH in these soils can be explained by the high clay content in soil 1, high nitrate concentration in soils 3 and 4, and high extractable sulfur (thus concomitant Fe(III)) concentration in soils 2, 6 and 7.

4.3. *Other observations*

Iron oxides that occurred on the inner container wall and the soil surface during the experiment particularly in treatment +C + pH (also suggested by the pH–Eh diagram (Fig. S3)) were likely the result of re-oxidation of Fe^{2+} that was reduced from Fe(III) in the soil and diffused into the overlying water [10,41]. Bacteria oxidizing Fe(II) to Fe(III) are chemolithotrophic [13,46], but Fe(III) reduction requires organic carbon. This explains why iron oxides occurred only or to a larger extent in treatments +C + pH and + C. The decrease in pH of the overlying water in treatment +C + pH during the first 4–8 weeks in most soils could be due to precipitation of iron oxides which releases H^+ [13]. Depletion of oxygen by decomposition of organic carbon added and re-oxidation of Fe^{2+} in the overlying water can explain the lower DO_2 concentrations in treatments +C + pH and +C compared to the control.

5. Conclusion

This study showed that in the sulfuric materials used low pH and low availability of native organic matter limited sulfate reduction. The results have implications for management of sulfuric ASS because the effectiveness of the combined treatment of raising pH to >5 and adding organic matter is likely to be greater in light-textured soils than in clay-rich soils and further influenced by the concentrations of competing electron acceptors.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jhazmat.2015.05.013

References

[1] R.W. Fitzpatrick, P. Shand, R.H. Merry, Acid sulfate soils, in: J.T. Jennings (Ed.), Natural History of the Riverland and Murraylands, Royal Society of South Australia (Inc.), Adelaide, South Australia, 2009, pp. 65–111.
[2] A. Wim, M.E.F.V. Mensvoort, Acid sulfate soils: distribution and extent, in: R. Lal (Ed.), Encyclopedia of Soil Science, CRC Press, 2005, pp. 14–19.

[3] B.C.T. Macdonald, O.T. Denmead, I. White, G. Byrant, Gaseous nitrogen Losses from coastal acid sulfate soils: a short-term study, Pedosphere 21 (2011) 197–206.

[4] R. Fitzpatrick, S. Marvanek, B. Powell, G. Grealish, R. Gilkes, Atlas of Australian acid sulfate soils: recent developments and future priorities, in: R. Gilkes, N. Prakongkep (Eds.), Proceedings of the 19th World Congress of Soil Science: Soil Solutions for a Changing World, Brisbane, Australia, 1–6 August, 2010, 2010, Published on DVD; ISBN 987-0-646-53783-2; http//www.iuss.org; Symposium WG 3.1 Processes in acid sulfate soil materials, pp. 24–27.

[5] R.A. Berner, Sedimentary pyrite formation: an update, Geochim. Cosmochim. Acta 48 (1984) 605–615.

[6] D. Rickard, J.W. Morse, Acid volatile sulfide (AVS), Mar. Chem. 97 (2005) 141–197.

[7] G.W. Luther III, Acid volatile sulfide—a comment, Mar. Chem. 97 (2005) 198–205.

[8] D. Rickard, Sedimentary pyrite, in: D. Rickard (Ed.), Developments in Sedimentology, Elsevier, 2012, pp. 233–285 (Chapter 6).

[9] G. Muyzer, A.J.M. Stams, The ecology and biotechnology of sulphate-reducing bacteria, Nature Rev. Microbiol. 6 (2008) 441–454.

[10] L.M. Mosley, R.W. Fitzpatrick, D. Palmer, E. Leyden, P. Shand, Changes in acidity and metal geochemistry in soils, groundwater, drain and river water in the Lower Murray River after a severe drought, Sci. Total Environ. 485-486 (2014) 281–291.

[11] L.M. Mosley, B. Zammit, A.M. Jolley, L. Barnett, Acidification of lake water due to drought, J. Hydrol. 511 (2014) 484–493.

[12] S.-E. Dear, N.G. Moore, S.K. Dobos, K.M. Watling, C.R. Ahern, Soil Management Guidelines, Queensland Acid Sulfate Soil Technical Manual, Department of Natural Resources and Mines, Indooroopilly, Queensland, Australia, 2002.

[13] C.-M. Neculita, G.J. Zagury, B. Bussiere, Passive treatment of acid mine drainage in bioreactors using sulfate-reducing bacteria: critical review and research needs, J. Environ. Qual. 36 (2007) 1–16.

[14] F. Ponnamperuma, The chemistry of submerged soils, Adv. Agron. 24 (1972) 29.

[15] L.A. Sullivan, R.T. Bush, N.J. Ward, D.M. Fyfe, M. Johnston, E.D. Burton, P. Cheeseman, M. Bush, C. Maher, M. Cheetham, K.M. Watling, V.N.L. Wong, M.R.E. Webe, Lower Lakes Laboratory Study of Contaminant Mobilisation under Seawater and Freshwater Inundation, Prepared by Southern Cross GeoScience for the SA Department of Environment and Natural Resources, Adelaide, 2010.

[16] M.C. Costa, E.S. Santos, R.J. Barros, C. Pires, M. Martins, Wine wastes as carbon source for biological treatment of acid mine drainage, Chemosphere 75 (2009) 831–836.

[17] M. Martins, M.L. Faleiro, R.J. Barros, A.R. Verissimo, M.C. Costa, Biological sulphate reduction using food industry wastes as carbon sources, Biodegradation 20 (2009) 559–567.

[18] M.A. Fraser, D.S. Baldwin, G.N. Rees, E.J. Silvester, K.L. Whitworth, Rehabilitation options for inland waterways impacted by sulfidic sediments–field trials in a south-eastern Australian wetland, J. Environ. Manage. 102 (2012) 71–78.

[19] D. Palmer, E. Leyden, M. Fogg, P. Mettam, K. Fradley, L.M. Mosley, Lower Murray Reclaimed Irrigation Area (LMRIA) Acidification Risk Project: Scoping and Trialling of Remediation Options, Environment Protection Authority, South Australia, 2013.

[20] M. v. Lützow, I. Kögel-Knabner, K. Ekschmitt, E. Matzner, G. Guggenberger, B. Marschner, H. Flessa, Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions – a review, Eur. J. Soil Sci. 57 (2006) 426–445.

[21] P. Roychand, P. Marschner, Respiration in a sand amended with clay – effect of residue type and rate, Eur. J. Soil Biol. 58 (2013) 19–23.

[22] A. Shi, P. Marschner, Addition of a clay subsoil to a sandy top soil alters CO_2 release and the interactions in residue mixtures, Sci. Total Environ. 465 (2013) 248–254.

[23] L.M. Mosley, D. Palmer, E. Leyden, F. Cook, B. Zammit, P. Shand, A. Baker, R.W. Fitzpatrick, Acidification of floodplains due to river level decline during drought, J. Contam. Hydrol. 161 (2014) 10–23.

[24] R., Fitzpatrick, P., Shand, S., Simpson, S., Grocke, M., Raven, A., Baker, L., Mosley, P. Self, Assessment of re-flooded acid sulfate soil environments at Long Flat, Jervois, Toora and Pompoota in the Lower Murray Reclaimed Irrigation Area (LMRIA), CSIRO Land and Water Science Report 2013. 03/12. 250 pp. (In press), 2013.

[25] R., Fitzpatrick, M., Raven, P., Self, P., Shand, L., Mosley, S., Simpson, A. Baker, Occurrence, genesis and environmental significance of schwertmannite in re-flooded Acid Sulfate Soils. In: the Lower Murray Reclaimed Irrigation Area, in: Combined Australian Regolith Geoscientists Association and Australian Clay Minerals Conference Mildura, Australia, 2012.

[26] R.W. Fitzpatrick, Demands on soil classification and soil survey strategies: special-purpose soil classification systems for local practical use, in: S.A. Shahid, F.K. Taha, M.A. Abdelfattah (Eds.), Developments in Soil Classification Land Use Planning and Policy Implications, Springer, Netherlands, 2013, pp. 51–83.

[27] Soil Survey Staff, Keys to Soil Taxonomy, 12th ed., USDA-Natural Resources Conservation Service, Washington, DC, 2014.

[28] R. Isbell, The Australian Soil Classification, CSIRO Publishing, Australia, 2002.

[29] D.W. Nelson, L.E. Sommers, Total carbon, organic carbon, and organic matter, in: D.L. Sparks, A.L. Page, P.A. Helmke, R.H. Loeppert, P.N. Soltanpour, M.A. Tabatabai, C.T. Johnston, M.E. Sumner (Eds.), Methods of Soil Analysis Part 3—Chemical Methods, Soil Science Society of America and American Society of Agronomy, USA, 1996, pp. 961–1010.

[30] C.R. Ahern, A.E. McElnea, L.A. Sullivan, in: Acid Sulphate Soils: Laboratory Methods Guidelines, Department of Natural Resources, Mines and Energy, Indooroopilly, Queensland, Australia, 2004.

[31] D.R. Keeney, D.W. Nelson, Nitrogen—inorganic forms, in: A.L. Page (Ed.), Methods of Soil Analysis. Part 2, Chemical and Microbiological Properties, American Society of Agronomy, Soil Science Society of America, USA, 1982, pp. 643–698.

[32] T.A. Kettler, J.W. Doran, T.L. Gilbert, Simplified method for soil particle-size determination to accompany soil-quality analyses, Soil Sci. Soc. Am. J. 65 (2001) 849–852.

[33] S.L. Simpson, A rapid screening method for acid-volatile sulfide in sediments, Environ. Toxicol. Chem. 20 (2001) 2657–2661.

[34] I. Sanchez-Andrea, J.L. Sanz, M.F. Bijmans, A.J. Stams, Sulfate reduction at low pH to remediate acid mine drainage, J. Hazard. Mater. 269 (2014) 98–109.

[35] M. Koschorreck, Microbial sulphate reduction at a low pH, FEMS Microbiol. Ecol. 64 (2008) 329–342.

[36] P.A. Hazelton, B.W. Murphy, Interpreting Soil Test Results: What do all the Numbers Mean? CSIRO Publishing, Australia, 2007.

[37] C. Hubert, G. Voordouw, Oil field souring control by nitrate-reducing *Sulfurospirillum* spp. that outcompete sulfate-reducing bacteria for organic electron donors Appl. Environ. Microbiol. 73 (2007) 2644–2652.

[38] K. Kaster, A. Grigoriyan, G. Jennneman, G. Voordouw, Effect of nitrate and nitrite on sulfide production by two thermophilic, sulfate-reducing enrichments from an oil field in the North Sea, Appl. Microbiol. Biotechnol. 75 (2007) 195–203.

[39] L. Zhang, J. Keller, Z. Yuan, Inhibition of sulfate-reducing and methanogenic activities of anaerobic sewer biofilms by ferric iron dosing, Water Res. 43 (2009) 4123–4132.

[40] D.R. Lovley, E.J. Phillips, Competitive mechanisms for inhibition of sulfate reduction and methane production in the zone of ferric iron reduction in sediments, Appl. Environ. Microbiol. 53 (1987) 2636–2641.

[41] K. Wendt-Potthoff, E. Bozau, R. Frommichen, J. Meier, M. Koschorreck, Microbial iron reduction during passive in situ remediation of an acidic mine pit lake mesocosm, Limnologica 40 (2010) 175–181.

[42] D.S. Baldwin, M. Fraser, Rehabilitation options for inland waterways impacted by sulfidic sediments – a synthesis, J. Environ. Manage. 91 (2009) 311–319.

[43] R. Frommichen, K. Wendt-Potthoff, K. Friese, R. Fischer, Microcosm studies for neutralization of hypolimnic acid mine pit lake water (pH 2.6), Environ. Sci. Technol. 38 (2004) 1877–1887.

[44] C. Yuan, L.M. Mosley, R. Fitzpatrick, P. Marschner, Amount of organic matter required to induce sulfate reduction in sulfuric material after re-flooding is affected by soil nitrate concentration, J. Environ. Manage. 151 (2015) 437–442.

[45] N. Creeper, R. Fitzpatrick, P. Shand, A simplified incubation method using chip-trays as incubation vessels to identify sulphidic materials in acid sulphate soils, Soil Use Manage. 28 (2012) 401–408.

[46] D. Emerson, E.J. Fleming, J.M. McBeth, Iron-oxidizing bacteria: an environmental and genomic perspective, Annu. Rev. Microbiol. 64 (2010) 561–583.

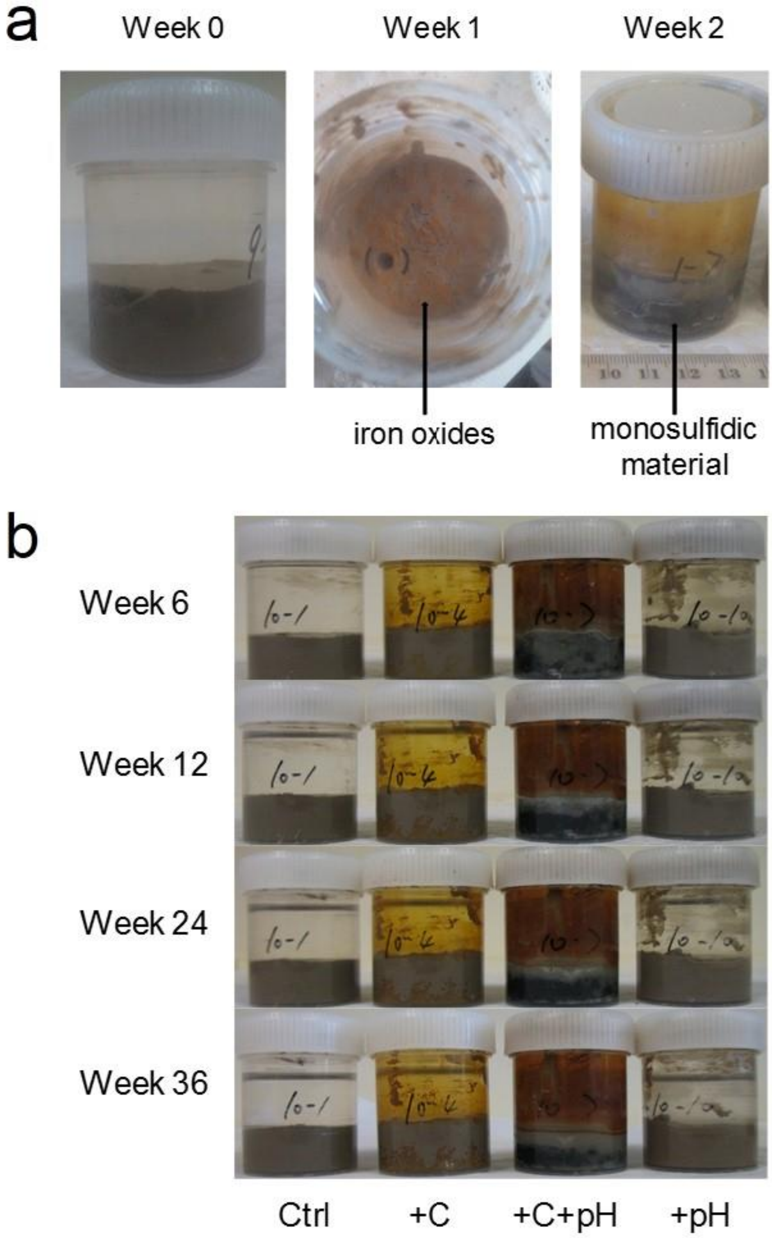

Fig. S1. a) Iron oxides observed after one week, and black (likely monosulfidic) material after two weeks. b) Images of soil 10 in weeks 6, 12, 24, and 36 with the four treatments: control (Ctrl); organic carbon addition (+C); organic carbon addition and pH increase (+C+pH); pH increase to 5.5±0.1 (+pH).

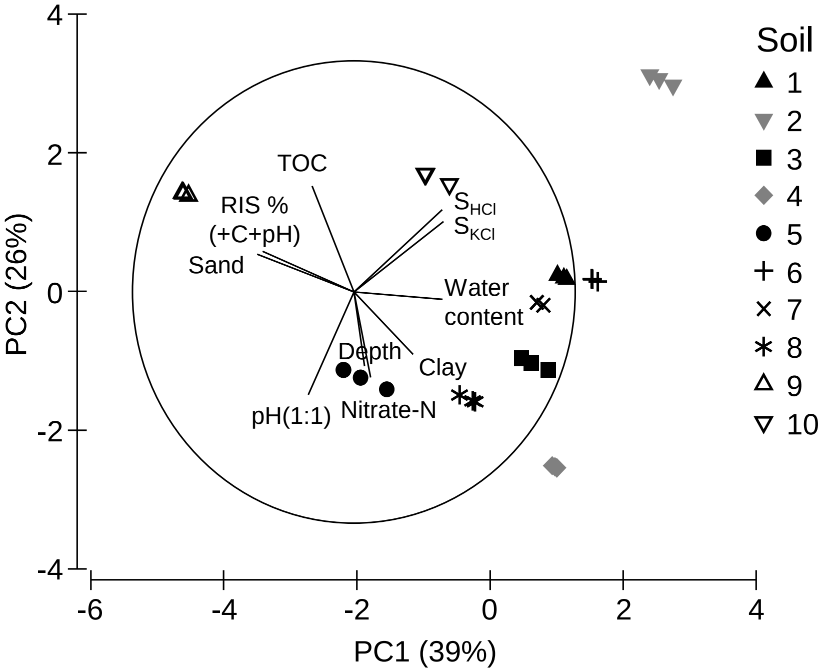

Fig. S2. Principal component analysis (PCA) plot of the relationships between concentration of soil reduced inorganic sulfur in treatment +C+pH (organic carbon addition and pH increase) as percentage of the control (%RIS) and initial soil properties after 36 weeks. TOC, total organic carbon; S_{HCl}, 4 M HCl extractable sulfur; S_{KCl}, 1 M KCl extractable sulfur.

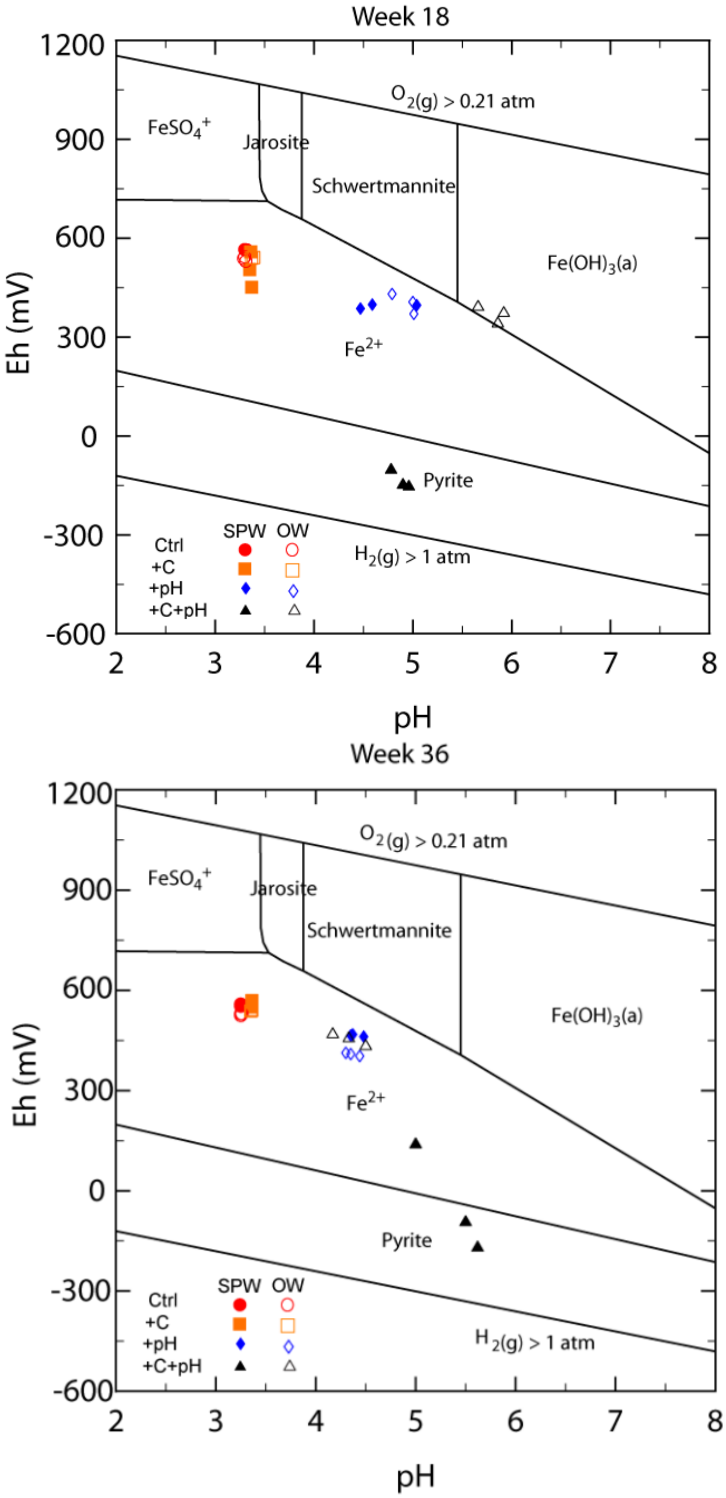

Fig. S3. pH and Eh of soil pore water (SPW) and overlying water (OW) in soil 10 in weeks 18 and 36 as superimposed into a geochemical model for the iron−sulfur system. Treatments: control (Ctrl); organic carbon addition (+C); pH increase to 5.5±0.1 (+pH); organic carbon addition and pH increase (+C+pH).

CHAPTER 3

Amount of organic matter required to induce sulfate reduction in sulfuric material after re-flooding is affected by soil nitrate concentration

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Author Contributions

By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Name of Principal Author (Candidate)	Chaolei Yuan		
Contribution to the Paper	Conducted experiment and data analysis and wrote manuscript		
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Name of Co-Author	Petra Marschner		
Contribution to the Paper	Supervised development of work, helped in data interpretation and manuscript evaluation, and acted as corresponding author		
Signature		Date	22/01/2015

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Amount of organic matter required to induce sulfate reduction in sulfuric material after re-flooding is affected by soil nitrate concentration

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ABSTRACT

Acid sulfate soils (ASS) with sulfuric material can be remediated through microbial sulfate reduction stimulated by adding organic matter (OM) and increasing the soil pH to >4.5, but the effectiveness of this treatment is influenced by soil properties. Two experiments were conducted using ASS with sulfuric material. In the first experiment with four ASS, OM (finely ground mature wheat straw) was added at 2–6% (w/w) and the pH adjusted to 5.5. After 36 weeks under flooded conditions, the concentration of reduced inorganic sulfur (RIS) and pore water pH were greater in all treatments with added OM than in the control without OM addition. The RIS concentration increased with OM addition rate. The increase in RIS concentration between 4% and 6% OM was significant but smaller than that between 2% and 4%, suggesting other factors limited sulfate reduction. In the second experiment, the effect of nitrate addition on sulfate reduction at different OM addition rates was investigated in one ASS. Organic matter was added at 2 and 4% and nitrate at 0, 100, and 200 mg nitrate-N kg^{-1}. After 2 weeks under flooded conditions, soil pH and the concentration of FeS measured as acid volatile sulfur (AVS) were lower with nitrate added at both OM addition rates. At a given nitrate addition rate, pH and AVS concentration were higher at 4% OM than at 2%. It can be concluded that sulfate reduction in ASS at pH 5.5 can be limited by low OM availability and high nitrate concentrations. Further, the inhibitory effect of nitrate can be overcome by high OM addition rates.

1. Introduction

Acid sulfate soils (ASS) have a large distribution in the world, with a total estimated area of coastal ASS of 10^7–10^8 ha (Macdonald et al., 2011; Wim and Mensvoort, 2005). Upon oxidation, ASS with sulfidic material ($pH > 4.0$) transform to sulfuric material ($pH < 4.0$) (Isbell, 2002) and release acidity and toxic metals, which can have severe negative impacts on the environment (Fitzpatrick et al., 2009). Stimulation of microbial sulfate reduction has been proposed as an efficient strategy for remediating ASS with sulfuric material as the process generates alkalinity (Baldwin and Fraser, 2009; Fitzpatrick et al., 2009). This could be particularly useful when chemical amelioration, such as liming, is costly or ineffective (Dear et al., 2002). For effective management of sulfuric ASS It is important to understand the factors that may limit or enhance sulfate reduction.

Following re-flooding and soil submersion, soil oxygen is quickly depleted and other oxidized components used as electron acceptors in anaerobic respiration are reduced according to thermodynamics in the sequence: NO_3^-, Mn(IV), Fe(III), SO_4^{2-}, CO_2 (Borch et al., 2009; Ponnamperuma, 1972). The presence of electron acceptors ranked higher in the sequence retards the reduction of electron acceptors ranked lower (Ponnamperuma, 1972) because the former can: (i) outcompete the latter for electron donors (organic carbon) (Hubert and Voordouw, 2007; Lovley and Phillips, 1987), and (ii) oxidize the reduction products of the latter (Canfield et al., 1993; Carlson et al., 2013; Hubert and Voordouw, 2007; Myers and Nealson, 1988; Zhang et al., 2009). Additionally, nitrate inhibits sulfate reduction because the product of nitrate reduction, nitrite,

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inhibits the dissimilatory sulfite reductase (Haveman et al., 2004; Kaster et al., 2007). The inhibitory influence of Mn(IV) and Fe(III) oxides on sulfate reduction (Canfield et al., 1993; Lovley and Phillips, 1987; Myers and Nealson, 1988; Zhang et al., 2009) is weaker because of (i) their low solubility, (ii) lower standard reduction potential of these redox couples than the NO_3^-/N_2 couple, and/or (iii) the limited number of microbes using these electron acceptors (Ponnamperuma, 1972).

Large-scale exposure and oxidation of ASS occurred in the Lower Murray region of South Australia during a recent prolonged and severe drought (Mosley et al., 2014b). Despite several years of re-submergence after the end of the drought, neutralization of the acidity in these soils via sulfate reduction does not appear to be occurring (Mosley et al., 2014a). In our previous study (Yuan et al., unpublished) we showed that low soil pH and availability of organic matter (OM) may limit sulfate reduction in these ASS with sulfuric material after re-flooding. But the ameliorative effect of the treatment with increased pH and OM addition on sulfate reduction differed among soils (Yuan et al., unpublished). Possible reasons for a small increase of sulfate reduction compared to the other soils could be high concentrations of competing electron acceptors, particularly nitrate as a large proportion of ASS in the LMRIA are under intensive pasture with high nitrogen fertilizer use. High nitrate concentrations have also been reported for other ASS (Macdonald et al., 2010). The influence of competing electron acceptors, such as nitrate has not been adequately studied in a systematic manner and is currently not taken into account for ASS management. The aim of the present study was to investigate the effects on sulfate reduction in sulfuric material after pH adjustment to 5.5 and re-flooding of: (i) addition rates of OM (Experiment 1) and (ii) combinations of amendment with nitrate and OM (Experiment 2). Our hypotheses were: (i) sulfate reduction increases with OM addition rate, and (ii) nitrate can inhibit sulfate reduction but this can be overcome by high OM addition rates.

2. Materials and methods

2.1. Soils

Five soils with sulfuric material (soils 1–5; Table 1) collected in the Lower Murray Reclaimed Irrigation Area (LMRIA), South Australia (35°7′28.05″S, 139°17′55.17″E) were used. They come from two profiles described in Fitzpatrick et al. (2013): DSa01A (soils 1–4) and DSa01B (soil 5). The soil is a sulfuric clay soil (Fitzpatrick et al., 2010; Fitzpatrick, 2013), Typic Sulfaquert or Sulfic Sulfaquert (Soil Survey Staff, 2014). The area is used for intensive cattle grazing with flood irrigation (for further information see Mosley et al., 2014a). Selected soil properties were determined (Table 1).

2.2. Experimental procedure

Soils 1–4 were used in Experiment 1. In our previous study (Yuan et al., unpublished), these clayey soils had a smaller increase in sulfate reduction compared with other soils after pH adjustment to 5.5 and OM addition. Twenty grams of moist soil (12–15 g oven dry soil) was mixed with 20 ml reverse osmosis (RO) water in 70 ml polypropylene containers. Organic matter (ground and sieved mature wheat straw to <2 mm with 313 g kg^{-1} total organic carbon and 7 g kg^{-1} total nitrogen) was then added to the soil paste at three rates: 2, 4 or 6% (w/w on oven dry basis). Controls were soils without OM addition. For soil 1 only, addition rates of 0.5 and 1% were also included, because in our previous study (Yuan et al., unpublished) OM was added at 2% only and more black material (likely monosulfidic, FeS) was observed in this soil compared to soils 2–4. It was unclear if this apparent sulfate reduction would occur with lower OM additions. After adjusting the soil pH in all treatments to 5.5 ± 0.1 by adding 1 M NaOH, the soils were incubated under 40 ml RO water for 36 weeks. There were three replicates per treatment. During incubation, pH of the overlying water and soil pore water were measured weekly from weeks 0–12 and every two weeks from weeks 12–36 (except in week 34) (Figs. S1 and S2). The concentration of O_2 in the overlaying water was determined at the end of the experiment.

Only one soil (Soil 5) was used in Experiment 2. This soil was selected because it had low initial nitrate concentration and in our previous study with 2% OM added and pH increased to 5.5 black (likely monosulfidic) material occurred early when incubated under water (Yuan et al. unpublished). There were three factors: organic matter addition (OM), nitrate addition (Nitrate), and time (Time). Twenty grams of moist soil (15 g oven dry soil) was mixed with 20 ml RO water in 70 ml polypropylene containers. Ground and sieved mature wheat straw (similar as in Experiment 1) was then added at two rates: 2% and 4% (w/w on oven dry basis). After 1.1, and 2.1 ml 0.1 M KNO_3 was added to achieve 100, and 200 mg Nitrate-N kg^{-1}. The control was without nitrate addition. The lower nitrate addition rate represents approximately the highest nitrate-N concentration in the soils used (Table 1) and the higher addition rate twice that concentration. To compensate the change of salinity caused by KNO_3 addition, 2.1, 1.1, and 0 ml 0.091 M KCl, which has the same salinity as 0.1 M KNO_3 (Weast et al., 1988), was added to the soil for control, 100, and 200 mg Nitrate-N kg^{-1}, respectively. Soil pH for all the treatments was then increased to 5.5 ± 0.1 by adding 1 M NaOH. The soil was incubated under 40 ml RO water for 2 weeks. This short incubation period was chosen firstly because nitrate can be reduced within a few days after submergence (Ponnamperuma, 1972), therefore the effect of nitrate as competing electron acceptor for sulfate reduction is likely to be greatest initially. Secondly, we observed that the difference in extent of black material among nitrate addition rates that developed in the

Table 1
Texture, pH and concentrations of TOC (total organic carbon), S_{HCl} (4 M HCl extractable sulfur), and S_{KCl} (1 M KCl extractable sulfur) of soils used in this study.

No.	Depth (cm)	Sand (%)	Clay (%)	Water content (g g^{-1})	pH (1:1)	TOC (%)	Nitrate-N (mg kg^{-1})	Ammonium-N (mg kg^{-1})	S_{HCl} (%)	S_{KCl} (%)
Experiment 1[a]										
1	57–95	1.3	75.3	0.33	4.30	3.0	13.0	48.3	0.32	0.26
2	95–190	1.9	53.7	0.54	3.09	2.3	0.7	117.7	0.64	0.42
3	190–280	1.6	56.1	0.63	3.21	1.6	16.5	125.2	0.55	0.37
4	280–350	1.8	58.7	0.40	4.23	1.5	93.9	51.2	0.40	0.29
Experiment 2[b]										
5	76–190	5.5	56.2	0.33	4.06	1.5	10.6	40.2	0.24	0.14

[a] Profile: DSa01A (Fitzpatrick et al., 2013).
[b] Profile: DSa01B.

first week became weaker in week 2 by the end of which the extent of black material became similar in all nitrate treatments (Fig. S3), suggesting that the nitrate effect became weaker. Acid volatile sulfur (AVS, comprising dissolved sulfides, solid FeS phases, and perhaps pyrite (Rickard, 2012)) concentration was used to estimate sulfate reduction due to these observations and because FeS tends to form more rapidly than pyrite (Rickard, 2012). During incubation, pH of the overlying water (pHow) and soil pore water (pHspw) were measured weekly. Soil subsamples were taken from the containers at the end of weeks 1 and 2 to determine AVS, nitrate, and ammonium concentrations.

2.3. Methods

Total organic carbon (TOC) was measured by the Walkley-Black method (Nelson and Sommers, 1996). Sulfur was extracted by shaking for 4 h with 1 M KCl (S_{KCl}) or for 16 h with 4 M HCl (S_{HCl}) according to Ahern et al. (2004) and sulfur in the extract was measured by ICP-AES. S_{KCl} represents mainly soluble and exchangeable sulfate, while S_{HCl} also recovers relatively insoluble sulfate in iron oxyhydroxysulfate minerals such as jarosite and natrojarosite that are commonly found in sulfuric material (Ahern et al., 2004; Fitzpatrick et al., 2013). Reduced inorganic sulfur (RIS) in the soil was measured as the sum of acid volatile sulfide (AVS) and chromium reducible sulfur (Scr, mainly pyrite), which were determined according to Simpson (2001) and Ahern et al. (2004).

Nitrate and ammonium concentrations were determined in the 1 M KCl extracts using the cadmium reduction method (Keeney and Nelson, 1982), and according to Willis et al. (1996), respectively. Soil texture (sand and clay content) was analyzed after Kettler et al. (2001).

The pH of the soil pore water was measured by inserting the pH electrode approximately 2 cm into the soil. The concentration of dissolved oxygen (DO_2) in the overlaying water was measured with a DO_2 electrode.

2.4. Data analysis

For Experiment 1, properties of soil and overlying water at the end of the experiment (week 36) were analyzed by two-way ANOVA (soil × OM addition rate). Significant differences ($P \leq 0.05$) among soils and treatments were determined by Fisher's protected least significant difference test using GenStat 16.2 (VSN International Ltd., UK). For Experiment 2, three-way ANOVA (OM addition rate, nitrate addition rate, and Time as a repeated-measure factor; Table S1) was conducted for the change in pHow and pHspw compared to values at week 0 and for concentrations of soil AVS, nitrate-N, and ammonium-N using IBM SPSS 20 (IBM, USA). If the three-way interaction was significant, further analyses for OM × Nitrate interactions were performed at each time point, and the main effect of Time was checked by multivariate tests and pairwise comparisons with Bonferroni adjustment at each OM × Nitrate combination. If three-way interaction was not significant, further analyses were carried out for significant two-way interactions and main effects (Cohen, 2013).

3. Results

3.1. Soil properties

The clay content of the soils ranged from 54 (soil 2) to 75% (soil 1) (Table 1). All soils were strongly acidic (pH 3–4) before pH adjustment and classified as sulfuric material (Isbell, 2002). The TOC content was lowest in soils 4 and 5 (1.5%) and highest in soil 1 (3%). Nitrate-N concentration was highest in soil 4 (94 mg kg^{-1}) and lowest in soil 2 (1 mg kg^{-1}). Ammonium-N concentration ranged from 40 (soil 5) to 118 mg kg^{-1} (soil 2). S_{HCl} and S_{KCl} concentrations were highest in soil 2 and lowest in soil 1.

3.2. Experiment 1

OM addition, soil, and their interaction significantly influenced pHow, pHspw, and RIS, DO_2, nitrate and ammonium concentrations after 36 weeks (Fig. 1), as well as the difference of these properties in the treatments with OM addition compared to the control (0% OM addition) (Fig. S4).

With ≥2% OM added, pHspw increased in the first 4 weeks, but decreased later with 2% OM whereas it continued to increase with 4 and 6% OM (Figs. S1 and S2). After 36 weeks (Fig. 1a, c) compared with the un-amended control, 2% OM addition increased pHow and pHspw significantly only in soil 1 (by 1.3 and 1.1 pH units) and soil 4 (by 0.4 and 1.2 units), but in all soils pHow and pHspw were significantly increased at OM addition rates 4% (by 1.6–2.9 units) or 6% (by 1.5–3.7 units). The pH increase induced by 4% and 6% OM addition was greater in soils 2–4 (by 2.5–3.7 units) than in soil 1 (by 1.5–1.8 units). Organic matter addition rates between 2 and 4% (w/w) increased soil RIS concentration significantly compared to the control (no OM added) (Fig. 1 d). Adding 2% OM caused an increase of RIS concentration by 0.09%–0.14% in all soils. The increase induced by 4% OM addition was greater in soils 2 and 3 (by 0.37%) than in soils 1 and 4 (by 0.24%). When 6% OM was added, the increase in RIS concentration was largest in soils 2 and 3 (by around 0.51%), followed by soil 4 (by 0.31%), and smallest in soil 1 (by 0.22%). In all soils, the increase in RIS concentration was greater from 2% to 4% OM addition than from 4% to 6%. In soil 1, where OM addition rates ranged between 0.5% and 6% (Fig. S5), RIS concentrations were higher than the un-amended control only at ≥2% OM added.

Compared to the control, 2% OM addition significantly decreased DO_2 in the overlying water only in soils 1 and 4 (by 1.97 and 0.66 ppm), but in all soils DO_2 concentration was significantly decreased with addition of 4% (by 0.67–3.76 ppm) and 6% OM (by 1.43–4.25 ppm) (Fig. 1b). The 4% and 6% OM addition caused greater DO_2 decrease in soil 1 (by 3.76 and 4.25 ppm) and soil 4 (by 1.30 and 2.74 ppm) than in soils 2 and 3 (by 0.67–1.86 ppm).

After 36 weeks, nitrate-N was detected in the controls (no OM added) of soils 1, 2 and 4. With OM addition nitrate-N concentrations were very low (<0.2 mg kg^{-1}) (Fig. 1e). Compared to the control, the concentration of ammonium-N was higher at 4% and 6% OM addition in soil 1 (by 14.3 and 30.0 mg kg^{-1}), lower with OM addition in soil 2 (by 36.5–46.6 mg kg^{-1}) and soil 3 (by 15.0–68.2 mg kg^{-1}), and not affected by OM addition in soil 4 (Fig. 1f).

3.3. Experiment 2

The increase in pHow and pHspw compared to values in week 0 was greater in week 2 than in week 1 (Fig. 2a, b). The pH increase was greater without nitrate addition than when 200 mg kg^{-1} nitrate was added in week 1 with 2% OM addition and in week 2 at both OM addition rates. With nitrate added, the increase in pHow and pHspw in week 2 was greater with 4% than with 2% OM added.

The AVS concentration was higher in week 2 than in week 1 when 4% OM was added, but did not change over time with 2% OM addition (Fig. 2c). With addition of 2% OM, the AVS concentration was higher without nitrate added than when nitrate was added. With 4% OM added, the AVS concentration was lowest when 200 mg kg^{-1} nitrate added. At all nitrate addition rates, the AVS concentration was higher with 4% than with 2% OM addition.

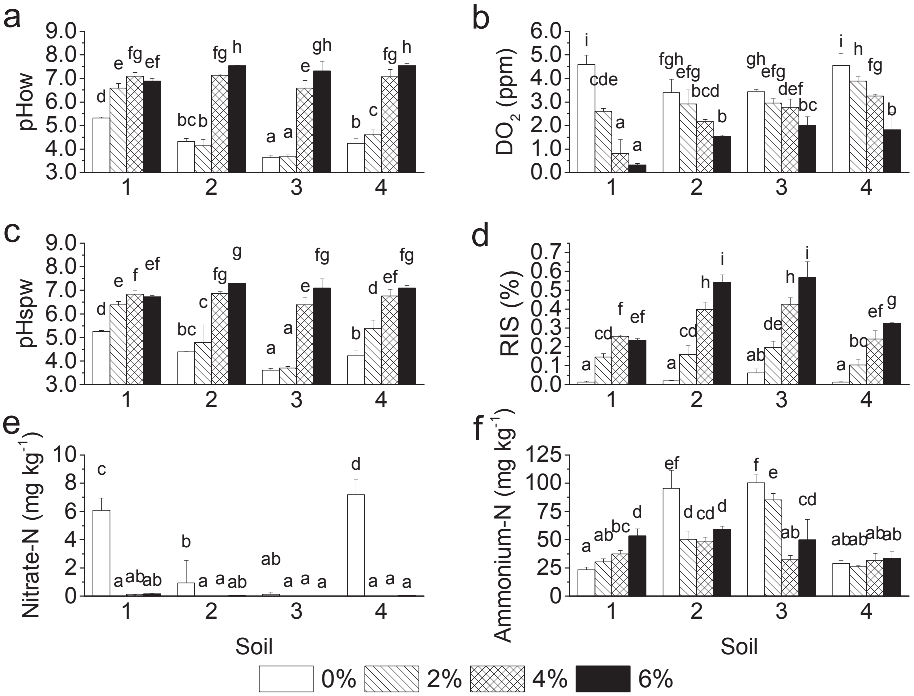

Fig. 1. Properties of overlying water and soil in soils 1, 2, 3 and 4 amended with 0, 2, 4 and 6% (w/w) organic matter after 36 weeks in Experiment 1. (a) pHow, pH of overlying water; (b) DO_2, dissolved oxygen concentration in overlying water; (c) pHspw, pH of soil pore water; and soil concentrations of (d) RIS, reduced inorganic sulfur, (e) nitrate-N, and (f) ammonium-N. Vertical lines show standard deviation (n = 3); values that do not share the same letter are significantly different at $P \leq 0.05$.

The nitrate-N concentration was higher in week 1 than in week 2 (Fig. 2d). The nitrate concentration was not influenced by nitrate addition rate with 2% OM added. With 4% OM addition, the nitrate-N concentration was higher without nitrate added than when nitrate was added.

In week 1 at both OM addition rates, the ammonium-N concentration was lower in the treatment without nitrate addition than with 200 mg kg^{-1} nitrate (Fig. 2 e). But in week 2 the ammonium-N concentrations was lower with 200 mg kg^{-1} nitrate added than without nitrate addition.

4. Discussion

This study showed that sulfate reduction in sulfuric material after re-flooding can be influenced by both OM availability and nitrate concentration when the pH is adjusted to 5.5. In agreement with our previous study (Yuan et al. unpublished), sulfate reduction was very low without OM addition (Fig. 1d) which indicates that native OM was poorly available for sulfate reducers although the native OM content was 1.5–3% (Table 1). This suggests that native OM was not available to sulfate reducers, probably due to binding to clay and complexion by dissolved metals (Lützow et al., 2006) in these soils which have a high clay content (Table 1) and high concentrations of dissolved metals (Fitzpatrick et al., 2013; Mosley et al., 2014a). In soil 1, lower OM addition rates (0.5% and 1%) did not increase RIS concentrations compared to the un-amended soils (Fig. S5). This suggests that only when the OM addition rate is above a certain threshold sulfate reduction can occur, possibly because added OM can also be bound by clay (Roychand and Marschner, 2013; Shi and Marschner, 2013). In addition, after flooding, oxygen, NO_3^-, Mn(IV) and Fe(III) are sequentially used as electron acceptors during OM decomposition (Borch et al., 2009; Ponnamperuma, 1972). Lower concentrations of dissolved oxygen in the overlying water and nitrate in the soil when OM was added (Fig. 1b) also suggest that competing electron acceptors (oxygen, nitrate) were used. If there is still OM available after these electron acceptors have been reduced, sulfate reduction will occur (Dugdale et al., 1977; Lovley and Phillips, 1987; Ontiveros-Valencia et al., 2012; Ponnamperuma, 1972) and the pH will increase (Fig. 1c). If sufficient OM remains for sulfate reduction, RIS formation can be limited by sulfate and dissolved Fe concentrations and therefore further addition of OM will not result in higher RIS concentrations. For all soils, at 4% OM added, the RIS concentrations after incubation were close to the initial S_{KCl} concentrations (Table 1 and Fig. 1d), suggesting that almost all soluble and exchangeable sulfate was reduced. We can therefore assume that when 6% OM was added, only the relatively insoluble sulfate ($S_{HCl}-S_{KCl}$) (Table 1 and Fig. 1d) was available as electron acceptor for the decomposition of the extra 2% OM. Thus the increase in RIS concentration was smaller from 4% to 6% OM addition than from 2% to 4%. This interpretation is supported by the finding that in soil 1, where the $S_{HCl}-S_{KCl}$ concentration was low (0.06%), RIS concentration did not increase from 4% to 6% OM addition.

It is known that nitrate inhibits sulfate reduction (Hubert and Voordouw, 2007; Ponnamperuma, 1972), however to our knowledge the influence of nitrate has not been considered in ASS management. The inhibitory effect of nitrate on sulfate reduction was confirmed in Experiment 2. Soil AVS concentrations were lower in the treatments with nitrate added, particularly at the higher nitrate rate (Fig. 2c). Nitrate concentrations after two weeks did not differ significantly among nitrate addition rates (Fig. 2d). This indicates that at high nitrate addition rate, more OM was utilized with nitrate as electron acceptor leaving less OM available for sulfate reduction. Compared to 2% OM, addition of 4% OM increased sulfate reduction at all nitrate addition rates (Fig. 2c), which suggests that high OM availability can overcome the inhibitory effect of nitrate. The stimulation of sulfate reduction by the higher OM addition rate is also evident in the greater increase in

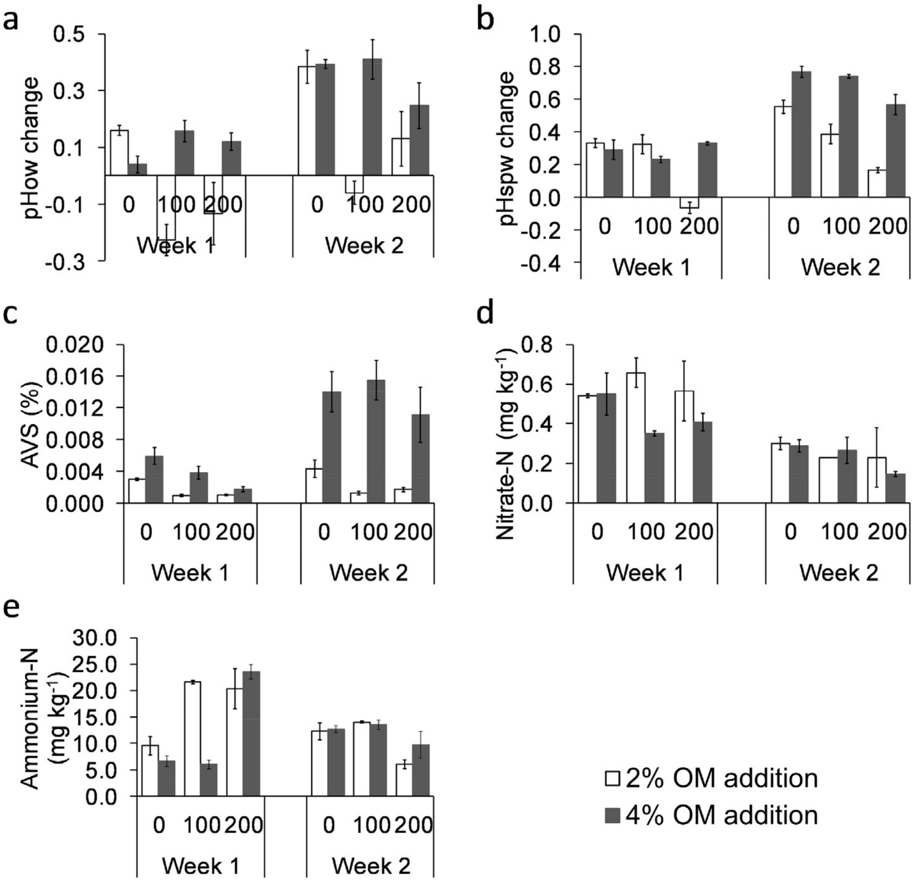

Fig. 2. Properties of overlying water and soil in soil 5 amended with 2 or 4% organic matter and 0, 100 or 200 mg kg^{-1} nitrate-N after 1 and 2 weeks in Experiment 2. (a) pHow change, change in pH of overlying water compared to the values at week 0; (b) pHspw change, change in pH of soil pore water compared to the values at week 0; and soil concentrations of (c) AVS, acid volatile sulfur, (d) nitrate-N, and (e) ammonium-N. Vertical lines show standard deviation (n = 3).

AVS concentration with time with 4% than 2% OM addition (Fig. 2c). Mn(IV) or Fe(III) oxides as other potential competing electron acceptors were not considered in this study because (i) their inhibitory effects on sulfate reduction are weaker compared to nitrate as mentioned above, and (ii) Fe and Mn oxide concentrations are typically much lower than nitrate concentrations in similar soils in the LMRIA [see Mosley et al. (2014a)]. Nevertheless, the addition of OM to achieve sulfate reduction would need to be sufficient to reduce these compounds and leave OM for sulfate reduction.

In the field, remediation of sulfuric material through sulfate reduction may be complicated by many factors, notably pH < 5.5 typically found in ASS, low availability of organic C and the presence of nitrate as found in our study. Field trials could be conducted to test if it is possible to overcome low availability of native OM and inhibition of sulfate reduction by competing electron acceptors through addition of organic carbon to acidified submerged layers.

5. Conclusions

This study confirmed that OM addition with pH adjustment can stimulate sulfate reduction and thus increase soil pH. However, we showed that OM addition has to be above a threshold (2% in this study) to stimulate sulfate reduction. A factor contributing to this threshold is the presence of competing electron acceptors, particularly nitrate in our soils. Only if sufficient OM remains available after nitrate has been reduced, sulfate reduction can occur. Consequently, more OM is needed for remediation of acid sulfate soils with high nitrate concentrations. On the other hand, adding large amounts of OM (>4% in this study) does not increase sulfate reduction further indicating other limiting factors such as sulfate concentration.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jenvman.2015.01.016.

References

Ahern, C.R., McElnea, A.E., Sullivan, L.A., 2004. Acid Sulphate Soils: Laboratory Methods Guidelines. Department of Natural Resources, Mines and Energy, Indooroopilly, Queensland, Australia.

Baldwin, D.S., Fraser, M., 2009. Rehabilitation options for inland waterways impacted by sulfidic sediments – a synthesis. J. Environ. Manag. 91, 311–319.

Borch, T., Kretzschmar, R., Kappler, A., Cappellen, P.V., Ginder-Vogel, M., Voegelin, A., Campbell, K., 2009. Biogeochemical redox processes and their impact on contaminant dynamics. Environ. Sci. Technol. 44, 15–23.

Canfield, D.E., Thamdrup, B., Hansen, J.W., 1993. The anaerobic degradation of organic matter in Danish coastal sediments: iron reduction, manganese reduction, and sulfate reduction. Geochim. Cosmochim. Acta 57, 3867–3883.

Carlson, H.K., Clark, I.C., Blazewicz, S.J., Iavarone, A.T., Coates, J.D., 2013. Fe(II) oxidation is an innate capability of nitrate-reducing bacteria that involves abiotic and biotic reactions. J. Bacteriol. 195, 3260–3268.

Cohen, B.H., 2013. Explaining Psychological Statistics, fourth ed. John Wiley & Sons, New Jersey.

Dear, S.-E., Moore, N.G., Dobos, S.K., Watling, K.M., Ahern, C.R., 2002. Soil Management Guidelines, Queensland Acid Sulfate Soil Technical Manual. Department of Natural Resources and Mines, Indooroopilly, Queensland, Australia.

Dugdale, R.C., Goering, J.J., Barber, R.T., Smith, R.L., Packard, T.T., 1977. Denitrification and hydrogen sulfide in the Peru upwelling region during 1976. Deep Sea Res. 24, 601–608.

Fitzpatrick, R., Marvanek, S., Powell, B., Grealish, G., Gilkes, R., 2010. Atlas of Australian acid sulfate soils: recent developments and future priorities. In: Gilkes, R., Prakongkep, N. (Eds.), Proceedings of the 19th World Congress of Soil Science: Soil Solutions for a Changing World. Brisbane, Australia, 1–6 August, 2010, pp. 24–27. Published on DVD; ISBN 987-0-646-53783-2. www.iuss.org. Symposium WG 3.1 Processes in acid sulfate soil materials.

Fitzpatrick, R., Shand, P., Simpson, S., Grocke, S., Raven, M., Baker, A., Mosley, L., Self, P., 2013. Assessment of Re-flooded Acid Sulfate Soil Environments at Long Flat, Jervois, Toora and Pompoota in the Lower Murray Reclaimed Irrigation Area (LMRIA). CSIRO Land and Water Science Report 2013. 03/12. 250 pp.

Fitzpatrick, R.W., 2013. Demands on soil classification and soil survey strategies: special-purpose soil classification systems for local practical use. In: Shahid, S.A., Taha, F.K., Abdelfattah, M.A. (Eds.), Developments in Soil Classification, Land Use Planning and Policy Implications. Springer, Netherlands, pp. 51–83.

Fitzpatrick, R.W., Shand, P., Merry, R.H., 2009. Acid sulfate soils. In: Jennings, J.T. (Ed.), Natural History of the Riverland and Murraylands. Royal Society of South Australia (Inc.), Adelaide, South Australia, pp. 65–111.

Haveman, S.A., Greene, E.A., Stilwell, C.P., Voordouw, J.K., Voordouw, G., 2004. Physiological and gene expression analysis of inhibition of *Desulfovibrio Vulgaris* Hildenborough by nitrite. J. Bacteriol. 186, 7944–7950.

Hubert, C., Voordouw, G., 2007. Oil field souring control by nitrate-reducing *Sulfurospirillum* spp. that outcompete sulfate-reducing bacteria for organic electron donors. Appl. Environ. Microbiol. 73, 2644–2652.

Isbell, R., 2002. The Australian Soil Classification. CSIRO Publishing, Australia.

Kaster, K., Grigoriyan, A., Jennneman, G., Voordouw, G., 2007. Effect of nitrate and nitrite on sulfide production by two thermophilic, sulfate-reducing enrichments from an oil field in the North Sea. Appl. Microbiol. Biotechnol. 75, 195–203.

Keeney, D.R., Nelson, D.W., 1982. Nitrogen—inorganic forms. In: Page, A.L. (Ed.), Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. American Society of Agronomy, Soil Science Society of America, USA, pp. 643–698.

Kettler, T.A., Doran, J.W., Gilbert, T.L., 2001. Simplified method for soil particle-size determination to accompany soil-quality analyses. Soil Sci. Soc. Am. J. 65, 849–852.

Lovley, D.R., Phillips, E.J., 1987. Competitive mechanisms for inhibition of sulfate reduction and methane production in the zone of ferric iron reduction in sediments. Appl. Environ. Microbiol. 53, 2636–2641.

Lützow, M.v., Kögel-Knabner, I., Ekschmitt, K., Matzner, E., Guggenberger, G., Marschner, B., Flessa, H., 2006. Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions – a review. Eur. J. Soil. Sci. 57, 426–445.

Macdonald, B., White, I., Denmead, T., 2010. Gas emissions from the interaction of iron, sulfur and nitrogen cycles in acid sulfate soils. In: 19th World Congress of Soil Science, Soil Solutions for a Changing World. 1–6 August 2010, Brisbane, Australia, pp. 80–83.

Macdonald, B.C.T., Denmead, O.T., White, I., Byrant, G., 2011. Gaseous nitrogen losses from coastal acid sulfate soils: a short-term study. Pedosphere 21, 197–206.

Mosley, L.M., Fitzpatrick, R.W., Palmer, D., Leyden, E., Shand, P., 2014a. Changes in acidity and metal geochemistry in soils, groundwater, drain and river water in the Lower Murray River after a severe drought. Sci. Total Environ. 485–486, 281–291.

Mosley, L.M., Palmer, D., Leyden, E., Cook, F., Zammit, B., Shand, P., Baker, A., Fitzpatrick, R.W., 2014b. Acidification of floodplains due to river level decline during drought. J. Contam. Hydrol. 161, 10–23.

Myers, C.R., Nealson, K.H., 1988. Microbial reduction of manganese oxides: interactions with iron and sulfur. Geochim. Cosmochim. Acta 52, 2727–2732.

Nelson, D.W., Sommers, L.E., 1996. Total carbon, organic carbon, and organic matter. In: Sparks, D.L., Page, A.L., Helmke, P.A., Loeppert, R.H., Soltanpour, P.N., Tabatabai, M.A., Johnston, C.T., Sumner, M.E. (Eds.), Methods of Soil Analysis Part 3—Chemical Methods. Soil Science Society of America and American Society of Agronomy, USA, pp. 961–1010.

Ontiveros-Valencia, A., Ziv-El, M., Zhao, H.-P., Feng, L., Rittmann, B.E., Krajmalnik-Brown, R., 2012. Interactions between nitrate-reducing and sulfate-reducing bacteria coexisting in a hydrogen-fed biofilm. Environ. Sci. Technol. 46, 11289–11298.

Ponnamperuma, F., 1972. The chemistry of submerged soils. Adv. Agron. 24, 29.

Rickard, D., 2012. Sedimentary pyrite. In: Rickard, D. (Ed.), Developments in Sedimentology. Elsevier, pp. 233–285.

Roychand, P., Marschner, P., 2013. Respiration in a sand amended with clay – effect of residue type and rate. Eur. J. Soil Biol. 58, 19–23.

Shi, A., Marschner, P., 2013. Addition of a clay subsoil to a sandy top soil alters CO_2 release and the interactions in residue mixtures. Sci. Total Environ. 465, 248–254.

Simpson, S.L., 2001. A rapid screening method for acid-volatile sulfide in sediments. Environ. Toxicol. Chem. 20, 2657–2661.

Soil Survey Staff, 2014. Keys to Soil Taxonomy, twelfth ed. USDA-Natural Resources Conservation Service, Washington, DC.

Weast, R.C., Astle, M.J., Beyer, W.H., 1988. CRC Handbook of Chemistry and Physics. CRC Press, Boca Raton, FL.

Willis, R.B., Montgomery, M.E., Allen, P.R., 1996. Improved method for manual, colorimetric determination of total Kjeldahl nitrogen using salicylate. J. Agric. Food Chem. 44, 1804–1807.

Wim, A., Mensvoort, M.E.F.V., 2005. Acid sulfate soils: distribution and extent. In: Lal, R. (Ed.), Encyclopedia of Soil Science, second ed. CRC Press, pp. 14–19.

Zhang, L., Keller, J., Yuan, Z., 2009. Inhibition of sulfate-reducing and methanogenic activities of anaerobic sewer biofilms by ferric iron dosing. Water Res. 43, 4123–4132.

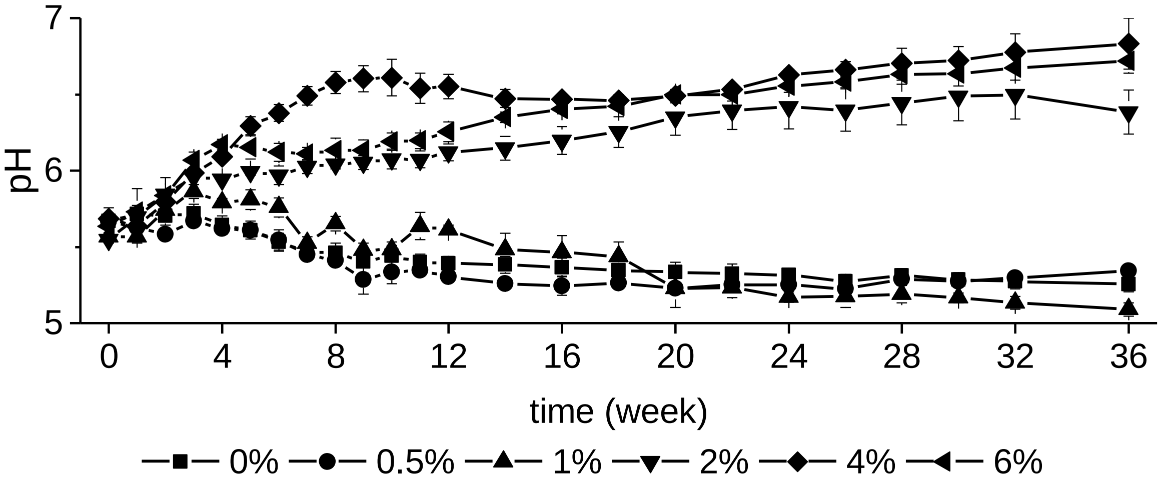

Figure S1. pH of soil pore water over 36 weeks in soil 1 amended with 0, 0.5, 1, 2, 4 and 6% (w/w) organic matter in Experiment 1. Vertical lines show standard deviation (n=3).

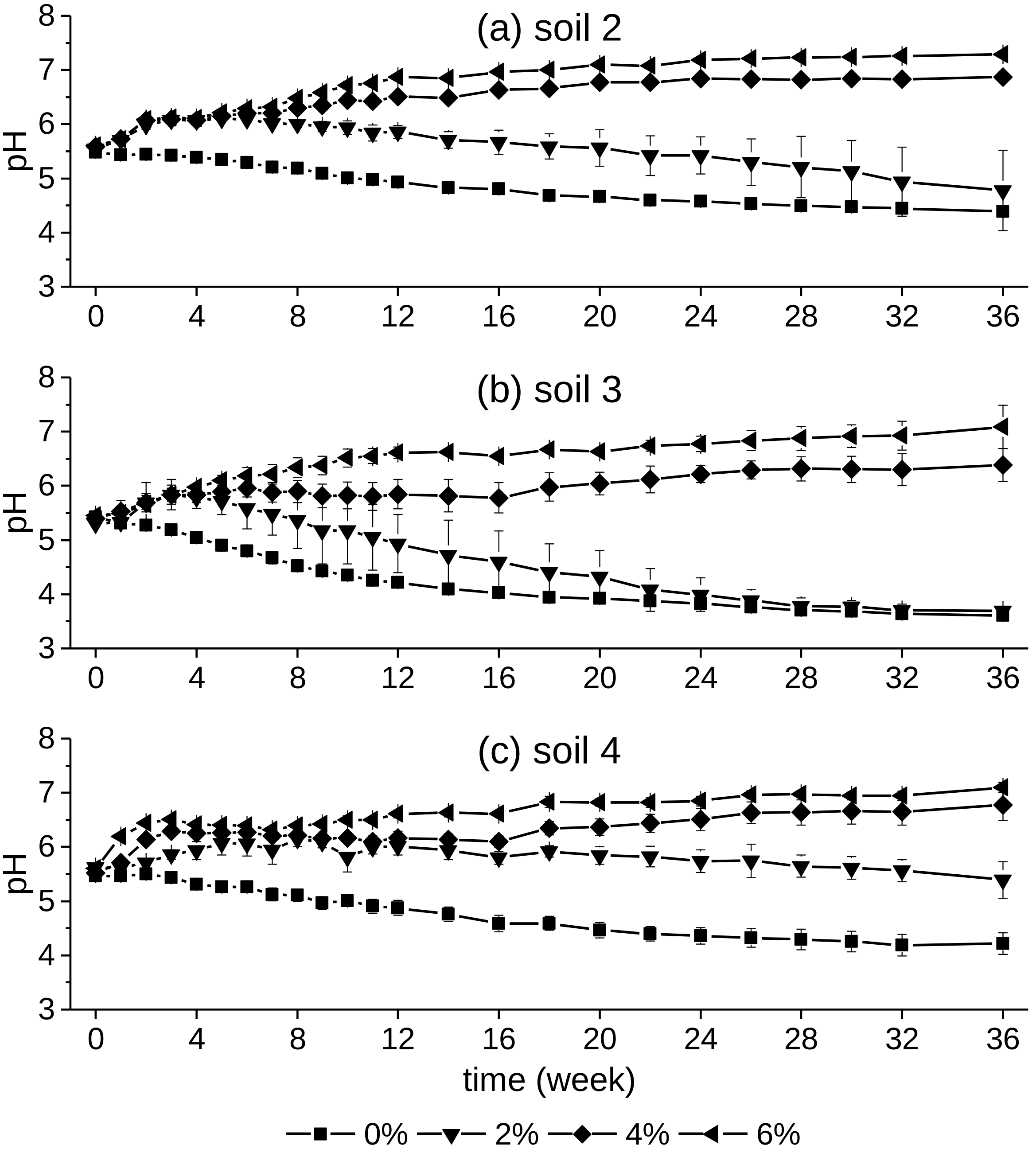

Figure S2. pH of soil pore water over 36 weeks in soils 2 (a), 3 (b), and 4 (c) amended with 0, 2, 4 and 6 % (w/w) organic matter in Experiment 1. Vertical lines show standard deviation (n=3).

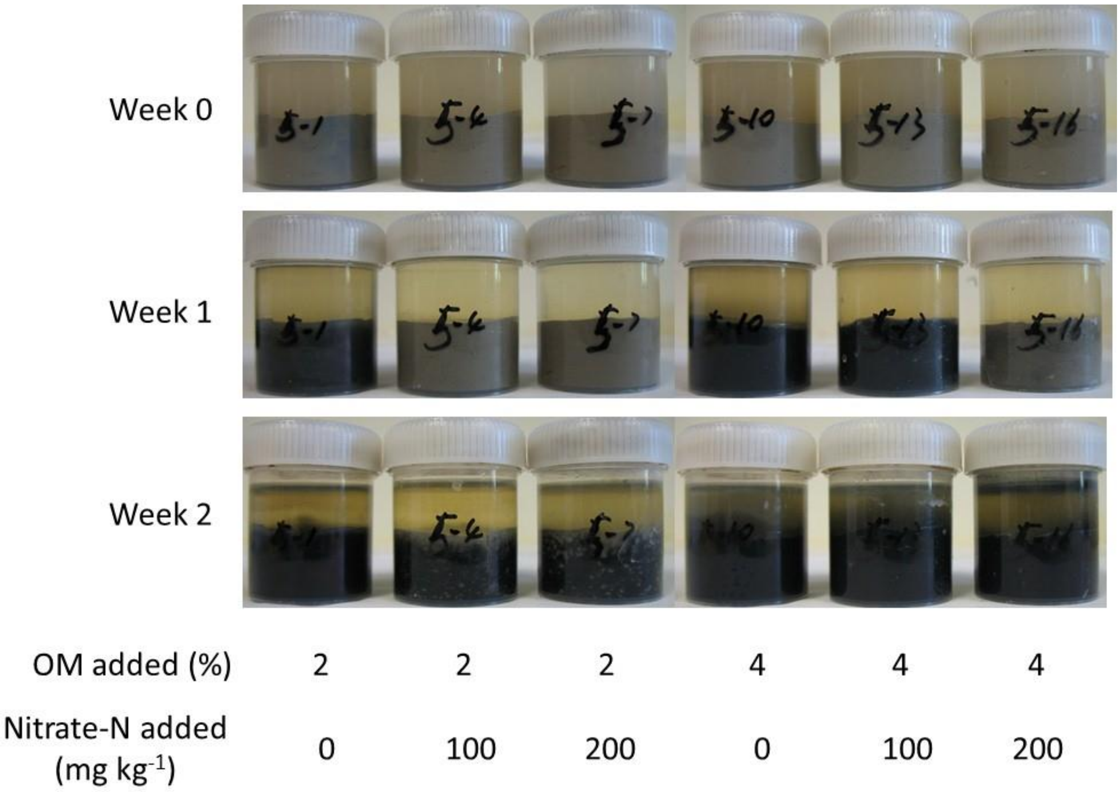

Figure S3. Morphology of soil 5 in weeks 0, 1 and 2 at different addition rates of organic matter (OM) and nitrate-N in Experiment 2.

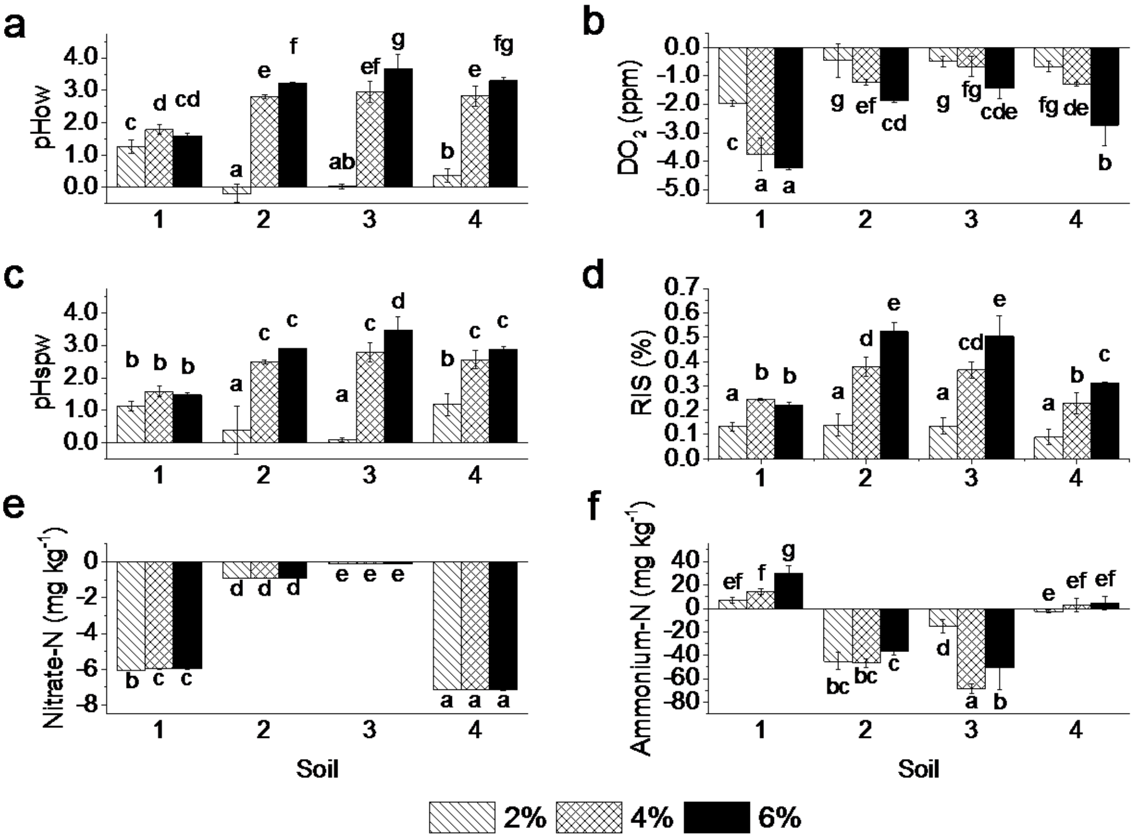

Figure S4. Difference of properties of overlying water and soil compared to the unamended control after 36 weeks in soils 1, 2, 3 and 4 amended with 2, 4 and 6 % (w/w) organic matter in Experiment 1. (a) pHow, pH of overlying water; (b) DO$_2$, dissolved oxygen concentration in overlying water; (c) pHspw, pH of soil pore water; and soil concentrations of (d) RIS, reduced inorganic sulfur, (e) nitrate-N, and (f) ammonium-N. Vertical lines show standard deviation (n=3); values that do not share the same letter are significantly different at $P \leq 0.05$.

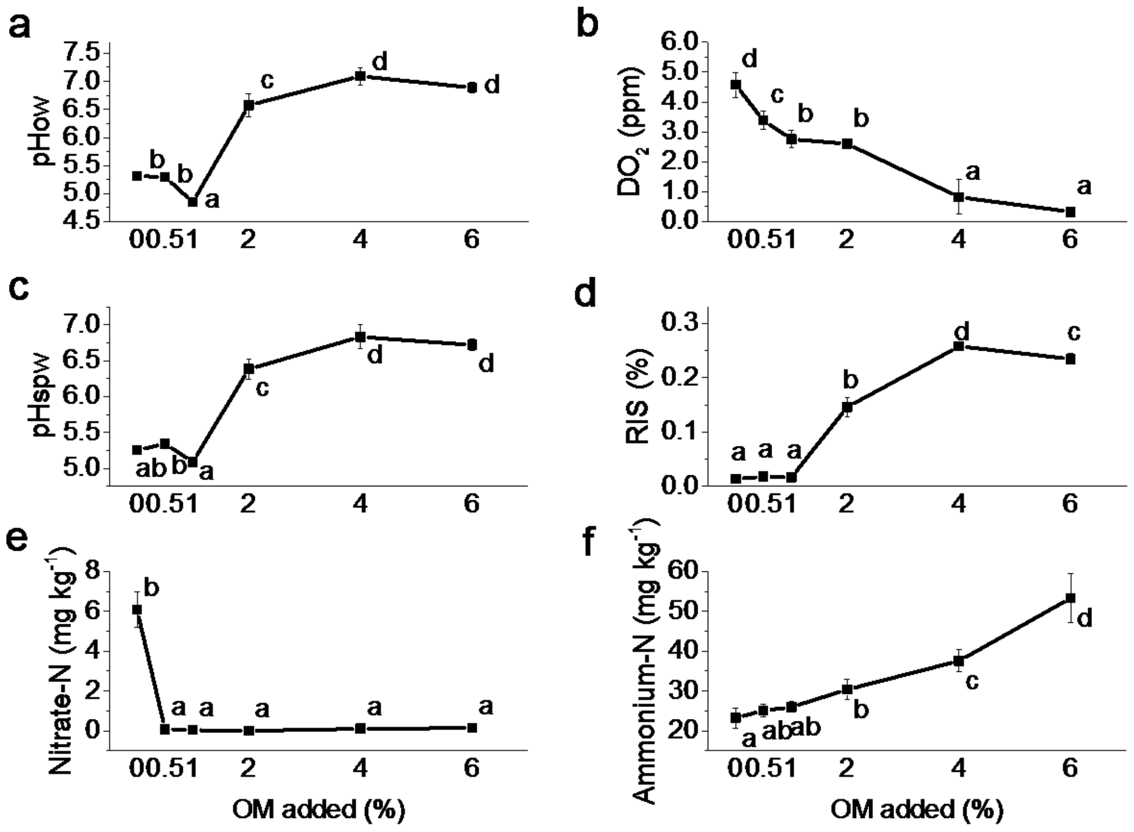

Figure S5. Properties of overlying water and soil in soil 1 amended with 0, 0.5, 1, 2, 4 and 6 % (w/w) organic matter after 36 weeks in Experiment 1. (a) pHow, pH of overlying water; (b) DO_2, dissolved oxygen concentration in overlying water; (c) pHspw, pH of soil pore water; and soil concentrations of (d) RIS, reduced inorganic sulfur, (e) nitrate-N, and (f) ammonium-N. Vertical lines show standard deviation (n=3); values that do not share the same letter are significantly different at $P \leq 0.05$.

CHAPTER 4

Organic matter addition reduces acidification during oxidation of sulfidic material: effect of application form and rate

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Author Contributions

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Contribution to the Paper	Supervised development of work, helped in data interpretation and manuscript evaluation, and acted as corresponding author		
Signature		Date	22/01/2015

Organic matter addition reduces acidification during oxidation of sulfidic material: effect of application form and rate

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Abstract

Acid sulfate soils with sulfidic material are widespread and can have detrimental impacts on ecosystems after acidification induced by pyrite oxidation. Conventional methods to prevent acidification of sulfidic material such as flooding or burying are costly. Adding organic matter (OM) may be an economical and environment-friendly remedial option because OM could retard oxidation of pyrite in sulfidic material through oxygen consumption by decomposing microbes, complexation of dissolved Fe(III), and coating of pyrite. OM can also buffer acid generated. But systematic investigations are needed to test this and optimize the effectiveness of OM application. Two experiments were carried out with sulfidic material incubated under oxidizing conditions for 6 weeks. The aim of Experiment 1 was to determine the effect of OM application form on acidification. There were three treatments: without OM addition (Ctrl) and with 30 g kg^{-1} OM (finely ground mature wheat straw) mixed into the soil (OMM) or placed as a layer on the soil surface (OML). The aim of Experiment 2 was to assess the effect of the rate of OM mixed into the soil (10–40 g kg^{-1} OM) on acidification. In Experiment 1, soil pH after 6 weeks was in the order OMM>OML>Ctrl. Mixing OM also minimized acidification in Experiment 2: after 6 weeks soil pH was in the order: 40=30>20>10>Ctrl. In both experiments when OM was added compared to the control, the decrease in soil concentration of total organic carbon was greater but the increase in soil sulfate concentration was smaller. However, the sulfate increase was not different between OM application forms or among OM addition rates. These indicate OM addition retarded soil acidification by increasing microbial decomposition and thereby competition with pyrite oxidizers for oxygen and by pH buffer capacity.

Keywords: acidification; acid sulfate soils; addition rate; application form; organic matter; pyrite oxidation; sulfidic material

1. Introduction

Acid sulfate soils (ASS) , soils containing iron sulfide minerals (predominantly pyrite) and/or their oxidation products (Fitzpatrick et al., 2009), are widespread, e.g. 10^7−10^8 ha coastal ASS world-wide (Andriesse and van Mensvoort, 2007; Macdonald et al., 2011). In Australia where ASS cover 215,000 km^2 (Fitzpatrick et al., 2010), the climate is dry and more frequent droughts are expected in the future (Hobday and McDonald, 2014; Stokes et al., 2008). Therefore oxidation of ASS with sulfidic material (containing iron sulfide minerals) is of great environmental concern.

After exposure to oxygen, pyrite can be oxidized according to the following equations (Ahern et al., 2004; Chandra and Gerson, 2010; Evangelou, 1995):

$$FeS_2+7/2O_2+H_2O \rightarrow Fe^{2+}+2SO_4^{2-}+2H^+ \text{ (1),}$$

$$Fe^{2+}+1/4O_2+H^+ \rightarrow Fe^{3+}+1/2H_2O \text{ (2),}$$

$$Fe^{3+}+3H_2O \rightarrow 3Fe(OH)_3\downarrow+3H^+ \text{ (3),}$$

$$FeS_2+14Fe^{3+}+8H_2O \rightarrow 15Fe^{2+}+2SO_4^{2-}+18H^+ \text{ (4).}$$

Oxygen initiates pyrite oxidation but in most situations dissolved ferric iron (Fe^{3+}) is the primary oxidant, and the transformation of dissolved ferrous iron (Fe^{2+}) to ferric iron (Equation 2), mainly catalyzed by chemolithotrophic acidophilic iron-oxidizing bacteria, is considered as the rate-limiting step in pyrite oxidation (Ahern et al., 2004; Emerson et al., 2010; Johnson and Hallberg, 2005).

The overall equation for the pyrite oxidation and the hydrolysis of Fe^{3+} is:

$$4FeS_2+15O_2+14H_2O \rightarrow 4Fe(OH)_3+8SO_4^{2-}+16H^+ \text{ (5).}$$

This reaction leads to significant release of acid and resulting dissolved metals (Ahern et al., 2004; Johnson and Hallberg, 2005; Neculita et al., 2007), which can have detrimental effects on soil and water quality and ecosystem services (Bronswijk et al., 1993; Fitzpatrick et al., 2009; Mosley et al., 2014a; Mosley et al., 2014b).

The conventional way to prevent oxidation or acidification of sulfidic material is by covering with water or non-ASS soil, but this can be costly or not practically feasible due to the large amount of water and soil required (Baldwin and Fraser, 2009; Dear et al., 2002; Fitzpatrick et al., 2009). A more economical and environmentally friendly potential option is the application of organic matter (OM). OM could influence oxidation of pyrite through oxygen consumption by decomposing microbes, complexation of dissolved ferric iron, and coating of pyrite (Bronswijk et al., 1993; Bush and Sullivan, 1999; Rigby et al., 2006; Ward et al., 2004), and buffer the generated acid (Paul and Ulf, 2011). Mitigation of oxidation of pyritic fines by OM was observed in a reaction vessel (Rigby et al., 2006), and mulching with OM has been suggested as a method to prevent oxidation or acidification of sulfidic material by maintaining anoxia and providing pH buffer capacity (Baldwin and Fraser, 2009; Cook et al., 2004; Fitzpatrick et al., 2009). However, to our knowledge the influence of OM addition form and rate on acidification during oxidation has not been demonstrated in ASS with sulfidic material.

The aim of this study is to determine the effect of OM addition by mixing or as a layer (Experiment 1) and of OM addition rate (Experiment 2) on acidification of sulfidic material under oxidizing conditions. Our hypotheses are (i) OM addition can retard acidification of sulfidic material, (ii) OM addition by mixing is more effective than as a layer because the former allows more contact of OM with soil, and (iii) the effectiveness of OM addition by mixing increases with OM addition rate.

2. Materials and methods

2.1. Soil

The sulfidic material used was a sandy loam from a wetland at Banrock Station, South Australia (34°12'4.8"S, 140°20'14.5"E). Before experiment, the soil was dried in a fan-forced oven at 40°C for 48 hours, ground, and sieved to < 2 mm. The ground and sieved soil had the following properties: pH (1:1) 5.0, pH (1:5) 5.5, total organic carbon (TOC) 4.8 g kg^{-1}, water holding capacity (WHC) 0.18 g g^{-1}, 1 M KCl extractable sulfur (S_{KCl}) 81 mg kg^{-1}, and chromium reducible sulfur (Scr; mainly pyrite) 0.02%.

2.2. Experimental procedure

2.2.1. Experiment 1

In Experiment 1 there were three treatments (with three replicates): control (Ctrl), OM mixed into the soil (OMM), and OM placed as a layer on the soil surface (OML). Thirty five grams of dry soil was filled into 70 ml polypropylene containers. Reverse osmosis (RO) water was added to and mixed with soil to adjust the soil water content to 100% of WHC (i.e., approximately field capacity, which is considered as the optimum moisture for oxidation of sulfidic material (Creeper et al., 2012)). After wetting and mixing, the height of soil layer was around 2 cm. For treatments OMM and OML, mature wheat straw (ground to <2 mm; pH(1:5) 6.24; TOC 313 g kg^{-1}; total nitrogen 7 g kg^{-1}) was added at 30 g kg^{-1}. For the treatment OMM, slightly more water was added due to the higher WHC in soil mixed with OM at 30 g kg^{-1} (0.22 g g^{-1} compared to 0.18 g g^{-1} in the control). For the treatment OML, the soil water content was adjusted to 100% WHC and dry wheat straw was placed on the soil surface, then RO water was added to the wheat straw so that the total amount of water added in treatment OML was the same as in OMM. The height of the moist OM layer was approximately 0.5 cm. The degree of saturation of the soil, i.e. the fraction of soil pores filled with water, was 80–90% in all treatments.

The containers were incubated for 6 weeks in the dark at room temperature (around 25°C) covered loosely with the lids. Soil moisture was kept constant by adding RO water to maintain the weight every one or two days based on the observation that in all treatments the water loss rate was around 0.1 g per day. Soil pH was measured weekly by inserting a spear-point pH electrode (Model IJ44C, Ionode, Australia) into the soil. For the treatment OML, a small hole was made in the OM layer to allow inserting the pH electrode into the soil. After pH measurement, the hole was refilled. Concentrations of soil S_{KCl}, TOC, particulate organic carbon (POC), and mineral-associated organic carbon (MaOC) were determined at the start and the end of the experiment (weeks 0 and 6).

2.2.2. Experiment 2

Wheat straw was mixed into soil at 10, 20, 30 and 40 g kg^{-1} (on oven dry basis), and unamended soil served as control. The soil was incubated as described above in Experiment 1. Soil pH was determined over 6 weeks, and S_{KCl} and TOC were measured in weeks 0 and 6.

2.3. Methods

Soil texture was analyzed after Kettler et al. (2001). The maximum water holding capacity (WHC) of the soils was measured by using a sintered glass funnel connected to a 1 m water column (Ψ_m= -10 kPa). The soil was placed in cores on a porous plate in a sintered glass funnel, thoroughly wetted and allowed to drain for two days. The drained soil was weighed before and after oven-drying at 105°C for 48 hours to determine the water content. 1 M KCl extractable sulfur (S_{KCl}), containing mainly soluble and exchangeable sulfate, was extracted by shaking for 4 h with 1 M KCl according to Ahern et al. (2004) and sulfur in the extract was measured by ICP-AES. Chromium reducible sulfur (Scr; mainly pyrite) was measured after Ahern et al. (2004). The pH buffer capacity of the soil without OM addition and with 30 g kg^{-1} OM mixed was measured after Aitken and Moody (1994). Total organic carbon (TOC) was measured by the Walkley-Black method (Nelson and Sommers, 1996). Soil particulate organic carbon (POC) and mineral-associated organic carbon (MaOC) were extracted after Skjemstad et al. (2004) and Cambardella and Elliott (1992). After fractionation organic carbon was measured using the Walkley-Black method (Nelson and Sommers, 1996). The POC and MaOC content was expressed in percentage of total organic C recovered, which is the sum of organic C in the two soil fractions ((TOC concentration of MaOC fraction × weight of MaOC fraction) + (TOC concentration of POC fraction × weight of POC fraction)). The degree of soil saturation (Sw) was estimated based on soil water content and bulk density (White, 2009). Soil pH was measured by inserting an electrode into the soil. In week 0 in Experiment 1, three replicates were used for the analysis of TOC, POC and MaOC but only two replicates for S_{KCl}. In Experiment 2, two replicates were used for soil analysis in week 0.

2.4. Data analysis

One-way analysis of variance (ANOVA) was performed for soil properties in week 0 (except S_{KCl} in Experiments 1 and 2 and TOC in Experiment 2 because only two replicates were analyzed) and in week 6, and for the change of these properties. Significant differences ($P \leq 0.05$) among treatments were determined by Fisher's protected least significant difference test. Statistics were carried out using GenStat 16.2 (VSN International Ltd., UK).

3. Results

3.1. Experiment 1

In week 0, soil pH was around 5.0 in all treatments (Figure 1). In the first three weeks, the pH decreased by 0.9 units in the control but increased by 0.4 units in OML and by 0.7 units in OMM. After that, pH stabilized in the control and OML, but continued to increase slightly in OMM. Consequently, in week 6 soil pH was in the order Ctrl<OML<OMM. Mixing of 30 g kg^{-1} OM into the soil increased the pH buffer capacity compared to the

unamended soil from 8.7 to 50.5 mmol kg^{-1} pH^{-1} in the pH range ±1 unit of the initial pH (data not shown).

In week 6, S_{KCl} concentrations did not differ significantly among treatments. Soil S_{KCl} concentration increased from week 0 to week 6 (Figure 2 (a)), with a greater increase in the control (approximately 3-fold) than in OML (by 70%) and OMM (by 30%).

The TOC content was about 3-fold higher in the treatments with OM addition than the control in week 0, and was lowest in the control and highest in OMM in week 6 (Figure 2 (b)). From week 0 to week 6, the TOC content did not change in the control but decreased by 24% in OMM and greater by 32% in OML.

In week 0, addition of OM increased the proportion of POC about 3-fold and consequently decreased the proportion of recovered organic carbon as MaOC (Figure 3). In the control the proportions of the OC fractions did not change from week 0 to week 6. In treatments OMM and OML the POC proportion decreased by 20–30% whereas the MaOC proportion increased by 70–80%.

3.2. Experiment 2

In week 0, soil pH was around 5.0 regardless of OM addition rates (Figure 4). In the unamended control, the pH decreased in the first three weeks by 1 unit and then stabilized. When OM was added, the soil pH in the first two weeks was stable with 10 g kg^{-1} OM addition or increased by around 0.5 units with 20–40 g kg^{-1} OM added. The pH dropped by around 0.4 units from week 2 to week 3 and then stabilized in most OM treatments (except a further pH decline by 0.3 units from week 4 to week 6 with 20 g kg^{-1} OM). After 6 weeks the pH was lower in the control than in treatments with OM addition, where it was highest with 30 and 40 g kg^{-1} OM added, followed by 20 g kg^{-1} OM addition and lowest with 10 g kg^{-1} OM added.

The S_{KCl} concentration in week 6 was higher in the control than in the treatments with OM addition. The increase in S_{KCl} concentration from week 0 to week 6 was greater in the control (around 4-fold) than in the treatments with OM addition (by 60–100%) (Figure 5(a)).

In weeks 0 and 6, the TOC concentration increased with OM addition rate (Figure 5 (b)). The TOC content decreased from week 0 to week 6, by 15% in the control and by 20–25% in the OM treatments.

4. Discussion

As expected, the unamended sulfidic material acidified when incubated under oxidizing conditions (Figure 1). The results confirmed our first (OM addition can retard acidification of sulfidic material) and second hypothesis (OM addition by mixing is more effective than as a layer because the former allows more contact of OM with soil).

Acidification was prevented by 30 g kg^{-1} OM addition by mixing into the soil or as a layer placed on the soil surface (Figure 1). The pH decrease in the control was associated with a strong increase in S_{KCl} from week 0 to week 6 (Figure 2), indicating that pyrite was oxidized. In the treatments with OM there was a small increase in S_{KCl}, but the pH did not decrease. The lack of decrease in pH may be explained by the additional pH buffer capacity provided by OM. OM contains carboxyl groups which can bind protons at low pH if the pH is above their pKa (Curtin and Trolove, 2013; Paul and Ulf, 2011). The decrease in TOC in the amended soils from week 0 to week 6 shows that the added OM was decomposed (Figure 2). This decrease in TOC is likely to be mainly due to decomposition of POC, as the proportion of POC in the organic carbon recovered decreased from week 0 to week 6 (Figure 3). The absolute changes in soil POC proportion and in pH over 6 weeks were strongly correlated (r=-0.96, n=9). Therefore the lower acidification rate and smaller increase in S_{KCl} in amended soil is also due to oxygen depletion by microbes decomposing the added OM. In the control the TOC content did not change from week 0 to week 6 (Figure 2), indicating that native OM was poorly available. Therefore little oxygen was used by decomposers and more was available to sulfide-oxidizing bacteria.

The results in Experiment 1 suggested that adding OM for prevention of acidification was more effective by mixing than as a layer on the soil surface. The TOC decrease during the experiment was smaller in OMM than OML (Figure 2), suggesting lower decomposition rate and thus oxygen consumption in the former. However, the inhibition of pyrite oxidization in these two treatments was not significantly different (Figure 2). This may be due to the direct competition for oxygen between OM decomposers and sulfide oxidizers in OMM whereas decomposers and sulfide oxidizers were spatially separated in OML. In addition, OM mixed into soil may complex dissolved Fe(III) generated during pyrite oxidation (Equation 2) thereby preventing it from oxidizing pyrite (Bronswijk et al., 1993) more efficiently than a OM layer. But the pH in week 6 was higher in OMM than in OML, probably because OM mixed into soil has more contact with soil components and thus can also buffer pH change more efficiently than an OM layer. However, the OM layer was only 0.5 cm thick in our experiment. A thicker layer may be more effective for prevention of acidification.

The second experiment confirmed the potential of mixing OM into the soil in retarding acidification during oxidation of sulfidic material, but also showed that effect of mixing OM depends on the addition rate (Figure 4). However, the third hypothesis (the effectiveness of OM addition by mixing increases with OM addition rate) has to be partly declined. After 6 weeks, the pH was lowest with 10 g kg^{-1} OM added and highest with 30 or 40 g kg^{-1} (Figure 4). The absolute decrease in TOC content from week 0 to week 6 was greater at 30 and 40 g kg^{-1} than with the lower OM addition rates (Figure 5), which suggests greater decomposition rate and thus oxygen consumption in the former. The greater decomposition rates could also increase the concentration of ferric iron chelating compounds such as organic acids which are produced during decomposition of OM (Tipping, 2002). However the S_{KCl} increase in the treatments with OM added was similar (Figure 5(a)). The lack of relationship between S_{KCl} increase and OM addition rate suggests that for the soil used, which has a low initial pyrite concentration (0.02%), 10 g kg^{-1} OM added was enough to reduce pyrite oxidation. However, higher OM addition rates mitigated acidification to a greater extent, probably due to the greater pH buffer capacity by the higher OM concentrations. OM addition rates of 30 and 40 g kg^{-1} did not differ in pH in week 6, suggesting that the pH buffer capacity provided by 30 g kg^{-1} OM was enough to buffer the acid generated by pyrite oxidation in the soil used.

5. Conclusion

This study showed that mixing OM at 30 g kg^{-1} into soil can prevent the acidification of the sulfidic material used, which can be explained by consumption of oxygen by OM decomposers and the pH buffer capacity of the OM. Lower OM addition rates and placing OM on the soil surface appear to be less effective. However effectiveness of OM addition may depend on OM properties (decomposition rate, pH buffer capacity) and soil properties (sulfide concentration, native pH buffer capacity). Further, feasibility and costs of OM treatments would have to be tested under field conditions.

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References

Ahern, C. R., McElnea, A. E., and Sullivan, L. A. (2004). "Acid Sulphate Soils: Laboratory Methods Guidelines," Department of Natural Resources, Mines and Energy, Indooroopilly, Queensland, Australia.

Aitken, R., and Moody, P. (1994). The effect of valence and Ionic-strength on the measurement of pH buffer capacity. *Soil Research* **32**, 975-984.

Andriesse, W., and van Mensvoort, M. (2007). Acid Sulfate Soils: Distribution and Extent. *In* "Encyclopedia of Soil Science, Second Edition", pp. 14-19. Taylor & Francis.

Baldwin, D. S., and Fraser, M. (2009). Rehabilitation options for inland waterways impacted by sulfidic sediments - A synthesis. *Journal of Environmental Management* **91**, 311-319.

Bronswijk, J. J. B., Nugroho, K., Aribawa, I. B., Groenenberg, J. E., and Ritsema, C. J. (1993). Modeling of Oxygen-Transport and Pyrite Oxidation in Acid Sulfate Soils. *Journal of Environmental Quality* **22**, 544-554.

Bush, R. T., and Sullivan, L. A. (1999). Pyrite micromorphology in three Australian Holocene sediments. *Soil Research* **37**, 637-654.

Cambardella, C. A., and Elliott, E. T. (1992). Particulate Soil Organic-Matter Changes across a Grassland Cultivation Sequence. *Soil Sci. Soc. Am. J.* **56**, 777-783.

Chandra, A. P., and Gerson, A. R. (2010). The mechanisms of pyrite oxidation and leaching: A fundamental perspective. *Surface Science Reports* **65**, 293-315.

Cook, F. J., Dobos, S. K., Carlin, G. D., and Millar, G. E. (2004). Oxidation rate of pyrite in acid sulfate soils: in situ measurements and modelling. *Australian Journal of Soil Research* **42**, 499-507.

Creeper, N., Fitzpatrick, R., and Shand, P. (2012). A simplified incubation method using chip-trays as incubation vessels to identify sulphidic materials in acid sulphate soils. *Soil Use and Management* **28**, 401-408.

Curtin, D., and Trolove, S. (2013). Predicting pH buffering capacity of New Zealand soils from organic matter content and mineral characteristics. *Soil Research* **51**, 494-502.

Dear, S.-E., Moore, N. G., Dobos, S. K., Watling, K. M., and Ahern, C. R. (2002). Soil Management Guidelines. *In* "Queensland Acid Sulfate Soil Technical Manual". Department of Natural Resources and Mines, Indooroopilly, Queensland, Australia.

Emerson, D., Fleming, E. J., and McBeth, J. M. (2010). Iron-Oxidizing Bacteria: An Environmental and Genomic Perspective. *Annual Review of Microbiology* **64**, 561-583.

Evangelou, V. P. (1995). "Pyrite Oxidation and Its Control," CRC Press.

Fitzpatrick, R., Marvanek, S., Powell, B., Grealish, G., and Gilkes, R. (2010). Atlas of Australian Acid Sulfate Soils: recent developments and future priorities. *In* "Proceedings of the 19th World Congress of Soil Science: Soil solutions for a changing world. Brisbane, Australia, 1-6 August, 2010" (R. Gilkes and N. Prakongkep, eds.), pp. 24-27. Published on DVD; ISBN 987-0-646-53783-2; http//www.iuss.org; Symposium WG 3.1 Processes in acid sulfate soil materials.

Fitzpatrick, R. W., Shand, P., and Merry, R. H. (2009). Acid sulfate soils. *In* "Natural History of the Riverland and Murraylands" (J. T. Jennings, ed.), pp. 65-111. Royal Society of South Australia (Inc.), Adelaide, South Australia.

Hobday, A. J., and McDonald, J. (2014). Environmental Issues in Australia. *Annual Review of Environment and Resources* **39**, 1-28.

Johnson, D. B., and Hallberg, K. B. (2005). Acid mine drainage remediation options: a review. *Sci Total Environ* **338**, 3-14.

Kettler, T. A., Doran, J. W., and Gilbert, T. L. (2001). Simplified method for soil particle-size determination to accompany soil-quality analyses. *Soil Science Society of America Journal* **65**, 849-852.

Macdonald, B. C. T., Denmead, O. T., White, I., and Byrant, G. (2011). Gaseous Nitrogen Losses from Coastal Acid Sulfate Soils: A Short-Term Study. *Pedosphere* **21**, 197-206.

Mosley, L. M., Palmer, D., Leyden, E., Cook, F., Zammit, B., Shand, P., Baker, A., and Fitzpatrick, R. W. (2014a). Acidification of floodplains due to river level decline during drought. *Journal of Contaminant Hydrology* **161**, 10-23.

Mosley, L. M., Zammit, B., Jolley, A. M., and Barnett, L. (2014b). Acidification of lake water due to drought. *Journal of Hydrology* **511**, 484-493.

Neculita, C.-M., Zagury, G. J., and Bussiere, B. (2007). Passive treatment of acid mine drainage in bioreactors using sulfate-reducing bacteria: Critical review and research needs. *Journal of Environmental Quality* **36**, 1-16.

Nelson, D. W., and Sommers, L. E. (1996). Total Carbon, Organic Carbon, and Organic Matter. *In* "Methods of Soil Analysis Part 3—Chemical Methods" (D. L. Sparks, A. L. Page, P. A. Helmke, R. H. Loeppert, P. N. Soltanpour, M. A. Tabatabai, C. T. Johnston and M. E. Sumner, eds.), pp. 961-1010. Soil Science Society of America and American Society of Agronomy, USA.

Paul, R. B., and Ulf, S. (2011). Soil pH and pH Buffering. *In* "Handbook of Soil Sciences" (P. M. Huang, Y. Li and M. E. Sumner, eds.). CRC Press.

Rigby, P. A., Dobos, S. K., Cook, F. J., and Goonetilleke, A. (2006). Role of organic matter in framboidal pyrite oxidation. *Science of The Total Environment* **367**, 847-854.

Skjemstad, J. O., Spouncer, L. R., Cowie, B., and Swift, R. S. (2004). Calibration of the Rothamsted organic carbon turnover model (RothC ver. 26.3), using measurable soil organic carbon pools. *Australian Journal of Soil Research* **42**, 79-88.

Stokes, C. J., Ash, A., and Howden, S. M. (2008). Climate Change Impacts on Australian Rangelands. *Rangelands* **30**, 40-45.

Tipping, E. (2002). "Cation Binding by Humic Substances," Cambridge University Press, Cambridge, UK.

Ward, N. J., Sullivan, L. A., and Bush, R. T. (2004). Soil pH, oxygen availability, and the rate of sulfide oxidation in acid sulfate soil materials: implications for environmental hazard assessment. *Australian Journal of Soil Research* **42**, 509-514.

White, R. E. (2009). "Principles and practice of soil science: the soil as a natural resource," 4/Ed. John Wiley & Sons.

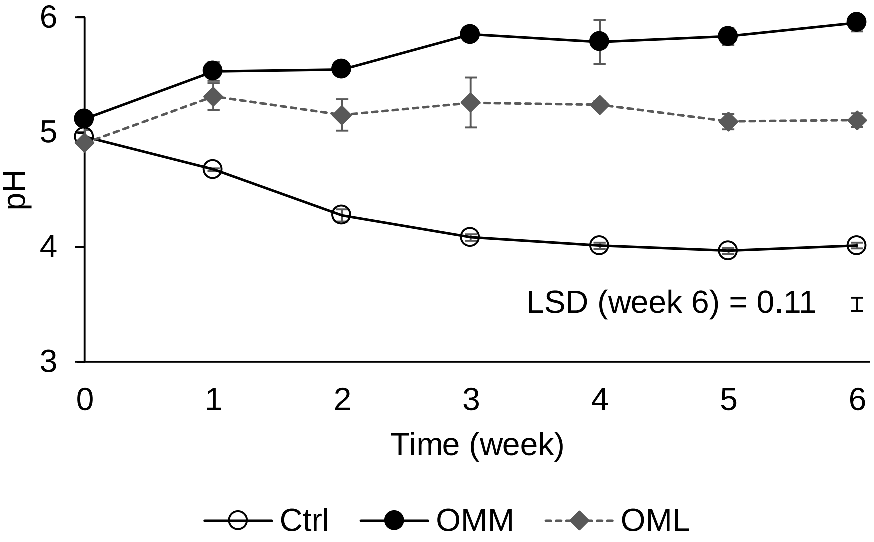
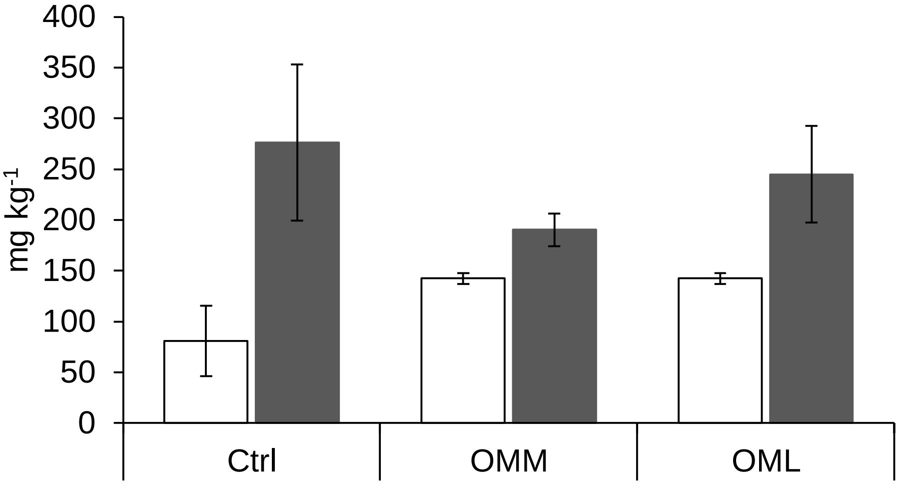
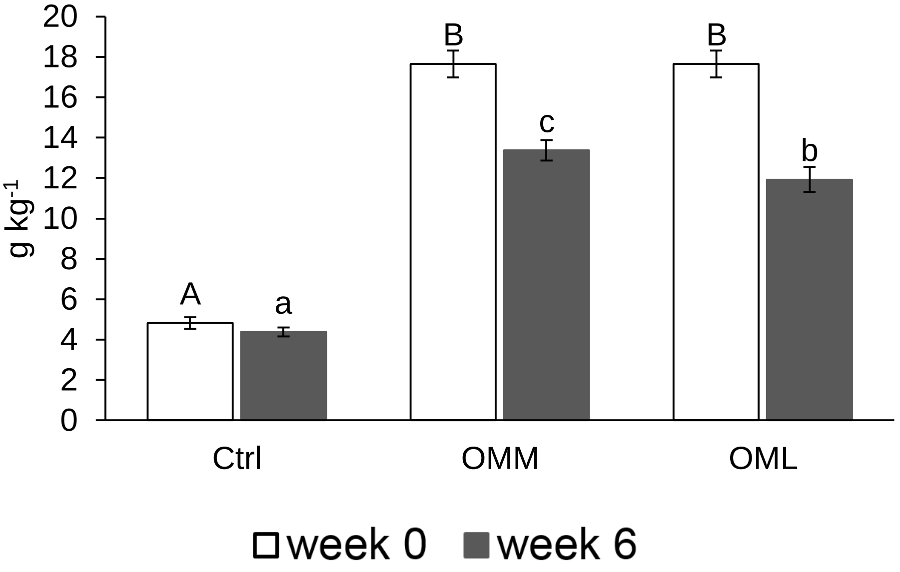


Figure 1. Soil pH over 6 weeks in the control (Ctrl) and treatments with OM addition (30 g kg^{-1}) by mixing (OMM) or as a layer (OML) in Experiment 1 (n=3). Vertical lines on values show standard deviation (may be too short to be visible).



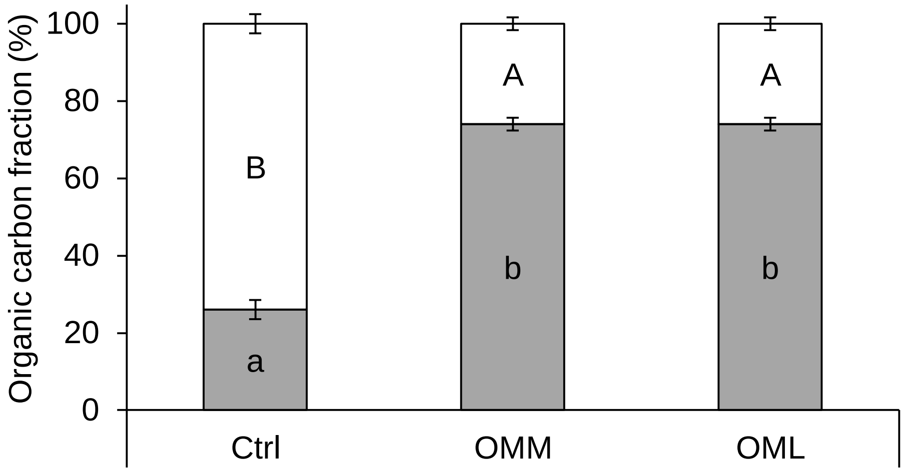
(a) S_{KCl}



(b) TOC

Figure 2. (a) 1 M KCl extractable sulfur (S_{KCl}) and (b) total organic carbon (TOC) in weeks 0 and 6 in the control (Ctrl) and treatments with OM addition (30 g kg^{-1}) by mixing (OMM) or as a layer (OML) in Experiment 1 (n=2 for S_{KCl} in week 0, otherwise n=3). Vertical lines show standard deviation. For S_{KCl} in week 6, treatments were not significantly different. For TOC, values that do not share the same letter are significantly different at P≤0.05, with capital letters for week 0 and small letters for week 6.

(a) Week 0



(b) Week 6

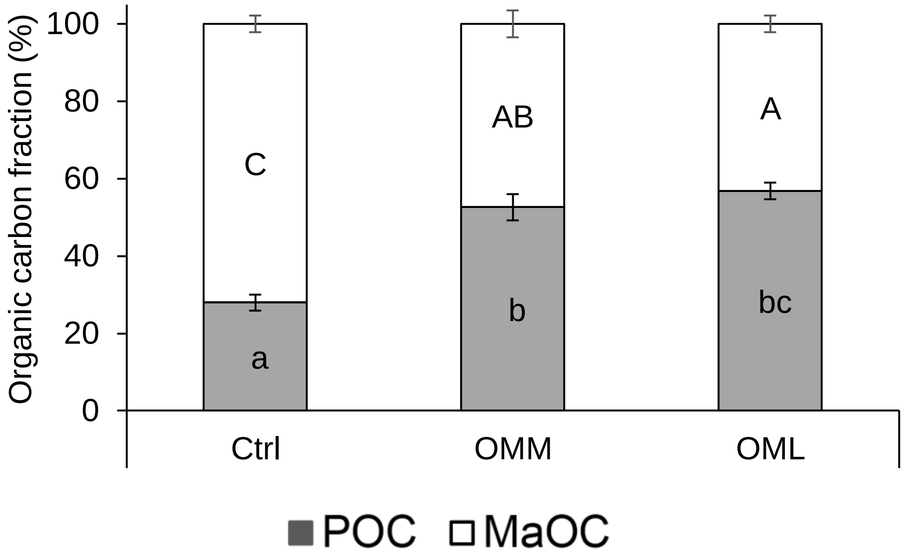


Figure 3. Soil organic carbon fractions (particulate organic carbon (POC) and mineral-associated organic carbon (MaOC)) in percentage of recovered organic carbon in weeks 0 (a) and 6 (b) in the control (Ctrl) and treatments with OM addition (30 g kg^{-1}) by mixing (OMM) or as a layer (OML) in Experiment 1 (n=3). Vertical lines show standard deviation. Values that do not share the same letter are significantly different at P≤0.05, with capital letters for MaOC and small letters for POC.

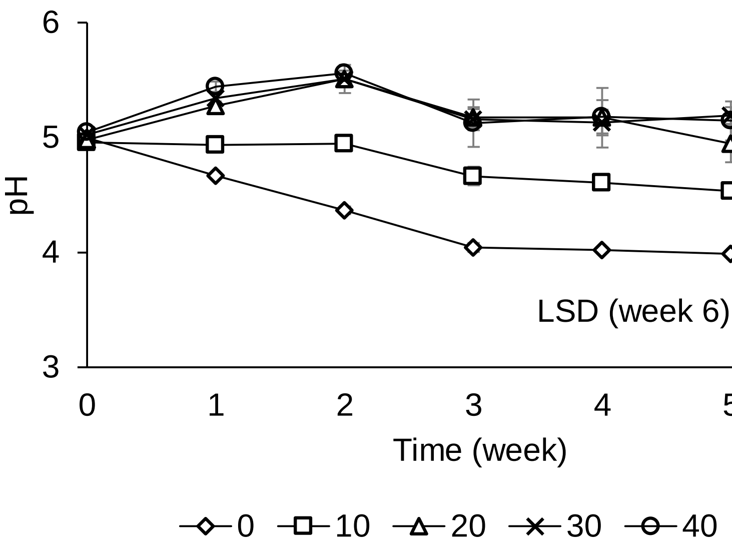
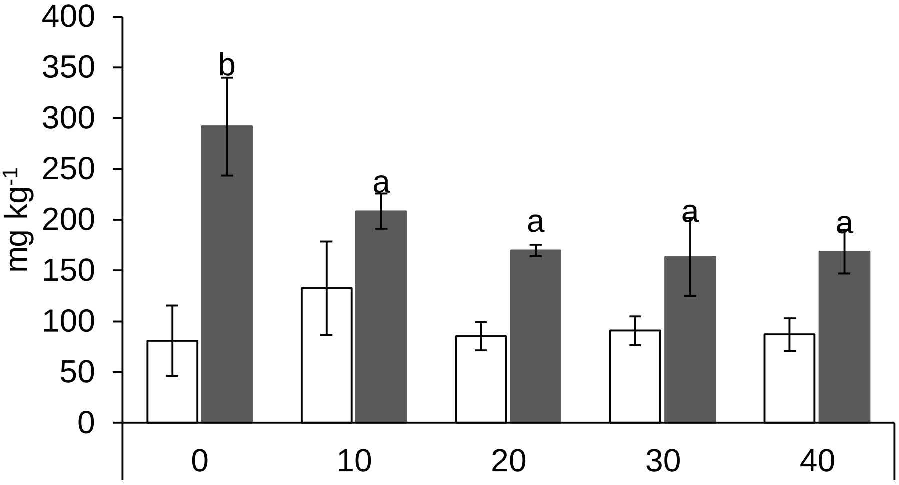


Figure 4. Soil pH over 6 weeks with 0-40 g kg^{-1} OM mixed into the soil in Experiment 2 (n=3). Vertical lines on values show standard deviation (may be too short to be visible).

(a) S_{KCl}



(b) TOC

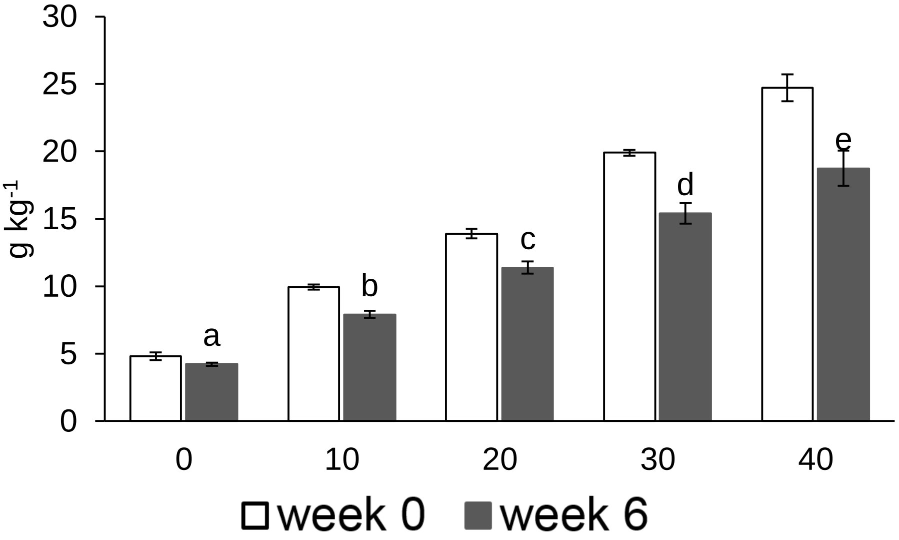


Figure 5. (a) 1 M KCl extractable sulfur (S_{KCl}) and (b) total organic carbon (TOC) in weeks 0 and 6 with 0-40 g kg^{-1} OM mixed into the soil in Experiment 2 (n=2 in week 0, n=3 in week 6). Vertical lines show standard deviation (may be too short to be visible). Values in week 6 that do not share the same letter are significantly different at P≤0.05.

CHAPTER 5

Conclusion and future research

Conclusion and future research

1. Conclusion

Organic matter (OM) is a vital component of the soil ecosystem and plays a critical role in biogeochemical cycles (Baldock, 2007). In acid sulfate soils (ASS), OM is the energy source for sulfate-reducing bacteria, which are responsible for the formation of sulfides in sulfidic material and generate alkalinity during sulfate reduction in submerged conditions (Baldwin and Fraser, 2009; Berner, 1984; Fitzpatrick et al., 2009). OM can also influence pyrite oxidation through oxygen consumption by OM decomposers, complexation of ferric iron, and coating of pyrite (Bronswijk et al., 1993; Bush and Sullivan, 1999; Rigby et al., 2006; Ward et al., 2004), and buffer acid generated (Paul and Ulf, 2011). However, native OM does not necessarily fulfill these roles. For example, ASS with sulfuric material may persist in the field even after several years of submergence (Baker et al., 2011; Mosley et al., 2014). Thus sulfate reduction may not necessarily occur after submergence of sulfuric material. This is particularly important in areas where future climate scenarios suggest more frequent droughts. Therefore, OM may need to be added to remediate ASS. However, the remedial effect of OM addition has not been systematically investigated in ASS. Information is required on the effects of addition rate and soil properties on the effectiveness of OM addition. The experiments described in this thesis are aimed at filling this research gap.

The experiment described in Chapter 2 confirmed submergence alone does not induce sulfate reduction in sulfuric material. Not only low pH but also low availability of native organic matter limits sulfate reduction in sulfuric material after reflooding. This can be concluded because sulfate reduction was increased compared to the control only in the treatment with pH adjustment to 5.5 and OM addition at 2%. However, the effectiveness of this combined treatment in stimulating sulfate reduction was influenced by soil properties. It was negatively correlated with clay content and initial nitrate concentration. The influence of clay content can be explained by binding of added OM to clay, which reduces its accessibility to microbes (Lützow et al., 2006; Roychand and Marschner, 2013; Shi and Marschner, 2013). Nitrate inhibits sulfate reduction because nitrate is a more energetically favorable acceptor for electrons derived from OM decomposition (Hubert and Voordouw, 2007; Ponnamperuma, 1972). Additionally, nitrate can oxidize reduced sulfur and indirectly inhibit a key enzyme involved in sulfate reduction (Hubert and Voordouw, 2007; Kaster et al., 2007).

The influence of OM addition rate and soil nitrate concentration was further studied in the experiments presented in Chapter 3. Sulfidic material was incubated under submerged conditions with different OM and nitrate addition rates. The first experiment showed that sulfate reduction was stimulated only at OM addition rates ≥ 2%. However, the increase in sulfate reduction between 4% and 6% OM addition was smaller than that between 2% and 4%, suggesting the presence of other limiting factors such as sulfate concentration. The second experiment confirmed the inhibition of sulfate reduction by nitrate, but this inhibition was overcome by a higher OM addition rate (4% compared to 2%). At a high OM addition rate, sufficient OM remains for sulfate reducers after utilization of OM by denitrifiers.

Another strategy to mitigate the environmental impact of ASS is to prevent or minimize acidification during oxidation of sulfidic material. The experiments described in Chapter 4 investigated the effects of different OM application forms and rates on pH changes during incubation of sulfidic material under oxidizing conditions. In the first experiment, acidification was minimized by mixing OM at 3% into the soil whereas placing OM on the soil surface was less effective, which can be explained by the greater contact between OM and soil in the former treatment. The second experiment confirmed that OM mixed into the soil can minimize acidification. Moreover, after incubation soil pH was lower with 1% and 2% OM addition than with 3% and 4% and there was no difference between the two higher rates, but soil sulfate concentration was the same with 1–4% OM addition. This suggest that, for the soil used, 1% OM was sufficient to limit pyrite oxidation, but ≥3% OM was needed to buffer the acid generated. The higher pH and loss of OM in the treatments with OM added compared to the control in both experiments indicated that organic matter addition prevented or minimized acidification through oxygen consumption by OM decomposers, which reduces the oxygen available for pyrite oxidation. Further, OM addition increased soil pH buffer capacity 6-fold, which can mitigate the acidification of soil.

In summary, the studies described in this thesis suggest that OM addition may be a strategy for remediation of ASS, by stimulating sulfate reduction under submerged conditions and preventing or minimizing acidification during oxidation. But they also showed that the effectiveness of OM addition can be influenced by soil properties and addition rates. However, more studies are required to investigate the efficiency and practicality of this strategy in the field.

2. Future studies

2.1. Greater range of ASS

In this thesis, only a limited number of ASS were used. However, there are many types of ASS (Fitzpatrick et al., 2011; Fitzpatrick et al., 2009), which differ in, for example, texture, pyrite content, pH buffer capacity, TOC content and nitrate concentration. Future experiments should be conducted under submerged conditions with a range of sulfuric materials occurring in a certain area with different properties. In the soils used in this thesis, OM addition stimulated sulfate reduction only when the soil pH was increased to 5.5. However, acidotolerant or acidophilic sulfate reducing bacteria have been found in lakes, rivers, wetlands, mine tailings and bioreactors (Koschorreck, 2008; Sanchez-Andrea et al., 2014). Recently, Michael et al. (2015) reported sulfate reduction after adding OM to a sulfuric material with initial pH 3.8. Soil microbial community composition and pH and pH buffer capacity of soil and OM added (also see Chapter 4) may determine whether pH adjustment of soil is needed to initiate sulfate reduction after OM addition. This could be investigated using a wide range of soils because increasing soil pH with chemical ameliorants is costly and not always possible. With a wider range of ASS, correlations between the stimulation of sulfate reduction by addition of OM without or with pH adjustment and soil properties would be more reliable. Further, in soils where the combined treatment was not effective, variations of the treatment could be tested (e.g. higher OM addition rates). With this information, specific remediation strategies for certain ASS could be developed. Monitoring pH and Eh during the experiments described in this thesis was very time consuming (for Eh 30 minutes per measurement) and might have introduced artifacts (oxygen diffusion into the soil during measurement, disturbance of the soil). A system with fixed probes that continuously monitor pH and Eh would allow a greater number of soils to be studied while minimizing artifacts.

2.2. Nitrate and sulfate reduction processes

In this thesis, the detailed processes underlying the results were inferred from knowledge gained from previous studies. For more detailed information about underlying processes, repeated measurements and mass-balance, stoichiometric calculations could be used. For example, the effect of nitrate on sulfate reduction under flooded conditions after OM addition could be examined by simultaneous determination of nitrate remaining in and N_2O and NO released from the soil in combination with measurement of sulfate reduction (reduction in SO_4^{2-}, increase in HS^- and AVS)**.** Isotopic tracers (e.g. ^{15}N labelled nitrate) and detection in emitted gases could also be employed (Peterson and Fry, 1987).

2.3. Wet-dry cycles

In the experiments described in the thesis, the soils were incubated under either reducing or oxidizing conditions for relatively short periods of time (6 to 36 weeks). In the field, ASS may be exposed to wet-dry cycles, which last several months or years (Fitzpatrick et al., 2011; Fitzpatrick et al., 2009). The effect of OM addition under such conditions may differ from those described in this thesis because OM is decomposed or bound more strongly to soil particles over time, which would limit its availability to sulfate reducers and decomposing microbes in general. Further, it is not clear if OM added at the start of the wet phase would be effective in minimizing oxidation in the following dry phase or if OM added at the start of the dry phase could still be available to sulfate reducers in the following wet phase. Therefore, longer term experiments with at least one wet-dry cycle are necessary to better understand the effectiveness of OM addition for remediation of ASS.

2.4. OM particle size and distribution in the soil

For the experiments described in the thesis, wheat straw was finely ground and (in most experiments) thoroughly mixed into the soil. This may not be possible in the field because of costs and available machinery. In the field, OM particle size is likely to be larger and OM will be placed on the soil surface, mixed with the top soil only or placed at depth in furrows that may be meters apart. Since field trials are expensive, incubation experiments could be conducted to inform the design of field experiments. In these incubation experiments, OM particles with various sizes could be added to the soil in different ways (mulching, mixing, or placing in furrows), which would then be exposed to wet-dry cycles.

2.5. C:N ratio of OM added

Our experiments in Chapter 4 suggest that competition between OM decomposition and pyrite oxidation for oxygen is an important mechanism by which OM addition retards acidification during oxidation of sulfidic material. We used only wheat straw, which has a high C:N ratio (> 50). Decomposition rates are higher of OM with low C:N ratios compared to OM with higher C:N ratios, especially in the early stages of decomposition (Chapin III et al., 2011). Therefore the prevention of acidification by OM addition may be influenced by its C:N ratio. An experiment in which OM with different C:N ratios is mixed into sulfidic material could be conducted. Soil pH and oxygen content during oxidizing incubation conditions should be monitored and the change in soil TOC and sulfate concentration after incubation should be measured.

2.6. Gene expression studies

From a basic research point of view, it is of interest to better understand microbiological processes in ASS during wet-dry cycles by studying expression of genes with certain functions. For example, the expression of genes involved in sulfate reduction (e.g. the *dsrAB* gene) could be investigated with qPCR (He et al., 2010) or microarray (He et al., 2007) in soils with and without OM addition at low and high nitrate concentrations. At the same time, expression of denitrification genes (e.g. *nirK*, *nirS* and *nosZ*) could be monitored to estimate the extent and timing of competition for electron donors between nitrate and sulfate reducers.

2.7. Field trials

The development of remediation strategies for ASS requires field trials, but they are expensive. Therefore before field trials are started the studies described above could be conducted to narrow the number of treatments to those most likely to be effective. However, there are issues that can only be studied properly in field trials such as (i) disturbance or compaction of the soil on the site following the treatment, (ii) poor accessibility of OM to sulfate reducers because of very localized OM placement, and (iii) drying of OM placed on the soil surface in the dry season which would reduce decomposition rates and thus competition for oxygen with pyrite oxidation. Further, addition of OM by soil amendments can be costly and may require repeated additions for long-term remediation. Planting of ASS may be a more sustainable and environmentally friendly option to increase OM availability.

References

Baker, A. K. M., Fitzpatrick, R. W., Simpson, S. L., and Merry, R. H. (2011). "Temporal variations in re-flooded Acid Sulfate Soil environments around Lakes Alexandrina and Albert, South Australia," CSIRO Land and Water Science Report, 04/11.

Baldock, J. (2007). Composition and Cycling of Organic Carbon in Soil. *In* "Nutrient Cycling in Terrestrial Ecosystems" (P. Marschner and Z. Rengel, eds.), Vol. 10, pp. 1-35. Springer Berlin Heidelberg.

Baldwin, D. S., and Fraser, M. (2009). Rehabilitation options for inland waterways impacted by sulfidic sediments - A synthesis. *Journal of Environmental Management* **91**, 311-319.

Berner, R. A. (1984). Sedimentary pyrite formation: An update. *Geochimica et Cosmochimica Acta* **48**, 605-615.

Bronswijk, J. J. B., Nugroho, K., Aribawa, I. B., Groenenberg, J. E., and Ritsema, C. J. (1993). Modeling of Oxygen-Transport and Pyrite Oxidation in Acid Sulfate Soils. *Journal of Environmental Quality* **22**, 544-554.

Bush, R. T., and Sullivan, L. A. (1999). Pyrite micromorphology in three Australian Holocene sediments. *Soil Research* **37**, 637-654.

Chapin III, F. S., Matson, P. A., and Vitousek, P. (2011). "Principles of Terrestrial Ecosystem Ecology," 2/Ed. Springer.

Fitzpatrick, R., Shand, P., and Hicks, W. (2011). "Technical Guidelines for Assessment and Management of Inland Freshwater Areas impacted by Acid Sulfate Soils," CSIRO Land and Water Science Report, 05/11. 171 pp.

Fitzpatrick, R. W., Shand, P., and Merry, R. H. (2009). Acid sulfate soils. *In* "Natural History of the Riverland and Murraylands" (J. T. Jennings, ed.), pp. 65-111. Royal Society of South Australia (Inc.), Adelaide, South Australia.

He, J.-Z., Liu, X.-Z., Zheng, Y., Shen, J.-P., and Zhang, L.-M. (2010). Dynamics of sulfate reduction and sulfate-reducing prokaryotes in anaerobic paddy soil amended with rice straw. *Biology and Fertility of Soils* **46**, 283-291.

He, Z., Gentry, T. J., Schadt, C. W., Wu, L., Liebich, J., Chong, S. C., Huang, Z., Wu, W., Gu, B., Jardine, P., Criddle, C., and Zhou, J. (2007). GeoChip: a comprehensive microarray for investigating biogeochemical, ecological and environmental processes. *ISME Journal* **1**, 67-77.

Hubert, C., and Voordouw, G. (2007). Oil Field Souring Control by Nitrate-Reducing *Sulfurospirillum* spp. That Outcompete Sulfate-Reducing Bacteria for Organic Electron Donors. *Applied and Environmental Microbiology* **73**, 2644-2652.

Kaster, K., Grigoriyan, A., Jennneman, G., and Voordouw, G. (2007). Effect of nitrate and nitrite on sulfide production by two thermophilic, sulfate-reducing enrichments from an oil field in the North Sea. *Applied Microbiology and Biotechnology* **75**, 195-203.

Koschorreck, M. (2008). Microbial sulphate reduction at a low pH. *FEMS Microbiology Ecology* **64**, 329-342.

Lützow, M. v., Kögel-Knabner, I., Ekschmitt, K., Matzner, E., Guggenberger, G., Marschner, B., and Flessa, H. (2006). Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions – a review. *European Journal of Soil Science* **57**, 426-445.

Michael, P. S., Fitzpatrick, R., and Reid, R. (2015). The role of organic matter in ameliorating acid sulfate soils with sulfuric horizons. *Geoderma* **255-256**, 42-49.

Mosley, L. M., Fitzpatrick, R. W., Palmer, D., Leyden, E., and Shand, P. (2014). Changes in acidity and metal geochemistry in soils, groundwater, drain and river water in the Lower Murray River after a severe drought. *Science of The Total Environment* **485–486**, 281-291.

Paul, R. B., and Ulf, S. (2011). Soil pH and pH Buffering. *In* "Handbook of Soil Sciences" (P. M. Huang, Y. Li and M. E. Sumner, eds.). CRC Press.

Peterson, B. J., and Fry, B. (1987). Stable isotopes in ecosystem studies. *Annual review of ecology and systematics*, 293-320.

Ponnamperuma, F. (1972). The chemistry of submerged soils. *Advances in Agronomy* **24**, 29.

Rigby, P. A., Dobos, S. K., Cook, F. J., and Goonetilleke, A. (2006). Role of organic matter in framboidal pyrite oxidation. *Science of The Total Environment* **367**, 847-854.

Roychand, P., and Marschner, P. (2013). Respiration in a sand amended with clay – Effect of residue type and rate. *European Journal of Soil Biology* **58**, 19-23.

Sanchez-Andrea, I., Sanz, J. L., Bijmans, M. F., and Stams, A. J. (2014). Sulfate reduction at low pH to remediate acid mine drainage. *J Hazard Mater* **269**, 98-109.

Shi, A., and Marschner, P. (2013). Addition of a clay subsoil to a sandy top soil alters CO_2 release and the interactions in residue mixtures. *Science of The Total Environment* **465**, 248-254.

Ward, N. J., Sullivan, L. A., and Bush, R. T. (2004). Soil pH, oxygen availability, and the rate of sulfide oxidation in acid sulfate soil materials: implications for environmental hazard assessment. *Australian Journal of Soil Research* **42**, 509-514.

APPENDIX

Descriptions of soil profiles mentioned in Chapters 2 and 3

Permission for Citation

I hereby give permission to Chaolei Yuan to use the material detailed below as an appendix in his thesis.

Rob Fitzpatrick

Date: 29/5/2015

Resource: Fitzpatrick RW, P. Shand, S. Simpson, S. Grocke, M. Raven, A.K.M Baker, L. Mosley and P.G. Self (2013) Assessment of re-flooded Acid Sulfate Soil environments at Long Flat, Jervois, Toora and Pompoota in the Lower Murray Reclaimed Irrigation Area (LMRIA), CSIRO Land and Water Science Report, 03/12. 250 pp. (In Press)

Portion: 3 pages

Format: both print and electronic

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Expected completion date: Jul 2015

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Descriptions of soil profiles mentioned in Chapters 2 and 3

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Fitzpatrick RW, P. Shand, S. Simpson, S. Grocke, M. Raven, A.K.M Baker, L. Mosley and P.G. Self (2013) Assessment of re-flooded Acid Sulfate Soil environments at Long Flat, Jervois, Toora and Pompoota in the Lower Murray Reclaimed Irrigation Area (LMRIA), CSIRO Land and Water Science Report, 03/12. 250 pp. (In Press)

References used in soil profile descriptions below

Fitzpatrick, R.W., Powell, B., Marvanek, S., (2008). Atlas of Australian acid sulfate soils. In: Fitzpatrick, R.W., Shand, P. (Eds.), Inland Acid Sulfate Soil Systems Across Australia. CRC LEME Open File Report No. 249. CRC LEME, Perth, Australia, pp. 75–89.

Fitzpatrick, R.W., (2013). Demands on soil classification and soil survey strategies: special purpose soil classification systems for local practical use. In: Shahid, S.A., Taha, F.K., Abdelfattah, M.A. (Eds.), Developments in Soil Classification, Land Use Planning and Policy Implications: Innovative Thinking of Soil Inventory for Land Use Planning and Management of Land Resources. Springer, Netherlands, pp. 51–83. DOI 10.1007/978-94-007-5332-7_2 http://link.springer.com/chapter/10.1007%2F978-94-007-5332-7_2

LONG FLAT **(DSa 01-A):** Sampling in June 2011

Australian acid sulfate soil classification (Fitzpatrick 2013; Fitzpatrick et al. 2008): **Sulfuric clay soil** or **Sulfuric cracking clay soil**

Soil Taxonomy (Soil survey Staff 2014): **Typic Sulfaquert or Sulfic Sulfaquert**

Sample ID Depth cm	Locality description and photographs	Morphology	Chip-tray photograph
DSa 01-A.1 0-57	Adjacent to drainage ditch located to the east of the paddock  View looking from west to east towards the River Murray (willow trees in background) of site DSa 01-A where the drilling rig is located and was used to obtain the 3.5 metre core approximately 15 metres from the salt drain ditch, which is located within the strip of long grass / sedges observed in the foreground.	Black (10YR 2/1), light clay; coarse sub-angular blocky breaking to fine polygonal cracks with high organic matter content throughout; many coarse roots and fine rootlets; few decomposing roots, sharp boundary.	
DSa 01-A.2 57-95		Dark greyish-brown (10YR 4/2), medium clay, strong planar vertical cracks and weaker horizontal planes/closed cracks, with slickensides comprising strong, medium, wedge-shaped peds (with long axis tilted 20 to 40 degrees from horizontal), remnant organic matter and fine roots maintaining the surfaces and preserving plans of weakness, clear, wavy boundary.	
DSa 01-A.3 95-190		Dark grey (2.5Y 4/1), heavy clay with 5 to 10% pale yellow (2.5Y 7/6) jarosite mottles especially along old root channels; sharp, wavy boundary; slickensides comprising moderate, fine, wedge-shaped peds (with long axis tilted 20 to 40 degrees from horizontal); clear, wavy boundary.	
DSa 01-A.4 190-280		Dark grey (2.5Y 4/1), heavy clay soft, sticky clear, wavy boundary.	
DSa 01-A.5 280-350		Dark grey (5Y 4/1) heavy clay, soft, sticky Few olive grey clay lenses	

LONG FLAT (**DSa 01-B):** Sampling in June 2011

Australian acid sulfate soil classification (Fitzpatrick 2013; Fitzpatrick et al. 2008): **Sulfuric clay soil** or **Sulfuric cracking clay soil**

Soil Taxonomy (Soil survey Staff 2014): **Typic Sulfaquert or Sulfic Sulfaquert**

Sample ID Depth cm	Locality description and photographs	Morphology	Chip-tray photograph
DSa 01-B.1 0-76	Located in the middle of the paddock  View of site DSa 01-B in the middle of the paddock looking from east to west towards the drilling rig observed at site DSa 01-A. Exposed deep cracks are readily visible in the black cracking clay in the foreground.	Black (2.5Y 2.5/1), light clay; fine sub-angular blocky breaking to fine polygonal cracks with very high organic matter content throughout; many coarse roots and fine rootlets; few decomposing roots, (pH=5-4.7) sharp boundary.	
DSa 01- B.2 76-190		Dark grey (2.5Y 4/1), medium clay, with 5% diffuse pale yellow (2.5Y 7/6) jarosite mottles strong planar vertical cracks and weaker horizontal planes/closed cracks, with slickensides comprising strong, medium, wedge-shaped peds (with long axis tilted 20 to 40 degrees from horizontal; few remnant organic matter and fine roots maintaining the surfaces and preserving plans of weakness; (pH=3.9) clear, wavy boundary.	
DSa 01- B.3 190-230		Dark grayish brown (2.5Y 7/6), heavy clay with 5 to 10% pale yellow (2.5Y 7/6) jarosite mottles especially along old root channels; soft and sticky; sharp, wavy boundary;	
DSa 01- B.4 230-290		Dark grayish brown (2.5Y 4/2), heavy clay soft, sticky with 5 to 10% pale yellow (2.5Y 7/6) jarosite mottles (pH3.9-4.2) clear, wavy boundary.	
DSa 01- B.5 290-340		Dark grey (5Y 4/1) heavy clay, soft, sticky with 5% pale yellow (2.5Y 7/6) jarosite mottles, especially along old root channels.	

LONG FLAT (**DSa 01-C):** Sampling in June 2011

Australian acid sulfate soil classification (Fitzpatrick 2013; Fitzpatrick et al. 2008): **Sulfuric clay soil** or **Sulfuric cracking clay soil**

Soil Taxonomy (Soil survey Staff 2014): **Typic Sulfaquert or Sulfic Sulfaquert**

Sample ID Depth cm	Locality description and photographs	Morphology	Chip-tray photograph
DSa 01-C.1 0-65	Adjacent to irrigation ditch located in the west of the paddock (near River Murray) 	Black (2.5Y 2.5/1), sandy clay loam lenses with light clay matrix; fine sub-angular blocky breaking to fine polygonal cracks with high organic matter content throughout; few, distinct, white 1-5mm diameter lime (calcite) segregations with iron oxide coatings, many coarse roots and fine rootlets; few decomposing roots (pH 6-7) sharp boundary.	
DSa 01- C.2 65-140		Black (2.5Y 2.5/1), medium to heavy clay, weak planar vertical cracks and weaker horizontal planes/closed cracks, with slickensides comprising strong, medium, wedge-shaped peds (with long axis tilted 20 to 40 degrees from horizontal), high remnant organic matter and very fine roots maintaining the surfaces and preserving plans of weakness (pH5-6); clear, wavy boundary	
DSa 01- C.3 140-200	View looking from west to east towards the River Murray (willow trees in extreme background) of grassed site DSa 01-C (peg) approximately 25 to 30 metres from the main irrigation cannel, which is located in the long grass / sedges observed in the backround.	Dark greyish brown (10YR 4/2), heavy clay with 5 to 10% pale yellow (2.5Y 7/6) jarosite mottles especially along old root channels (pH3.5); sharp, wavy boundary.	
DSa 01- C.4 200-255		Dark greyish brown (2.5Y 4/2), heavy clay with 10 to 20% pale yellow (2.5Y 7/6) jarosite mottles especially along old root channels (pH3.5 to 4); soft, sticky, clear wavy boundary;	
DSa 01- C.5 255-340		Dark grey (5Y 4/1) heavy clay, soft, sticky (pH7 in matrix) but with isolated mottles along roots at 3.40m with clear jarosite mottles along very few old root channels (pH3.5 to 4).	