Soil Microbial Investigation

Assessing microbial functioning in soils with different inundation contexts

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Executive summary

Riverine environments are increasingly threatened by global changes that include increasing drought, extreme weather events, and anthropogenic alterations to natural flows. The health of Australia's longest river system, the Murray-Darling, is dependent on the soil, microbial, and plant communities that underpin it. This report is part of a larger body of work undertaken by The University of Adelaide, on behalf of the Department of Environment's Riverine Recovery Program (RRP), to assess the impacts and management of acid sulfate soils and soil microbial communities to help improve the health of managed wetlands along South Australia's River Murray and its environs. This work is concerned primarily with acid sulfate soils and the effects of wetting and drying regimes on environmental values and water quality. The overall aim of this work is to inform and support ongoing wetland management in the riverine environment between the South Australian/Victorian border and Wellington (SA).

This specific report is a 'primer' document about the importance and interaction of soil microbial function to water management for ecological purposes. The information in this report is presented in two parts – Part 1 (management) and Part 2 (technical). Although management is the first part of the report, some technical knowledge is required to best consider the management implications. Consequently, the technical information (Part 2) is presented first in this executive summary.

Microbial organisms (AKA 'microbes') have a strong influence in shaping riverine and other ecosystems. Soil microbes regulate carbon and nutrient cycles (converting organic matter or rock minerals into nutrients available to plants and animals), partner with plants to provide nutrients, promote productivity, and yet can also limit plant populations. As sulfur and iron recyclers, soil microbes are also the main drivers in the formation and oxidation of acid sulfate soils. In submerged soils where iron (from soil minerals) and sulfate (e.g. from groundwater salts) are available, microbes enhance the production of pyritic minerals. If these soils are exposed to air, different microbes with high-acidity tolerance then enhance the production of sulfuric acid and thrive in the lower pH (<4) conditions. These microbially-mediated processes are therefore important targets for potential management interventions to promote the health of the River Murray system.

The development of strongly acidic soil conditions has negative effects on wetland productivity and water quality. Both nutrient deficiencies and toxicities develop in strongly acidic soils – these toxicities can infiltrate the water column (e.g. through seepage or runoff during rainfall events), and alter plant community composition, structure and dominance. Feedback loops form between organic matter, soil microbes and plants. The magnitude and direction of these loops (i.e. whether beneficial or negative) can also affect the carbon sequestration potential of soils, as organic matter chemistry affects organic matter decomposition. As such, they are also potentially important management 'levers' in such systems.

Acid sulfate soils can be remediated by increasing soil pH to more moderate levels, increasing nutrient availability, and providing carbon resources for beneficial microbes. Potential management options that can be used individually, or as complementary strategies to achieve these aims, include: maintaining vegetation cover; adding further organic matter; adding biochar and/or lime; flooding; restoring hydraulic connectivity; removing impediments to natural wetting and drying cycles; and regulating ‘optimal’ wetting and drying cycles. Maintaining a high level of native plant diversity, cover, and heterogeneity is an important management strategy for remediation of acid sulfate soils as it: (i) prevents soil erosion; (ii) can accumulate high concentrations of nutrients and metals in above- and below-ground tissues (with potential to harvest to remove from the system); (iii) provides organic matter (litter) for decomposition; and (iv) maintains soil health (e.g. aeration into flooded soils). Together, these typically lead to higher microbial activity and improved water quality. Renewing the connectivity of flows, from the main channel in and out of wetlands, can facilitate remediation of acid sulfate soils by: (i) increasing the transfer and availability of organic matter for beneficial microbes; and (ii) when water levels are high enough to fully inundate the soil surface, it can cause localised anoxia during decomposition of the organic matter and improve sulfate reduction in the sulfurous acid sulfate soils. A nuanced management strategy is required to prevent and remediate acid sulfate soils, particularly as altering carbon inputs, water availability, and/or disturbance may activate unfavourable, microbes that will take advantage of novel soil conditions.

The main management levers for change in wetland soil microbial ecosystems include water ingress, organic matter addition, and reduction in human-mediated disturbance or impediments to natural cycles. There is a paucity of scientific literature (e.g. lack of studies or contextually relevant studies) on improving beneficial soil microbial communities in these systems. Nevertheless, some informed suggestions based on known levers and direction of change are explored herein. We do however caution that without additional empirical data, these suggestions are speculative, and it would be premature to comment on the timing or magnitude of change.

We recommend the following management priorities: actions that increase (i) wetland and (ii) soil resilience, and (iii) minimise environmental extremes where possible. These actions include improving and maintaining wetland vegetation while preventing erosion and physical disturbance of soils. These actions also include preventing the excessive accumulation and oxidation of pyritic materials by preventing prolonged wetting scenarios. Further to these actions, we suggest enhancing environmental remediation of sulfurous soils; this requires the creation of soils that are anoxic, contain sulfate and simple organic matter, and have a pH greater than 5. While it may seem counter-intuitive to require an increase in pH to remediate pH, note that this only needs to be an initial increase to allow sulfate reducing bacteria to establish. As each wetland contains different environments, management practices with satisfactory outcomes in one wetland may not translate to the same results in another wetland. Taking this context dependency into consideration, we strongly recommend prioritising a monitoring program (pilot studies or trials) to identify wetland responses to management change and to prevent the breach of catastrophic tipping points.

In addition to these management priorities, we recommend a number of research priorities. These include basic research on (i) the relationships between primary productivity, water quality, and soil chemistry as affected by the activities and outputs of soil microbes; (ii) the biology, ecology, and relationships of microorganisms in acid sulfate soils; and (iii) the effects of native and added organic matter on microbial composition and function in acid sulfate soils. We further recommend applied research on (i) the relationships between managed inundations and organic matter energy dynamics in naturally formed wetlands that are at risk of developing sulfidic materials; (ii) the effects of biomass management decisions on acid sulfate prevention and remediation; and (iii) the effects of clay content (i.e. clay addition) and mineralogy on management options for acid sulfate soils.

Abbreviations

ASS	Acid sulfate soil	PMN	Potentially mineralisable nitrogen
DNA	Deoxyribonucleic acid	qPCR	Quantitative polymerase chain reaction
Eh	Redox potential	RIS	Reduced inorganic sulfur
FAME	Fatty acid methyl esters	SIP	Stable isotope probing
mV	Millivolts	SOM	Soil organic matter
OM	Organic matter	SRB	Sulfur reducing bacteria and archaea
PCR	Polymerase chain reaction		
pH	Acidity/alkalinity scale		
PLFA	Phospholipid fatty acids		

Element names and chemical formula

C	Carbon	Mo	Molybdenum
Ca	Calcium	N	Nitrogen
Cu	Copper	Ni	Nickle
Fe	Iron	O	Oxygen
H	Hydrogen	P	Phosphorus
K	Potassium	S	Sulfur
Mg	Magnesium	Zn	Zinc
Mn	Manganese		

$CaCO_3$	Calcium carbonate; calcite	H_2S	Hydrogen sulfide
$CaSO_4$	Calcium sulfate	H_2SO_4	Sulfuric acid
$CaSO_4.2H_2O$	Gypsum	HCO_3^-	Bicarbonate ion
CH_4	Methane	HS^-	Bisulfide ion
CO_2	Carbon dioxide	MnO_2	Manganese dioxide
Fe	Iron	N_2	Nitrogen gas
Fe^{2+}	Iron (II) ion	N_2O	Nitrous oxide
Fe_2O_3	Iron (III) oxide	NH_4^+	Ammonium ion
Fe^{3+}	Iron (III) ion	NO_2^-	Nitrite ion
Fe_3S_4	Iron (II,III) sulfide	NO_3^-	Nitrate ion
FeOOH	Goethite	NO_x	Nitrogen oxides
FeS	Iron monosulfide	O_2	Oxygen gas
FeS_2	Pyrite	$S_2O_3^{2-}$	Thiosulfate ion
H^+	Hydrogen ion/proton	SO_4^{2-}	Sulfate ion

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1 Scope and summary

This report is part of a larger body of work which researchers from The University of Adelaide have been contracted to perform to inform the Department of Environment and Water's Riverine Recovery Program (RRP). This body of work is concerned with: the risks that acid sulfate soils (ASS) pose to environmental values and water quality at RRP wetlands; confirming that the knowledge and assumptions made in RRP Wetland Management Plans reflect contemporary science; ensuring that appropriate management and mitigation actions are in place; and investigating the influence of wet-dry management regimes on soil microbial communities and processes. It is intended that investigation outcomes will inform and support ongoing wetland management. The relationships of the project's objectives and outcomes can be viewed in Figure 1.

Acid sulfate soils are a concern in RRP as almost all pool-connected SA River Murray wetlands have the potential to form ASS materials. From a historical context, prior to the Millennium Drought, potential ASS materials built up in the wetland, lake and river channel soils due to the presence of iron, sulfate, organic carbon, and permanently reducing conditions (Section 8.1) These conditions were due to the almost permanent inundation of the soils since river regulation. During the Millennium Drought, declining water levels led to the exposure and oxidation of accumulated potential ASS and the formation of sulfurous ASS throughout SA. After the break of the Millennium Drought, these soils were reflooded causing a substantial decline in the water quality of downstream waterbodies. The current state of ASS material in wetlands is relatively unknown.

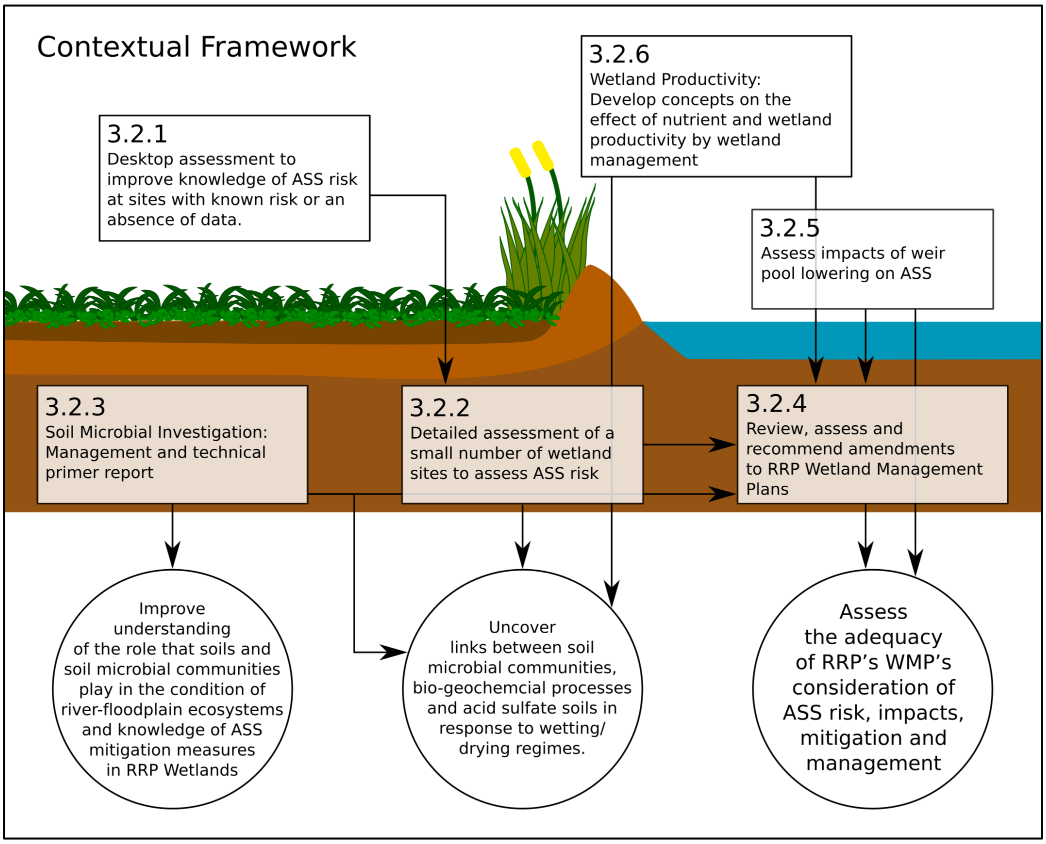

Figure 1: Contextual framework for the series of work being undertaken in the broader contracted project. Square boxes indicate objectives while round boxes indicate outcomes; arrows indicate connections between boxes. The primary outcome is in the far right round box. Figure made by E Stirling using Inkscape version 0.91 (The Inkscape Team, 2018).

1.1 3.2.3 Soil Microbial Investigation – assessing microbial functioning in soils with different inundation histories/contexts.

The scope of the work presented here is as follows: Prepare a 'primer' document about the importance and interaction of soil microbial function to water management for ecological purposes (e.g. wetland management, weir pool manipulation, managed floodplain inundation and wetland pumping) **(Section 7)**. This document will be based on a review of literature, and will synthesise this knowledge and knowledge gaps. The 'primer' will suggest a list of topics for discussion that may include reference to the following:

- The impact of ASS on soil microbial function **(Section 8)**.
- Soil microbial community composition in ASS affected soils relevant to nutrient concentration and availability **(Section 9)**.
- Are complementary measures desirable to facilitate recovery of healthy/balanced soil microbial communities (e.g. soil inoculation)? **(Section 5)**
- How floodplain soil microbial communities respond to restored flooding regimes **(Section 3)**:
    - Under variable grazing influence **(Section 3.1)**
    - Under variable salinity conditions **(Section 3.2)**
    - With different soil types (e.g. sand versus clay) **(Section 3.3)**
- If organic matter increases with microbial diversity **(Section 4)**.
- If there is a positive feedback loop incorporating soil microbial communities, soil organic content and vegetation condition. How do the variables highlighted above influence this loop? **(Section 4)**
- Do regular engineered inundations of the floodplain change the quality and quantity of allochthonous energy being transported to the channel and wetlands? If so, do the dynamics of soil microbial biomass and activity play a role in this change? What is the pattern and process? **(Section 2)**

Recommendations for management priorities and future research are further outlined in **Section 6**.

1.2 Summary of essential technical background information

The information in this report is presented in two parts – Part 1 (management) and Part 2 (technical). Although management is the first part of the main report, in this summary an overview of essential technical/background information is given first. Presenting the technical information first in this summary will provide all readers with essential background information necessary to better understand management options and issues. Please see individual sections in Part 2 (technical) for more detailed scientific information.

1.2.1 Soil microbes in wetland systems

Soil microbes are organisms that are too small to see without magnification. These organisms may be single celled individuals, single celled colonies, or relatively large multicellular organisms. At the highest classification, soil microbes are split into two groups based on cell physiology; they are defined by presence (Eukaryotes) or absence (Prokaryotes) of a defined nucleus and specialised organelles (Stahl et al., 2013). Due to the identification of two distinct and only distantly related groups within the prokaryotes, the most frequently used classification for discussing soil microbes further splits into archaea and bacteria (Woese et al., 1990). Soils have exceptionally high microbial abundance and diversity as they contain a wide variety of habitats and resources used by microbes (Curtis et al., 2002; Gans et al., 2005).

Soil functions that influence, and are influenced by, microbes include (i) biomass (including plants) production, (ii) elemental storage, filtering, and transformation, and (iii) habitat (Andrews et al., 2004). There are a variety of ways to measure microbial function, which are outlined in Section 7.3. The processes by which microbes influence these functions include organic matter production and decomposition and nutrient cycling. Concomitantly, organic matter can be produced by plants and autotrophic microbes as primary producers and by heterotrophic microbes during decomposition. The net consumption or production of organic matter affects soil health through its effect on soil aggregation (Section 7.1.2). Nutrient cycling is the transformation of elements from organic species to inorganic species and back, and is a biproduct of microbial growth and activity. The ecosystem benefit of nutrient cycling is the conversion of organic molecules containing plant nutrients (such as N, P, and S) into forms that are available for plant uptake. However, microbial nutrient cycling is responsible for the accumulation of pyrite minerals in soils, and is also responsible for rapid oxidation of pyrite to form acid sulfate soils (Section 8).

In wetlands, microbial activities affect energy flow and oxygen concentration in the soil; they close the cycle between soils, primary productivity and the atmosphere by decomposing organic matter, and, during this process, consume oxygen during cellular respiration (Wolf et al., 2013). In the absence of oxygen, anaerobic and facultatively anaerobic microbes may consume carbon dioxide, nitrate, sulfate or other electron acceptors as available (Muyzer and Stams, 2008). Both aerobic (in the presence of oxygen) and anaerobic (in the absence of oxygen) processes are important for nutrient cycling; the accumulation of pyrite minerals in soils and their subsequent oxidisation are due to anaerobic and aerobic processes (Section 8).

In environments with plentiful water, microbial colonies can form larger, visible, structures. Two such structures are biofilms and microbial mats. Biofilms are relatively simple congregations of microorganisms that attach to surfaces *via* a thin film while microbial mats are complexes of multiple species of microorganisms and organic matter (Stahl et al., 2013). The structural complexity of microbial mats allows the movement of resources between microbial colonies, which allow microbes to thrive in an environment that might otherwise be hostile to them (Section 7.1.4). Both biofilms and microbial mats can exist in low pH conditions, and may be the only organisms visible in environments with extremely low pH (Méndez-García et al., 2014).

1.2.2 Acid sulfate soil and soil microbes

Acid sulfate soils are the result of microbially enhanced redox reactions that occur under anaerobic (anoxic soils) and aerobic (oxic soils) conditions. Pyrite and other reduced inorganic sulfur minerals accumulate in anoxic soils due to the activities of sulfate reducing bacteria or archaea (Karimian et al., 2018). These organisms use sulfate during cellular respiration and produce waste products that become iron sulfide minerals when exposed to iron (Rabus et al., 2013). These minerals can be oxidised abiotically by exposure to oxygen, a reaction which is then rapidly increased by the activities of iron oxidising prokaryotes (Singer and Stumm, 1970). These organisms use the oxidation of iron (II) to iron (III) as an energy source, which increases the rate at which pyrite can be oxidised (Baker and Banfield, 2003; Ilbert and Bonnefoy, 2013). The oxidation of pyrite leads to the formation of sulfuric acid and the development of acid sulfate soils.

The development of acid sulfate soils completely changes the soil microbial assemblage from one that thrives under circumneutral (pH) conditions to one tolerant of acidic conditions (i.e. acidophilic microbial communities) (Hedrich et al., 2011; Su et al., 2017). This change in microbial community structure affects microbial functions such as nutrient cycling by increasing carbon limitation and promoting microbes with nutrient cycling capabilities (Jugsujinda et al., 1996). However, the exact effects of extreme soil acidification on microbial nutrient cycling remain unclear (Section 8.2.2).

1.2.3 Microbial interactions with nutrients and organic matter in acid sulfate soils

Both nutrient toxicities and deficiencies can occur in acid sulfate soils due to acid mediated solubilisation of soil minerals and organic matter. Nutrients that can be toxic to plants include aluminium, copper and manganese; however, increased availability of these metals may have a similar effect on non-adapted microbes (Golez and Kyuma, 1997; Panhwar et al., 2014a). Nutrients that are limiting include those that form insoluble compounds (such as calcium phosphate compounds) and nutrients that are easily leached. However, as microbes are also carbon limited, nutrient limitation can be addressed with organic matter amendments.

Organic amendments can be used as a remediation tool for acid sulfate soils. Soil pH can be increased by organic matter sorption of protons, by organic matter dissolution, and by increased activity of sulfate reducing bacteria and archaea (Section 9.1.2). Increased activity of these microbes may be induced by the initial increase of soil pH, the development of anoxia, or the provision of carbon resources (Ward et al., 2004; Creeper et al., 2015). In experiments assessing the response of sulfate reducing bacteria and archaea to organic matter amendments, increased nutrient content was associated with improved sulfate (and acidity) consumption (Section 9.1.2). However, when nutrients are added as mineral nutrients (i.e. no carbon), there appears to be no positive effect on microbial growth or activity (Michael et al., 2016).

1.2.4 Organic matter from managed inundations

Wetlands may be dominated by autochthonous or allochthonous organic matter inputs (i.e. formed on site or off site, respectively). The dominant source of organic matter is influenced by wetland connectivity; hydraulic connectivity therefore has an important effect on organic matter and sediment transfers (Leibowitz et al., 2016). Wetlands that receive allochthonous organic matter are able to capture, store and cycle nutrients from upstream sources (Newcomer Johnson et al., 2016). In these systems, nutrient cycling can be stimulated by the addition of mineralisable organic matter, nitrate, dissolved organic carbon, and soluble reactive phosphorus (Wolf et al., 2013). Nutrient cycling is more rapid in wetlands that receive allochthonous inputs as microbes immobilise less nutrients within their biomass when nutrients are not limiting (Wolf et al., 2013). From these abilities, nutrients in stream waters can be captured within wetlands with allochthonous organic matter inputs.

1.2.5 Microbial communities and restored flooding

After inundation with water, microbial community function in ASS may change to a sulfate reducing system or remain an iron oxidising system. However, the microbes responsible for sulfate reduction are not active at low pH, are sensitive to redox conditions, resource type, resource availability, and experience competition from other electron acceptors. Therefore, flooding does not necessarily promote a change in microbial community function. Factors that may be managed in addition to inundation include grazing, water salinity and to a small degree, soil clay content.

Maintaining a high level of vegetative cover is an important management strategy for preventing soil erosion and maintaining soil health in soils under a restored flood regime. Vegetation cover prevents erosion and provides soil organic matter, leading to improved water quality and higher microbial activity (Micheli and Kirchner, 2002). Cover can be affected by grazing and while it is obvious that grazing decreases vegetative biomass, and that overgrazing can eliminate aboveground vegetation entirely, it is not necessarily clear that grazing also affects the diversity of vegetation present (Jacobo et al., 2006; Todd, 2006; Liu et al., 2015). In addition, livestock grazing on acid sulfate soils are at risk of cadmium, lead and other metal toxicities (Cobb et al., 2000); livestock grazing in wetlands can lead to decreased soil surface structure and reduced vegetation cover, which may reduce the soil's capacity to cycle nutrients and resist erosive forces (Section 3.1).

Australian inland acid sulfate soils are associated with saline waters (Section 8.1.3) thus saline water can be used for inundation. In general, increasing soil salinity is associated with decreased microbial activity (Rath and Rousk, 2015); however, sulfate reducing bacteria and archaea are not negatively affected by saline water (Whitworth and Baldwin, 2011). These organisms may gain a competitive advantage over less desirable organisms in soils inundated with saline waters due to the presence of sulfate salts (Whitworth and Baldwin, 2011).

Clay particles are mineral particles that can pass through a 0.002 mm sieve. Clay particles have a large surface area to volume ratio, and their charged surfaces mean they can readily store and exchange nutrients. Clay content affects water movement, influences soil pH buffering capacity and interacts with soil organic matter amendments. Restrictions of water movement through soil due to clay can reduce the accumulation of pyrite in soil and also increase the recovery time of oxidised acid sulfate soils due to decreased sulfate inputs and decreased acid flushing, respectively (Wong et al., 2016; Mosley et al., 2017). Increasing clay content can increase soil pH buffering capacity by binding to protons or by dissolving into their constituent parts (Section 3.3.2). However, clay can also enhance or reduce the effectiveness of organic matter amendments through its effects on pH buffering and by binding organic matter into forms inaccessible to microbial decomposition (Section 3.3.3).

1.2.6 Feedback loops

Feedback loops occur between organic matter, soil microbes, and plants within riverine ecosystems. These loops may be beneficial or limiting, and their magnitude is constrained by resource and nutrient limitations that occur at both landscape and fine scales. Landscape scale feedback loops depend on the nutrient enrichment at a community level. Nutrient poor soils, for example, support nutrient poor plants that produce nutrient poor litter (i.e. high C:N), and these support microbial communities adapted to the low nutrient conditions and limited capability to cycle nutrients back into the soil (Zechmeister-Boltenstern et al., 2015). Fine scale feedback loops are strongly controlled by the physiology of individual plants. Plants with high nutrient demands provide nutrient rich organic matter, and plants with low nutrient demands provide nutrient poor organic matter (Section 4.3.1).

Feedback loops can also affect carbon sequestration potential of soils, as the chemistry of organic matter affects its decomposition. Although organic carbon can be categorised into varying levels of chemical complexity such as ‘labile’ carbon and ‘recalcitrant’ carbon, complexity does not directly correlate to time required for decomposition (residence time) (Han et al., 2016). Functional diversity can also affect carbon sequestration potential. For example, fungi are associated with greater sequestration potential due to their lower carbon requirements relative to bacteria (Trivedi et al., 2013). Overall diversity may also be important as high species richness of soil microbes has been associated with increased resilience to environmental perturbation (Fitter et al., 2005). A consideration for acid sulfate soils is that changes in carbon inputs, water availability, or disturbance may lead to microbes that were otherwise inactive in the soil becoming active to take advantage of new situations.

1.2.7 Complementary management options

The aim of complementary management options is to enhance the degree or speed of acid sulfate soil remediation; the options considered here include inoculation with microbes, inoculation with non-acid sulfate soil, and biochar amendments. Microbial inoculation has been highly successful in leguminous agricultural systems, but is largely uneconomical for soil health remediation (Abbott et al., 2018). Soil inoculation may involve inoculating seeds before planting or broad scale distribution of inoculant over an area. In systems that do not have highly specialised relationships between plants and microorganisms, inoculation is frequently ineffective (Abbott et al., 2018). Similarly, the transfer of soil from one location to another appears to change the soil that was moved rather than the soil it is moved to (Section 5.1.2).

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Part 1 of 2 - Management

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2 Managed wetting and drying cycles

This section refers to Project Brief **Attachment 5** section **3.2.3 Soil Microbial Investigation - assessing microbial functioning in soils with different inundation histories/contexts** point 7: "Do regular engineered inundations of the floodplain change the quality and quantity of allochthonous energy being transported to the channel and wetlands? If so, do the dynamics of soil microbial biomass and activity play a role in this change? What is the pattern and process?".

2.1 Concepts for managed wetting and drying cycles

In an unaltered drainage basin, water levels in streams and wetlands fluctuate over short timescales due to seasonal changes and over decadal timescales due to climatic changes. In areas with pronounced wet and dry seasons or in locations where wetland hydraulic connectivity relies on levee overflow, wetlands can experience complete drying and inundation events over a less than annual timescale. This is a likely scenario for the Lower Murray Basin before the streams were managed for transport and irrigation. In a system where stream levels are maintained at an optimal level for human activities, wetlands become permanently wet or dry depending on their connection to the stream. In a system where wetland hydraulic connectivity can be controlled, wetlands can be returned to wetting and drying cycles (Section 2.2.1). While there is a limited amount of literature surrounding the effects of reconnecting wetlands on nutrient cycling and on wetting and drying ASS under laboratory conditions, the literature on ASS does not capture the effects of managed wetting and drying events in the field. This is a substantial literature gap that will need to be addressed through future investigations. Here we provide some insights at the conceptual level as follows (Section 2.1.1).

2.1.1 Conceptual management options – cycle frequency

Three wetland scenarios will be discussed in this section: permanently dry (or hydraulically disconnected) with an occasional ingress of stream water; permanently wet with an occasional drying event; and managed wetting and drying regime with 'optimal' hydroperiods (timing and depth) that are currently unknown (Figure 2). Permanently dry wetlands can be expected to have low productivity and low organic matter inputs, low wetland plant diversity, and slow nutrient cycling. These sites will also have limited capacity to accumulate pyrite due to the inhospitable environment for sulfate reducing bacteria. Sulfate reduction may occur at depth if the water table is sufficiently high, but should be relatively slow due to carbon limitation of the microbes. Wetting or hydraulically reconnecting previously dry wetlands can increase wetland productivity and nutrient cycling, but may also lead to decreased water quality at downstream sites due to leaching (Section 2.2).

The permanently wet, occasionally dry scenario is an example of the events of the Millennium Drought (Figure 2). A decline in water levels caused the exposure of extensive areas of previously 'permanently' wet wetland soils and lake bed sediments that had accumulated high concentrations of iron sulfide minerals (Mosley et al., 2014b). The oxidation of these minerals caused a catastrophic pyrite oxidation event which caused the generation of sulphurous materials in 3,300 ha of floodplain soils (Mosley et al., 2014a). Severe acidification of some of these soils continues to be an issue well after the drought ended (Mosley et al., 2017).

An optimum (currently unknown) frequency of wetting and drying would prevent the accumulation of pyritic materials in soil by having small pyrite oxidation events where the acidity generated does not exceed the environmental buffering capacity (Figure 2). It would enhance nutrient capture within the wetland, leading to improved primary productivity and enhanced nutrient cycling (Section 2.2.2). Microbial community structure (i.e. species present) would not be strongly affected during drying phases as the carbonate developed during sulfate reduction, ion displacement from clay surfaces, and organic matter should provide sufficient neutralisation capacity to prevent pH driven microbial community shifts (Sections 9.1, 3.1.2, and 3.3). The best management practices to achieve this optimal condition are currently unknown.

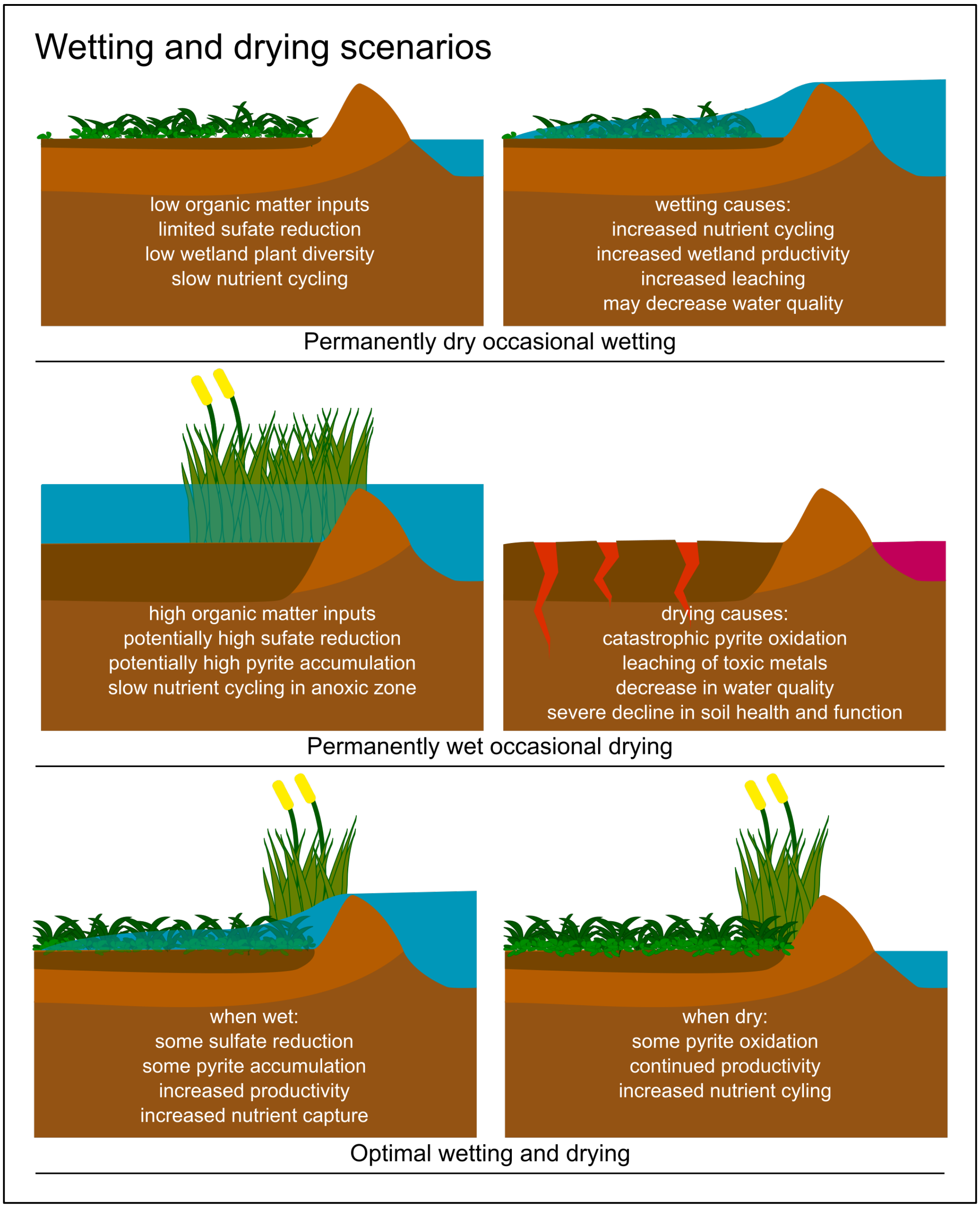

Figure 2: Wetting and drying scenarios for managed wetting and drying cycles in soils susceptible to pyrite accumulation via microbial sulfate reduction. Figure made by E Stirling using Inkscape version 0.91 (The Inkscape Team, 2018).

2.2 Allochthonous organic matter transport during managed inundations

2.2.1 Hydraulic connectivity

Hydraulically isolated wetlands such as those that form on perched aquifers or wetlands that have been isolated by anthropogenic activities are dominated by autochthonous (locally formed) organic matter. Hydraulically connected wetlands can be dominated by autochthonous or allochthonous (imported from external sources) organic matter (Ballantine and Schneider, 2009). Inundation of otherwise isolated wetlands allows the transfer of allochthonous organic matter and sediments from the stream to the wetland and, in the case of wetlands where water is then returned to the stream *via* overland flow, allows the transfer of organic matter and sediments from the wetland to the stream (see Walker et al. (1995) for more information). Connections between wetlands and streams allows the transfer of matter and energy between these two ecosystems, and therefore has an important effect on ecosystem services for these systems (Leibowitz et al., 2016). Reducing the artificial regularity of managed river discharges can also improve ecosystem functioning more broadly, as it limits the introduction and establishment of invasive species (e.g. *Pennisetum clandestinum* (kikuyu grass)) (Bunn and Arthington, 2002). It may also reduce riverine homogenisation from monodominant stands of cosmopolitan species with non-native haplotypes that can be cryptic invaders (e.g. *Phragmites* and *Typha*) (Ciotir and Freeland, 2016; Packer et al., 2017).

Restoring hydraulic connectivity between streams and wetlands is a common management practice to improve water quality of downstream reaches, as wetlands are able to retain nutrients and sediments from allochthonous sources (Kuwabara et al., 2012; Wolf et al., 2013). However, recently reconnected wetlands can be a substantial source of nutrients (N and P) and organic matter, particularly over the first three years before appropriate aquatic vegetation has established (Kuwabara et al., 2012). This increased nutrient load can cause algal blooms and a decrease in water quality. The effect of reconnecting wetlands with streams is influenced by the direction of flow; in situations where water spills (or is pumped) into the wetland, wetlands can contain high organism endemism while in situations where there is a continual connection with a stream, the wetland organism assemblage will be similar to the stream assemblage (Leibowitz et al., 2016).

2.2.2 Nutrient capture, storage, and cycling

In addition to different organism introductions, wetlands that receive continuous flow or pulsed flows can experience different sediment and nutrient loads. Wetlands with continuous flows tend to collect a greater fraction of organic matter relative to sediment, while wetlands with pulsed flow collect more sediments (Nahlik and Mitsch, 2008). This is most likely an effect of scouring at the connection site for pulsed flows. Increased availability of organic carbon can stimulate nutrient cycling within a wetland, leading to increased nutrient availability (Section 7.1) (Maynard et al., 2014). Fluctuations in water availability and scouring caused by stream water flows can also drive floristic succession by opening new niches and introducing new species (Nahlik and Mitsch, 2008). Isolated wetlands that receive no flows from external streams have low nutrient inputs and a reduced capacity to capture and cycle nutrients (Wolf et al., 2013).

Wetlands that receive allochthonous organic matter are able to capture, store and cycle nutrients from upstream sources when water retention time is high (Newcomer Johnson et al., 2016); i.e.

when there is sufficient time (or a sufficient decrease in flow energy) to release sediments and organic matter from the water column. Nutrient cycling can be stimulated by the addition of mineralisable organic matter, nitrate, dissolved organic carbon, and soluble reactive phosphorus (Wolf et al., 2013). Nutrient cycling is relatively efficient in wetlands, as nutrients and carbon resources are in close proximity and microbes do not tend to be water limited; in these systems redox state is an important variable controlling nutrient cycling (Section 8.1).

Although a large and active microbial biomass will process additional organic matter faster than a dormant microbial biomass, the initial size of the microbial biomass is unlikely to negatively affect nutrient capture or nutrient cycling over the long term as soil microbes are able to rapidly respond to available resources (Kalbitz et al., 2000). In laboratory experiments, for example, soil microbes are able reach maximum activity within two days of organic matter addition (Zhang and Marschner, 2017); microbes also display a decrease in carbon use efficiency (i.e. they consume more resources for the same amount of growth or activity) when large amount of organic matter are added to the soil (Rui et al., 2016). Nutrient cycling is more rapid in wetlands that receive allochthonous inputs as microbes immobilise less nutrients within their biomass when nutrients are not limiting (Wolf et al., 2013). In addition, in these systems, both microbial activity and sediment burial are responsible for removing organic matter from the water column; therefore, during periods of rapid accumulation of sediments or organic matter, excess organic matter may become inaccessible to microbes through physical means. Management options that may maximise nutrient capture, cycling, and storage include reintroducing hydraulic connectivity to streams and slowing water flow rates through wetland areas.

2.2.3 Climate change, organic matter and pulsed water flows

Due to the projected decrease in rainfall and increase in temperature, overall runoff and stream flow in are likely to decrease by approximately 45% of baseline values in the lower Murray Darling basin over the next 50 years (Austin et al., 2010). Although it is not clear how rainfall intensity will change over this area and period (Crosbie et al., 2012), two scenarios are relevant to allochthonous organic matter inputs in wetlands: (i) increased frequency of dry periods interspersed with high intensity rainfall and associated scouring, and (ii) increased frequency of dry periods interspersed with low intensity rainfall. In the first scenario, wetlands will experience pulsed water flows that may deliver large amounts of sediment and allochthonous organic matter. In the second scenario, water flow rates may not be high enough to dislodge and move materials from their source location, and wetlands may receive less sediment and allochthonous organic matter. Future management plans will therefore need to take into account changes in organic matter dynamics due to changed climate, biomass conditions, and water regimes.

2.3 Managed wetting and drying cycles

This section addresses questions and uncertainties around managed wetting and drying cycles for wetland and stream health. It discusses conceptual ideas for optimal management of wetting and drying (2.1.1), briefly outlines the effects of hydraulic isolation and reconnection (Section 2.2.1), and discusses wetland ability and capabilities to capture, store, and transform nutrients from streams (Section 2.2.2). There is also a short consideration of the effects that climate change may have on water dynamics and organic matter transport in the Murray Darling Basin (Section 2.2.3).

Wetlands that are managed as permanently wet sites are at risk of accumulating sulfidic materials in the soil profile if sulfate is available in the water. These sites are then at risk of pyrite oxidation and the production of sulfuric materials if the water level cannot be maintained (whether through drought or by other restrictions). An optimal management regime would allow sufficient water to enter the wetland so that it can retain wetland functions such as nutrient capture and storage, while also preventing the accumulation of excessive amounts of pyritic minerals. The best management practice for this optimal management approach is unknown at this point, however the literature surrounding hydraulic connectivity of wetlands may be a starting point.

Hydraulic connectivity affects the quantity of organic matter available to wetlands; wetlands that are hydraulically isolated rely on autochthonous organic matter, while wetlands that receive water from a stream or channel also receive allochthonous organic matter. Wetlands that rely on autochthonous organic matter experience similar nutrient limitations as non-wetland soils, while sites where allochthonous organic matter is available have a supply of externally sourced carbon and nutrients. Because of the co-occurrence of carbon resources and nutrients, wetlands can capture, store, and transform large amounts of organic matter through microbial nutrient cycles. It is possible that, over the next 50 years, wetlands in the Murray Darling Basin may experience increased or decreased allochthonous organic matter inputs depending on rainfall and streamflow intensity.

2.3.1 Research gaps

As far as we could determine, there is not literature available that specifically investigates the relationships between managed inundations and organic matter energy dynamics in naturally formed wetlands. Additional research gaps include:

- The optimal frequency of wetting and drying cycles to prevent pyrite accumulation and soil acidification events.
- Quantities, origins, and fate of allochthonous organic matter in regulated streams.
- Reconnection effects of organic matter inputs to acid sulfate soils.
- Microbial colonisation of soils following increased hydraulic connectivity.
- Energy pools and transfers during organic matter decomposition in wetlands.
- Nutrient capture capability of recently reconnected wetlands.
- Effect of these processes on higher trophic levels.

2.3.2 Management priorities

This section outlines the importance of wetting and drying cycles in managed wetlands. While the research gaps in this area of the literature are substantial, there are a number of management priorities that have been identified. These include:

- Preventing excessive accumulation of pyritic materials.
- Preventing catastrophic oxidation events.
- Improving hydraulic connectivity between wetlands and streams for improved vegetation and nutrient cycling activities.
- Anticipating changes in water flow and organic matter movement due to climate change.

3 Floodplain soil microbial communities under restored flooding regimes

This section refers to Project Brief **Attachment 5** section **3.2.3 Soil Microbial Investigation - assessing microbial functioning in soils with different inundation histories/contexts** point 4: How floodplain soil microbial communities respond to restored flooding regimes:

- Under variable grazing influence
- Under variable salinity conditions
- With different soil types (e.g. sand v clay).

In this section, we emphasise management implications and opportunities where possible.

3.1 Influence of biomass conditions and grazing after restored flooding

After inundation with water, microbial community function in ASS may change to a sulfate reducing system or remain an iron oxidising system (Section 8). Flooding for ASS remediation allows anoxia to develop in the soil and sulfate reducing bacteria and archaea (SRB) to consume Fe^{3+} and SO_4^{2-} to produce reduced inorganic sulfur (RIS) minerals (such as pyrite; Equation 5). Reduced inorganic sulfur minerals can immobilise Fe^{2+} (therefore preventing further iron sulfide oxidation) and sequester other toxic metals (such as Al^{3+}; Section 5.1.3) (Karimian et al., 2018). However, SRB have specific environmental requirements and are highly sensitive to environmental conditions. They are not active at low pH, are sensitive to redox conditions, sensitive to resource type and availability, and experience competition from other electron acceptors. Flooding, therefore, does not necessarily promote a change in microbial community function.

3.1.1 Maintaining cover

Maintaining a high level of vegetative cover is an important management strategy for preventing soil erosion and maintaining soil health in soils under a restored flood regime (Figure 3). Soil health is defined as the capacity of soil to function as a living system, to sustain primary productivity and maintain or enhance water and air quality. Soils with incomplete or absent vegetative cover have low organic matter inputs and are at risk of structural decline leading to low tensile strength of the soil and high particle detachment rates (Micheli and Kirchner, 2002). Vegetation cover, by littoral plants in particular, limits water erosion of soil after flooding through root reinforcement of the soil (Micheli and Kirchner, 2002) and prevents erosive scouring during rain events by reducing the impact force of raindrops and subsequent flooding. High organic matter inputs also encourage high soil microbe abundances as heterotrophic soil microbes require organic matter as a source of nutrients and energy; organic matter is added to the soil *via* plant primary productivity, animal wastes, and biomass turnover (Section 7.1).

Grazing management is another possible management option for these systems. Grazing pressure affects biomass condition by influencing plant survival rate and resource partitioning (such as partitioning resources to reproduce or to grow more biomass). Grazing also affects soil health by influencing vegetation cover rates and can change soil properties *via* compaction. While it is obvious that grazing decreases vegetative biomass, and that overgrazing can eliminate vegetation entirely, it is not necessarily clear that grazing also affect the diversity of vegetation present; continuous or intermittent grazing with large herbivores can increase, have no effect, or decrease plant diversity depending on the herbivore and the plant assemblages available (Jacobo et al., 2006; Todd, 2006; Liu et al., 2015). Regardless of the effect on biomass cover, livestock grazing on ASS with sulfuric materials are also at risk of cadmium, lead, and other metal toxicities as toxic metals in the soil solution can be translocated into the edible portions of plants (Cobb et al., 2000); crops that have been irrigated with acid mine drainage (AMD) show high rates of contamination from cadmium, lead, and other metals from both plant uptake through the soil and from dust deposition on the foliage (Lin et al., 2005; Pruvot et al., 2006).

3.1.2 Organic matter inputs

High quality and quantity of organic matter can be beneficial to soil microbial function, diversity and activity, and can be maintained under grazing systems that are appropriately managed (Figure 3) (Griffiths and Philippot, 2013; Vukicevich et al., 2016). Organic matter can be added *via* root exudates, plant litter (including dead roots), and animal wastes. Again, these inputs can be managed through improved water, vegetation, and animal management. Root exudates are released by plants and contain carbon rich and nitrogen containing compounds; these exudates attract and sustain a variety of soil microorganisms that may directly benefit the host plant by improving access to nutrients or by releasing anti-pathogen microbial exudates (van der Heijden et al., 2008; Vukicevich et al., 2016). The effects of plant litter on soil microbes varies with the quality of the litter; litters with a high proportion of complex carbon rich molecules are less decomposable than litters with a high proportion of simple carbon molecules or high nitrogen content. Plant litter inputs tend to be lower in continuously grazed systems than intermittently grazed systems (Griffiths and Philippot, 2013).

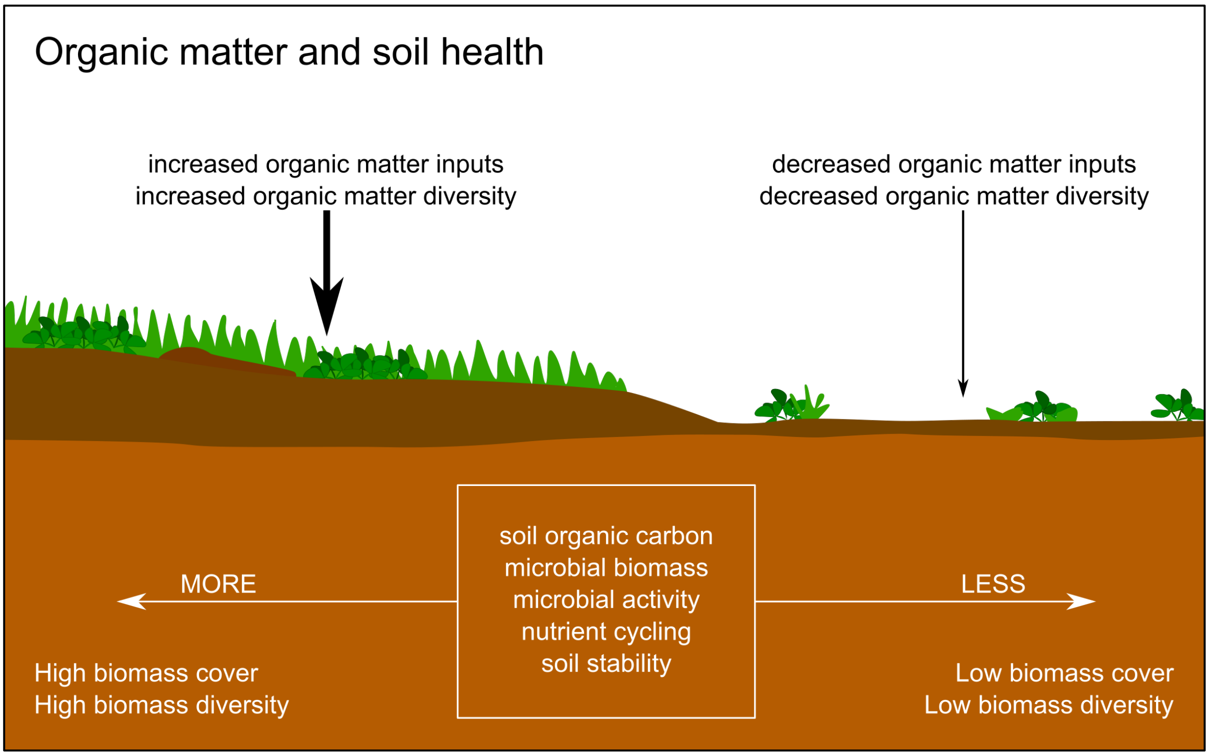

Figure 3: Organic matter and soil health; figure made by E Stirling using Inkscape version 0.91 (The Inkscape Team, 2018).

3.1.3 Livestock

Livestock wastes are a substantial source of organic matter in grazing systems, and, in systems with supplementary feeding, may be the dominant source of organic matter. Livestock that spend time in streams and wetlands may release wastes directly into water bodies, which can contribute to excessive nutrient inputs and high pathogen loads (Malan et al., 2018). Cattle, for example, are able to consume nutrients over a large area and then concentrate those nutrients into the areas where they spend time resting (Augustine, 2003). Ungulate livestock also affect soil structure by increasing compaction, breaking surface structure through pugging, and damaging riparian vegetation by wallowing or struggling in mud to reach drinking water. Damage to soil surface structure and vegetation cover reduce the soil's capacity to cycle nutrients and resist erosive forces. In ASS, stock damage can introduce oxygen deep into the soil profile, which may disturb anoxic sediments and enhance pyrite oxidation. Therefore, management of livestock to improve vegetation cover and soil surface condition is another important management option.

3.2 Influence of salinity

Depending on site location and history, inundation waters of different salinities are possible management options for remediating ASS (Table 1). While both fresh and saline water sources can stimulate the reformation of RIS minerals, there are a number of hydrological and geochemical differences between these two water sources when used for ASS remediation (Johnston et al., 2014). For example, freshwater inundation relies on streams levels and rainwater while seawater inundation relies on tidal movements. This means that freshwater wetlands may be exposed to extreme stochastic events such as floods or droughts, while coastal wetlands experience a more regular flooding and drying cycle. Another important differentiation between freshwater and seawater inundation is the availability of SO_4^{2-}; freshwater systems tend to have low availability of sulfate when compared to coastal systems which may lead to sulfate limitation during RIS formation (Karimian et al., 2018). In Australian inland wetlands, RIS is strongly associated with saline soils and waters due to the presence of sulfur salts (Section 8.1.3).

Table 1: Typical salinities (mg L^{-1}) of a variety of natural water sources. Morgan target acquired from Murray–Darling Basin Authority (2014).

Water source	Typical salinity (mg salt L^{-1} water)
Rain water	< 20
Freshwater	20 – 1,000
Morgan target	512 (EC < 0.8 mS cm^{-1})
Brackish water	1,000 – 35,000
Seawater	35,000
Saline water	35,000 – 100,000
Brine	>100,000

The use of high salinity water for irrigation (or flooding) can lead to soil salinisation and, if applied over a long period, can lead to the development of sodic structures. Soil salinisation is characterised by salt scalds and crusting, a decrease in primary productivity and an increase in erosion potential; soil salinity can be managed *via* deep drainage and salt leaching. Soil sodicity is characterised by a severe decrease in hydraulic conductivity, spontaneous dispersion of clay when wet, hardsetting when dry, poor drainage and poor root penetration. Soil sodicity is caused by the substitution of calcium ions (Ca^{2+}) by sodium ions (Na^{+}) on clay exchange sites. When exposed to lower salt solutions (such as rain), sodium ions are not able to hold the negatively charged clay particles together, and they spontaneously disperse. Soil sodicity is largely irreversible and leads to increased erosion risk, decreased water use efficiency, and decreased productivity (Qadir and Oster, 2004). Consequently, any management option that involves inundation will require consideration of salinity and salt species present.

3.2.1 Salinity effects on the soil microbial community

The main physiological challenge for soil microbes exposed to saline conditions is to maintain cell turgor. High salt concentrations outside the cell creates a diffusion gradient where water leaves the cell for the more concentrated extracellular solution. Microbes adapted to saline conditions maintain turgor by increasing the concentration of solutes within their cells; however, the ions available for consumption may also be toxic. Microbes may actively pump more toxic ions out of the cell, while accumulating less toxic ions to maintain the high intercellular solute concentration (Rath and Rousk, 2015). Adaption mechanisms to high salinity are energetically expensive and lead to slow growth rates; microbes unable to tolerate high salinity die or become inactive, which leads to community composition change.

Microbial activity (measured as respiration or enzyme activity) is inversely correlated with salinity in field samples. Although microbial activity decreases as salinity increases, it is not clear if salt concentration is directly responsible as soil organic matter inputs also tend to be low in saline environments (Rath and Rousk, 2015). However, in experiments where soils were amended with organic matter and exposed to high salinities, soil respiration was similarly supressed in the high salinity treatments. The decrease was less pronounced than in experiments that used the native soil organic matter (Rath and Rousk, 2015). The lower activity of enzymes in saline soils may be due to organisms requiring extra energy to maintain turgor, thereby reducing the resources allocated to proteins. Decreased activity in high salinity environments will affect microbially mediated biogeochemical cycling (Wong et al., 2010). Thus, in systems were we are managing for 'soil health' we need to take into account the effects of soil salinity and saline waters on the microbial biomass.

3.2.2 Flooding ASS with saline water

As discussed in Section 8.1.3, saline water is a considerable source of sulfate for inland waters and many of Australia's inland sulfidic soils are associated with saline soil. It can be assumed, therefore, that under such conditions salinity is not a major limiting factor for SRB. This is supported by the work of Whitworth and Baldwin (2011), where non saline wetland soil was incubated with a range of artificially salinised (sea salt) waters. Sulfate reduction was detected at all salinities tested, and proved to be sulfate limited at concentrations up to 10 mS cm^{-1} and carbon limited at 50 mS cm^{-1} (for reference, the electrical conductivity (EC) of seawater is approximately 55 mS cm^{-1}) (Whitworth and Baldwin, 2011). The development of alkalinity by SRB may be further limited by the availability of calcium and magnesium ions in ASS inundated by saline water (Whitworth and Baldwin, 2011). Although inundating wetlands with saline waters can lead to increased soil pH, it can also increase the accumulation of pyritic materials and subsequent risk of catastrophic oxidation events.

3.3 Influence of soil type

The effect of restored flooding regimes on soil microbial communities is affected by soil texture and soil structure. Soil texture is determined by the relative proportions of clay, silt and sand: clay content has the most influential effect on most soil properties (Figure 4). Soil structure ranges from single grain (such as beach sand) or massive (unstructured clay) to strongly structured soils (Figure 5). Soil structure is a description of the strength, size, and pattern of organisation of soil aggregates, and the proportion of soil that aggregates compose. Soil aggregates are self-forming clumps of soil mineral particles and organic matter. Organic matter within an aggregate may be protected from decomposition due to a lack of air, water, or microbial access. Soil structure affects water movement and aeration and therefore influences root growth and microbial spatial distribution in soils. This section will focus on the effect of clay content in ASS, as it is highly influential on water movement, acidity buffering, and the effectiveness of organic matter amendments.

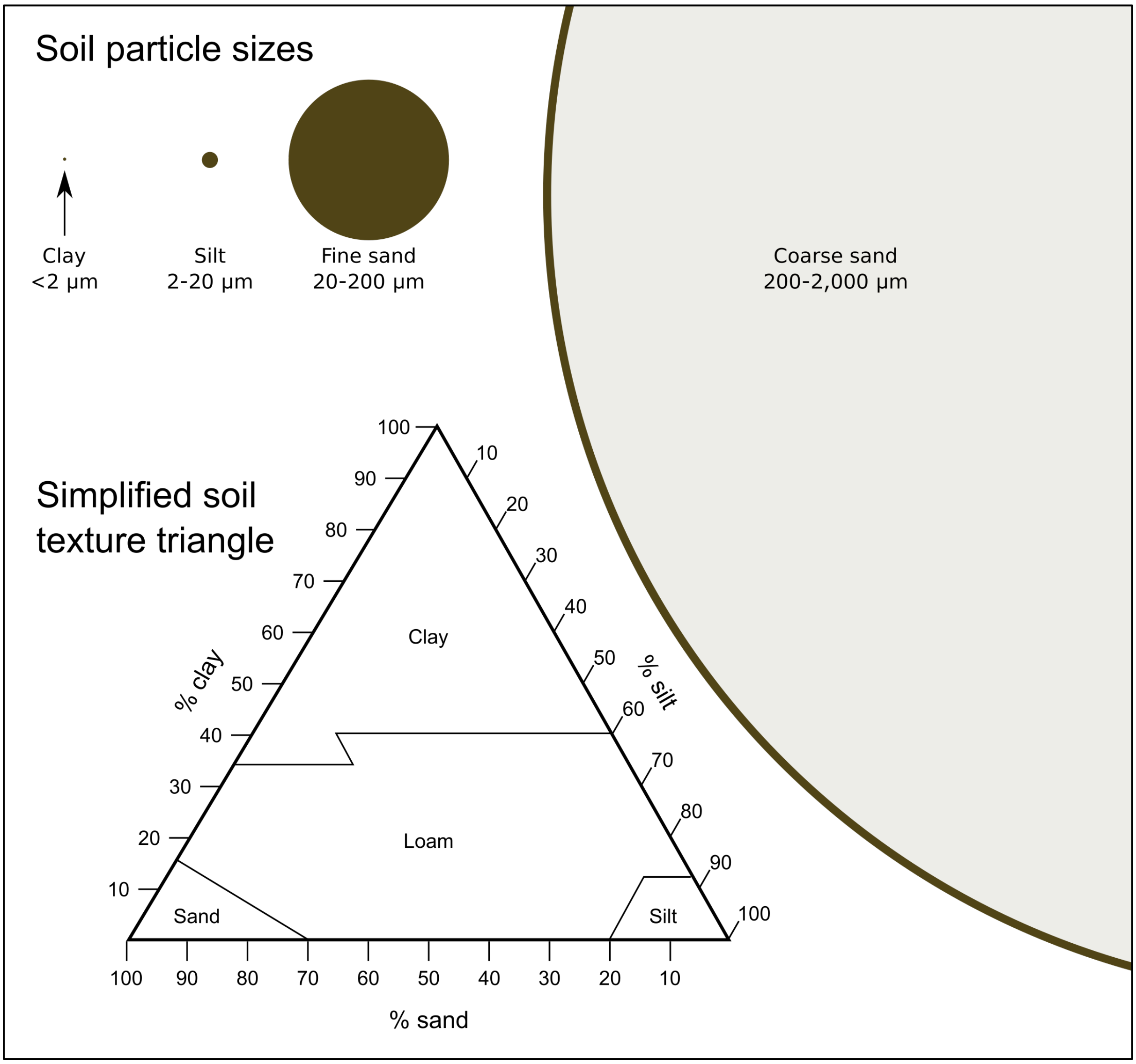

Figure 4: Soil particle sizes and simplified soil textural triangle. Soil particles are to scale for the largest particle in each class. Figure made by E Stirling using Inkscape version 0.91 (The Inkscape Team, 2018).

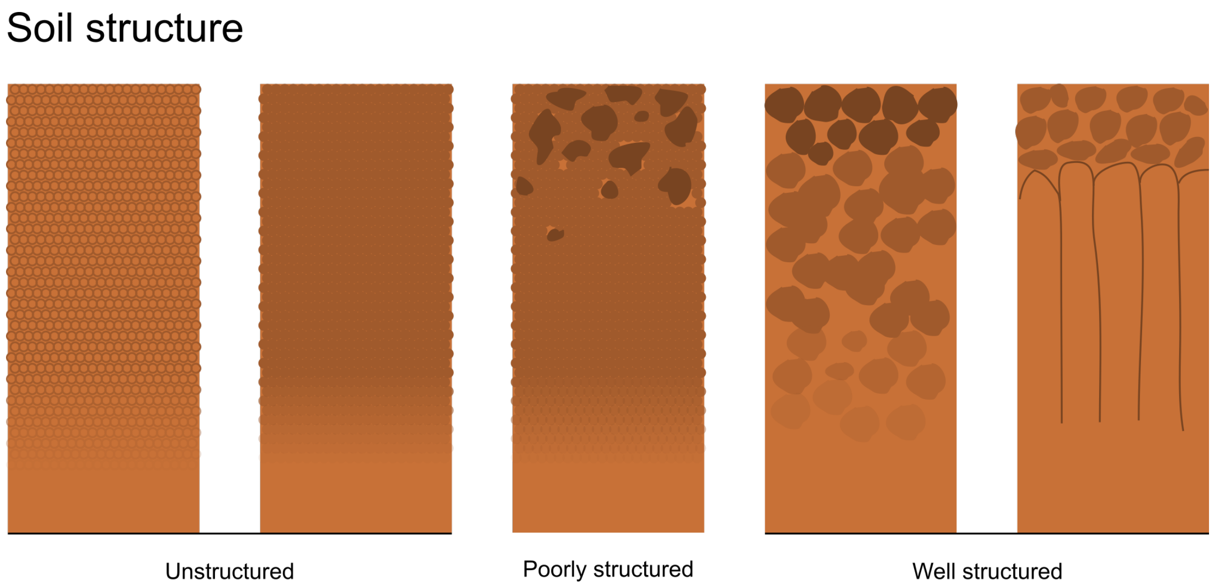

Figure 5: Examples of soil structure: unstructured soil are single grain (left) and massive (right); well-structured is granular (left) and granular above columnar (right). Figure made by E Stirling using Inkscape version 0.91 (The Inkscape Team, 2018).

3.3.1 Clay content and water movement

Soils with high clay content tend to have slow saturated hydraulic conductivity values; that is, water flows slower through soils with high clay contents than through soils with low clay contents. This restriction to mass water movement is due to the small pore sizes that are present between clay particles, which may be overcome in highly structured soils due to the formation of aggregates with pore channels between the aggregate surfaces that allow rapid movement of water. The distribution of RIS in wetlands and streams is affected by the clay content of soils or sediments, with increased groundwater flow occurring in areas of low clay content (Wong et al., 2016) and decreased groundwater flow occurring in areas of high clay content (Mosley et al., 2017). Clay content also affects the rate of recovery after a soil has acidified, with slow rates associated with slow flushing of acidity from the soil profile (Mosley et al., 2017). Nevertheless, limited flow rates in ASS with high clay contents can be overcome by the presence of macropores such as cracks or root channels, which must be considered as sources of both water and oxygen to the soil and subsoil (Johnston et al., 2009). Management of microbial communities in ASS with deep cracks is difficult, as oxygen penetrates deep into to soil profile, allowing iron oxidising microbes to thrive at depth rather than simply at the surface.

3.3.2 Clay as a buffer against the formation of acidity

In soils without a native or introduced supply of carbonate, clays can provide a buffer against the formation of acidity in ASS and may be the only significant neutralisation pathway (Whitworth et al., 2014). Clays are able to absorb excess H^+ at pH<5 by substitution of exchange cations (such as calcium) from clay surfaces; at pH<3, the aluminosilicate crystal structure of clays begins to dissolve, as hydroxyl groups (OH^-) combine with H^+ to form water (Shaw and Hendry, 2009). At lower pH values, dissolution continues until the clay dissolves into its component parts (Shaw and Hendry, 2009). Chemical weathering of phyllosilicate minerals (clays) *via* hydrolysis is initially rapid and produces water and metal ions, which may then precipitate as other minerals (Bibi et al., 2014). The relatively fast speed of clay dissolution may be due to the presence of ultrafine particles (with large surface areas) and surface defects or strains on large particles (Bibi et al., 2014).

In incubation experiments using ASS, clay addition treatments have shown capacity to maintain stable pH during incubated wetting and drying phases (Jayalath et al., 2016b). The type of clay soil used influenced the ability of a soil to maintain a stable pH; the best results were achieved with clays that had a large negative net acidity (i.e. a large capacity to absorb H^+). In addition to exchanging H^+ with other cations on clay surfaces, clay addition may prevent the formation of acidity by reducing the penetration of air into the soil (Section 3.3.1) that can maintain the anaerobic conditions required by SRB (Section 8.1.2). However, in addition to the substantial practical difficulties in mixing clay into a wetland soils, applying clay to ASS may have no net benefit if the soils are disturbed during the process, as this will introduce oxygen into the soil allowing the growth of iron oxidising bacteria (Section 8.1.5).

3.3.3 Clay interference with organic matter amendments

Although clay content increases the pH buffering capacity of soil (Section 3.3.2), it can also reduce the effectiveness of organic matter amendments to improve sulfate reduction rate by SRB. Accessibility of organic matter by microbes can be reduced by (Lützow et al., 2006): (i) the formation of organo-mineral complexes that bind organic molecules to the surfaces of clay (and other precipitated) minerals; (ii) intercalation of organic matter in clay layers (i.e. insertion of organic molecules between layers in clay crystal lattices); and (iii) occlusion of organic matter within aggregates (where it may then be inaccessible to microbes). In incubation experiments with organic matter amendments where clay content was a variable, sulphate consumption during the incubation negatively correlated with clay content (Yuan et al., 2015a; Jayalath et al., 2016a). In the field, high clay content of soil is known to decrease microbial decomposition of soil organic matter (Kögel-Knabner et al., 2010) and to increase the time required for ASS recovery (Mosley et al., 2017).

3.4 Concluding remarks on floodplain soil microbial communities under restored flooding regimes

This section discusses the literature on the relationships between floodplain soil microbial communities and grazing, salinity, and soil type under restored (i.e. managed) flooding regimes. Grazing (Section 3.1) factors, such as the importance of maintaining cover (Section 3.1.1), the influence of organic matter inputs (Section 3.1.2) and the effects of livestock (Section 3.1.3) are discussed. Salinity is discussed in general (Section 3.2) and as specific microbial responses to increase salinity (3.2.1) with reference to the effects of using saline water for inundating acid sulfate soils (Section 3.2.2). The effects of soil texture and structure are briefly introduced in Section 3.3, however the bulk of the discussion is focused on the effect of clay content in soils. In this section, the effect of clay content on water movement (Section 3.3.1), on soil pH buffering capacity (Section 3.3.2), and its interference with soil organic matter amendments (Section 3.3.3) are discussed.

Intermittent or permanent flooding acid sulfate soils is a management option that may be undertaken during remediation efforts; however, there are many variables that should be considered before flooding a soil. The variables considered in this report are grazing and the effects of livestock, floodwater salinity and its effect on soil microbes, and the effect of soil type (in particular, clay content). The effects of sheep and cattle grazing on wetlands include changes to organic matter inputs and the physical effects of large herbivores on soil structure. The reduction of biomass cover that may be caused by poorly managed grazing can lead to increased soil erosion, decreased water quality, and decreased soil organic matter which can then lead to structural decline of the soil. In acid sulfate soils, livestock are also at risk of consuming high levels of toxic metals that have accumulated in and on plants. While livestock wastes are a source of organic matter to the soil, the movement of livestock in and around wetlands can lead to organic matter and mineral nitrogen accumulating in specific sites and declining over the larger area. Livestock with access to wetlands and streams (e.g. for drinking) also negatively affect soil structure and decrease water quality.

In acid sulfate soils that are not already saline, inundation with saline water can lead to soil salinisation and may induce soil sodicity. However, as inland acid sulfate soils are frequently associated with saline wetlands, restored inundation regimes are likely to naturally include saline waters. Plants and soil microbes can struggle to survive in soils inundated with saline water due to osmotic stress; however, sulfur reducing bacteria and archaea do not appear to be negatively affected by high salinity water. Saline water may increase the microbial activity, due to the high concentration of sulfur salts within saline waters.

Soil texture and structure are additional soil properties that affect water movement, pH buffering, and organic matter decomposition. Soil texture is a description of the proportion of mineral particles in different size classes and includes particles less than 0.002 mm (clay) through to particles up to 2 mm (coarse sand). Texture is strongly influenced by the proportion of clay due to the charged surfaces found on clay particles and their large surface area to volume ratio. Soil structure is a description of the aggregation and self-organisation of soil mineral and organic particles into discrete peds (or units). Both clay content and structure affect water movement, which may then affect the activities of soil microbes; high clay content or poor structure can increase recovery time in acid sulfate soils under restored flooding regimes due to poor flushing of acidity from the soil profile. However, clay can also buffer acidity formed by pyrite oxidation by absorbing hydrogen ions onto their charged surfaces, or by dissolving in strongly acid pH to form metals, salts, and water. Clay can also interfere with organic matter amendments by preventing microbial access; microbes may be prevented from using organic matter as a resource if organic matter is bound to or within clay particle surfaces or when physically protected within soil aggregates.

3.4.1 Research gaps

Much of the information on grazing and biomass has been taken from general wetland and acid mine drainage literature, as few studies have specifically investigated their interaction with acid sulfate soils. In addition, the research available on the effect of clay content and clay type on acid sulfate soil production and remediation is also relatively limited. With this is mind, the following research gaps were identified during the literature review:

- The effect of acid tolerant, non-wetland plants on soil organic matter inputs and sulfate reduction in acid sulfate soils.
- The effect of animal disturbance (including compaction and pugging) and manures on sulfate reducing bacteria and archaea, and on plant diversity and cover.
- Bioaccumulation of metals in plants grown on acid sulfate soils.
- The influence of roots and root exudates on sulfate reduction.
- The effect of clay content and mineralogy on pH buffering and organic amendments.
- Best practices for applying clay to improve soil pH buffering capacity without increasing soil aeration.
- Determining organic amendments practices that are able to resist clay interference.

3.4.2 Management priorities

Management priorities for restoring flood regimes for wetland microbial health include the effects of livestock, salinity, and soil type. While the literature dealing directly with acid sulfate soil wetlands is limited, the following management activities are worthy of further consideration (e.g. via field trials):

- Preventing soil erosion by maintaining vegetative cover.
- Improving soil organic matter inputs by increasing vegetation cover.
- Reducing soil disturbance in wetlands through the exclusion of livestock.
- Assessing water quality (in particular, salinity) before embarking on wetland inundation activities.
- Assessing soil type when considering the potential hazard of oxidation events.
- Assessing the influence of clay materials before using organic amendments for acid remediation.

4 Interactions and feedback loops of soil microbial communities and organic matter

This section refers to Project Brief **Attachment 5** section **3.2.3 Soil Microbial Investigation - assessing microbial functioning in soils with different inundation histories/contexts** point 5: "If organic matter increases with microbial diversity" and point 6 "If there is a positive feedback loop incorporating soil microbial communities, soil organic content and vegetation condition. How do the variables highlighted above (flooding, grazing, salinity, and soil type) influence this loop?". Again, we emphasise management implications and opportunities where possible.

4.1 What are riverine feedback loops and why do they matter?

Riverine environments, like all ecosystems, are shaped by interdependent relationships between their abiotic (e.g. climate, minerals, nutrients, soil type, and water) and biotic (e.g. microbe and vegetation) characteristics (Corenblit et al., 2007; Vaughn). These mechanisms drive processes that circulate elements (e.g. nutrients), shape landforms, and influence communities through ongoing feedback loops (Power et al., 1988). Major contributors to these feedback loops are microbes, plants, and organic matter (i.e. non-living material from animals, microbes and plants). The most powerful riverine feedback processes include: (i) movement of substrates and fluvial landforms (Corenblit et al., 2007); (ii) cycling of gas (e.g. oxygen, carbon dioxide and methane), nutrients (e.g. carbon sequestration), and water (e.g. evapotranspiration); and (iii) community connectivity and structuring (Ward; Jackson et al., 2001).

Feedback loops are important because they drive the flow of energy and matter within an ecosystem. Together these processes can alter soil conditions (physical, (bio)chemical and/or biological), which in turn influence the productivity and fitness of individual microbes and plants, and subsequently influence the composition and diversity of microbial and plant communities within the ecosystem (Bever et al., 2010). The organic matter produced by these communities then influence subsequent cycle(s) of the feedback loop. By influencing the direction and pace of cycling, feedback loops can alter the biodiversity and functioning within wetland ecosystems.

4.2 Contributors to the feedback cycles – soil organic matter, microbes and vegetation

Organic matter within the soil is comprised of non-living material (excretions, deceased organisms and plant litter, root exudates, and roots) amongst abiotic elements. This soil organic matter provides resources for heterotrophic soil microorganisms (Section 3.1; Figure 3). Sources of organic matter have a strong effect on the rate of decomposition, and on the community of microbes able to use and contribute to its cycling.

Microbes are a major contributor to soil feedback loops and exist within complex biological systems that generate strong feedback mechanisms to resource availability within the soil community (Kaiser et al., 2015). They are an important driver of belowground biomass dynamics and contribute directly to feedback loops in at least three ways. Firstly, soil microbes are producers of organic matter *via* their extracellular excretions and their necromass (Schmidt et al., 2011). Secondly, they regulate the transformation of nutrients within the soil and are a major driver of soil nutrient cycling (Section 7.2). Finally, microbes influence the relative growth rates of their associated plant community and may mediate the partitioning of resources within the soil, through their mycorrhizal networks between plants (Bever et al., 2010).

A significant pathway in which soil microbes affect feedback cycles is through their actions as primary decomposers, and hence recyclers, of organic matter. They take organic matter from the environment, recycle the nutrients, and make them available for primary producers. Bacteria and fungi within the microbial community contribute to the decomposition and element cycling in different ways and timing. Bacteria can respond rapidly to changes in conditions and decompose more quickly; fungi tend to be slower growing and contribute to slower decomposition processes. However, microbes are influenced by the composition and source of organic matter within the soil, and diverse organic matter encourages a more diverse microbial community. Some microbes (such as SRB) are adapted to decomposing simple organic molecules and are not able to use complex organic matter (such as fresh leaves, roots, or woody material; Section 9.1.2). Nevertheless, although a diverse resource base can encourage greater microbial diversity, microbes are able to change their internal C, N, and P use efficiencies to adjust to resource limitation situations (Zechmeister-Boltenstern et al., 2015). From a management perspective, diverse plant communities and litter are preferable to promote diverse microbial communities, and manipulation of organic matter composition could also be used to facilitate particular outcomes.

In addition to consuming soil organic matter (SOM), microbes are capable of producing their own diverse range of SOM as excretions and necromass. In experiments where soil microbes were regularly given a single source of simple C, they were able to produce a wide range of stable organic matter within 18 months (Kallenbach et al., 2016). Microbes can also respond rapidly to changes in their environment (e.g. carbon availability, water availability, or disturbance), and altered conditions may activate otherwise dormant soil microbes. In summary, their major contributions are to produce organic matter (dead litter, roots and woody debris), influence the density and/or composition of the soil microbial community (through composition of plant community), share resources with the microbial community through their fungal partners, and to alter the conditions (physical, biochemical, and biological) within soil in ways that affect them and surrounding organisms (Bever et al., 2010). Feedback loops between microbes and plants are strongly influenced by their own metabolic by-products as well as abiotic resources (Section 7.2).

Although microbes are strong drivers of below ground dynamics, plants are a primary driver of aboveground feedback loops. Plants make up the majority of the aboveground biomass and also contribute to belowground biomass. Plants themselves are strongly influenced by limiting factors such as light, water, and soil. In particular, soil nutrient availability at the landscape scale determines overall nutrient content of plants, litter, and microbes (Zechmeister-Boltenstern et al., 2015). At smaller scales (individual plants or root systems), however, nutrient cycling is strongly controlled by the physiology of individual plants (Figure 6). The lifeform and associated physiology of riverine plant species strongly influences its role within the feedback cycle. For example, altering the soil conditions within their microhabitat can promote conditions that suit their preferred growing environment Bever et al. (2010). Monodominant stands of *Phragmites australis*, for example, drive a feedback loop within South Australian wetlands through water level reduction (evapotranspiration) and faster decomposition (production of deep, unflooded litter) that reinforce the living conditions preferred by this cosmopolitan species (Roberts, 2016). Like many waterways globally, the regulated River Murray hydrology has promoted homogenisation of plant communities through monocultures such as *Phragmites australis* and *Typha domingensis* (Packer et al. in review) that may cascade into altered feedback loops.

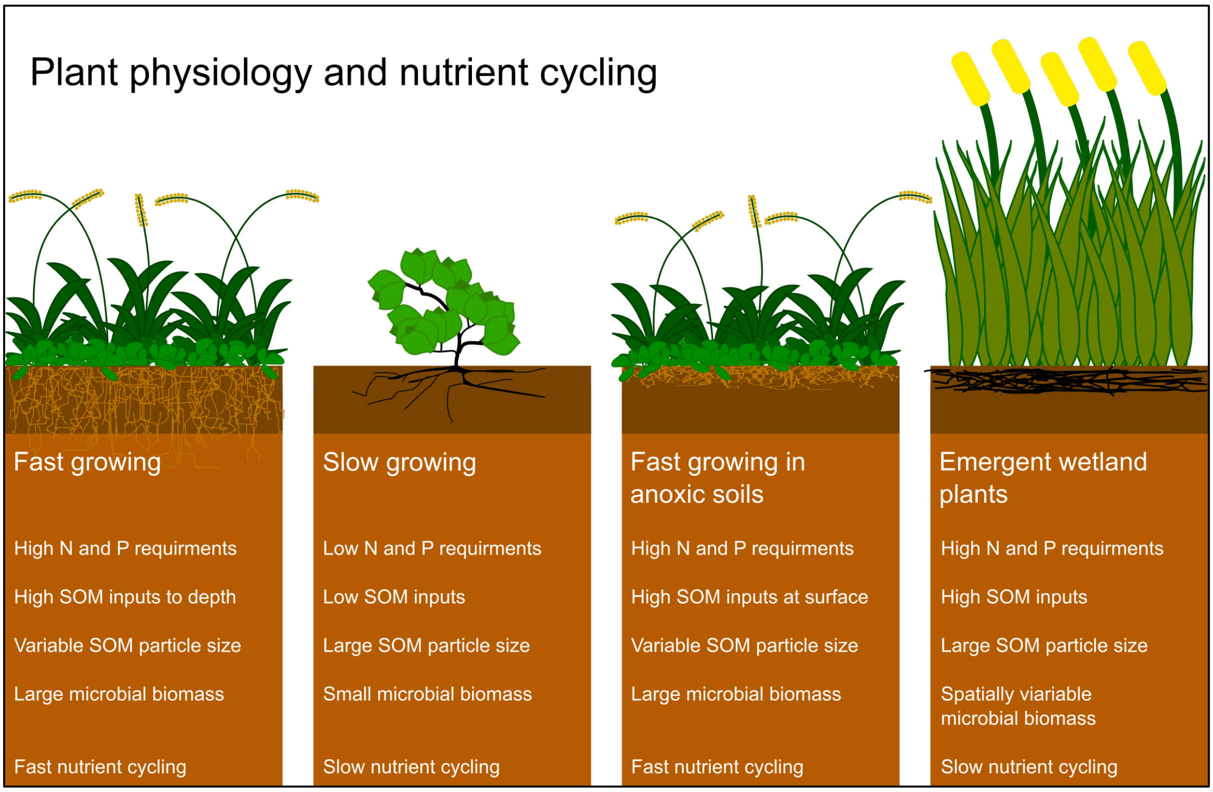

Figure 6: Plant physiology determines depth of root zone, N and P requirements, and nutrient cycling. Example species for fast growing plants might include herbs, forbs, and grasses such as *Pennisetum clandestinum* (kikuyu). Slow growing species might include shrubs and trees such as *Melaleuca* spp. and *Eucalyptus* spp. such as *E. camaldulensis*. Emergent wetland plants might include reeds and bulrushes such as *Typha sp.* and *Phragmites australis*. Figure made by E Stirling using Inkscape version 0.91 (The Inkscape Team, 2018).

4.3 Major feedback processes

4.3.1 Nutrient cycles

At the landscape level, nutrient cycling depends on the availability of nutrient enrichment; that is, nutrient poor soils support nutrient poor plants that produce nutrient poor litters (i.e. high C:N) that supports a nutrient limited microbial biomass with limited capability to cycle nutrients back into the soil (Zechmeister-Boltenstern et al., 2015). Although local climate can control soil nutrient content *via* weathering and leaching, plant N and P contents are strongly determined by soil nutrient availability rather than local climate (Vitousek, 1998). Wetlands and floodplains tend to be relatively nutrient rich compared to other ecosystems as they have inputs of organic matter and sediments from upstream catchments. However, wetlands can also experience high levels of N loss *via* leaching and denitrification (Section 7.2.2), and ASS experience low P availability due to the formation of insoluble P compounds at low pH (Section 7.2.3).

Feedback loops are also influenced at the community scale, or finer, by the nutrients available to primary producers and by plant physiology and growth strategy. Both plants and microbes are able to self-regulate their nutrient requirements to a degree; for example, plants or microbes that are phosphorus limited may spend extra energy and other nutrient resources in order to locate and uptake more difficult to access forms of phosphorus. Although nutrient content at the landscape scale determines nutrient availability for plants, litter, and microbes, at smaller scales (individual plants or root systems), nutrient cycling is strongly controlled by the physiology of individual plants.

Plants with high growth rates require more N and P than plants with slow growth rates when considered over the same amount of time. Plants also have different abilities to re-absorb nutrients from leaves before they fall to become litter (Zechmeister-Boltenstern et al., 2015). These differences can lead to substantial changes in the timing of nutrient availability as microbes immobilise (i.e. absorb) N and P under limiting conditions and mineralise (i.e. release) N and P when experiencing C limitation. During decomposition, C is lost from the soil as a by-product of respiration, and nutrients such as N and P are concentrated; this leads to a decrease in soil organic carbon during decomposition. In wetlands, functional traits that allow plants to survive in waterlogged soils (anoxic conditions) can also affect nutrient cycling in these soils by limiting C inputs; for example, roots tend to be thick, adventitious roots near the soil surface rather than the deep filamentous roots found in soils with good drainage (De Deyn et al., 2008). Plants adjust biomass proportions between above and below-ground depending on conditions and stability of the conditions.

4.3.2 Water cycles

The topography of the riverine landscape influences landscape-scale water flows (path, velocity and turbidity) and fine-scale biotic communities (e.g. few species can tolerate the velocities of mid-channel currents (Hart, 1992)). These communities can enhance nutrient cycling by collecting and creating further sediment; macrophytes such as *Phragmites* and *Typha*, for example, can slow stream flow and trap sediment (Rooth and Stevenson, 2000). Feedback loops also include the movement of water from substrate into the atmosphere. These hydrological cycles can be observed as water from soil moisture cycling plant roots and up into leaves, then into the atmosphere through evapotranspiration, and then precipitation returning it to soil moisture. Note though that the scale in which the hydrological cycle functions is regional to continental and beyond; a particular molecule of water evaporated from a specific wetland in South Australia is highly unlikely to fall as precipitation in the same wetland. In addition to the mass of water moved in hydrological cycling, water cycles also include the transfer of energy in and out of wetlands from the main channel via floodplains (Section 2.2).

Fluctuations in water levels (and associated stream velocity) influence feedbacks over seasonal, decadal, and longer time frames as they influence the life histories of many littoral species (Power et al., 1988). Important River Murray plant species that rely on recharge events include black box (*Eucalyptus largiflorens*), lignum (*Muehlenbeckia florulenta*), and river red gum (*E. camaldulensis*). These species all depend on flooding during their seed set for dispersal, followed by receding water levels to create muddy banks for germination (Jensen and Walker, 2017). Both *Phragmites* (Packer et al., 2017) and *Typha* (K. Mason, personal communication) can also take advantage of post-recharge conditions with moist, unflooded substrate to establish new populations.

4.3.3 Other cycles – sediment

Sediment cycles are also important functions of wetland ecosystems. In sediment cycling, scouring and erosion of substrates along and beyond the main channel during regular flows, and extreme flood events especially, alter riverine landforms and their connectivity to floodplains and wetlands (Corenblit et al., 2007). The disturbance from these fluvial cycles can also strongly influence vegetation (Grime; Corenblit et al., 2007) and soil microbial communities and their succession. Fluvial controls on vegetation succession are mainly determined by the flow patterns of sediment, substrate erosion and depositing, sediment texture (i.e. soil type), and the ever-changing topography (Corenblit et al., 2007). Therefore, the relative strength of water flows and streambanks are important variables to consider when managing inundation events for ecological health.

4.3.4 Community connectivity and structuring

Global changes are altering many species composition and distributions; these changing interactions can have cascading effects on how ecosystems function (Classen et al., 2015). Positive feedback loops link riverine connectivity with spatio-temporal heterogeneity, and this in turn influences biodiversity richness (Ward, 1998) and ecological functioning such as primary productivity, decomposition, respiration, and nutrient cycling (Cardinale et al., 2002). Richness may drive high ecological functioning, as different species partition or share resources (niche differentiation), facilitate resource opportunities for others, or strongly influence others within their system (e.g. as an apex predator or ecosystem engineer (Vaughn, 2010)). Both primary productivity of algal communities and respiration of benthic biofilms were increased when the heterogeneity of streambed substrate was increased experimentally through the addition or removal of different sized pebbles in a freshwater stream in North America (Cardinale et al., 2002). Streams and rivers are one of the most altered and homogenised ecosystems globally, including changes to the physical structure, flow, sedimentation, vegetation and woody debris (Cardinale et al., 2002). Thus, management decisions altering community connectivity and structuring need to take into account the flow on effects of these decisions.

4.4 Feedback direction and variability

Feedback loops operate at multiple scales, both spatially and over time (Liao et al., 2008) Spatially, they are affected by fine-scale adaptions of individual organisms (Section 4.2) through to landscape-scale conditions such as nutrient availability, and these different scales can influence each other (Suding et al., 2004). Spatial scale can influence the perception of whether abiotic or biotic influences are stronger in driving feedback: at the fine-scale, biotic effects such as competition are more evident, while at the landscape-scale abiotic conditions such as soil type may be emphasised (Jackson et al., 2001). Over time, feedback loops vary with daily and seasonal cycles, and over longer timeframes in response to major weather events such as 100 year floods and, increasingly, to global change. These human-mediated changes to climate (temperature, precipitation and extreme weather events), water availability, and disturbance (including grazing) can all affect the direction, magnitude, and pace of feedback processes within an ecosystem. Understanding feedback loops therefore requires a combination of multiple scales and ecological approaches (Vaughn, 2010). This highlights the complexity of developing management options in systems with multiple feedback loops operating at multiple scales.

Feedback loops occur in several stages, and resources can flow between them in either a positive or negative direction for microbial and plant communities (Figure 7). Changes in the environment that affect organic matter inputs can cause positive or negative feedback on organic matter dynamics *via* the effect of those changes on the soil microbial biomass. At the most fundamental level, decomposition of organic material (stage 1) can make nutrients, and hence active growing conditions, available for previously dormant organisms. Individual microbes or plants (e.g. clonal stands of *Phragmites australis*) can in turn respond to, and further alter, soil conditions within the habitat (stage 2). In a positive feedback loop, these changed conditions can provide the microbe or plant with an intraspecific advantage over individuals of the same species (Bever et al., 2010; Roberts, 2016). In a negative feedback loop, the changes alter the environment to less favourable growing conditions. These changes can extend to interspecific dynamics that can shape the diversity and structure of microbial and communities (stage 3; (Bever et al., 2010)). In a positive feedback loop, this provides an advantage over co-occuring species (e.g. *Typha* over *Phragmites* or *Cyperus*) and can lead to increasing monodominance and decreasing community diversity. In a negative feedback cycle, the dominant species is disadvantaged and other species may be promoted (Bever et al., 2010). Overall, feedback loops involving the soil microbial community tend to be negative through a combination of direct host-specific pathogens and indirect host-specific changes in mycorrhizal fungi and rhizosphere bacteria.

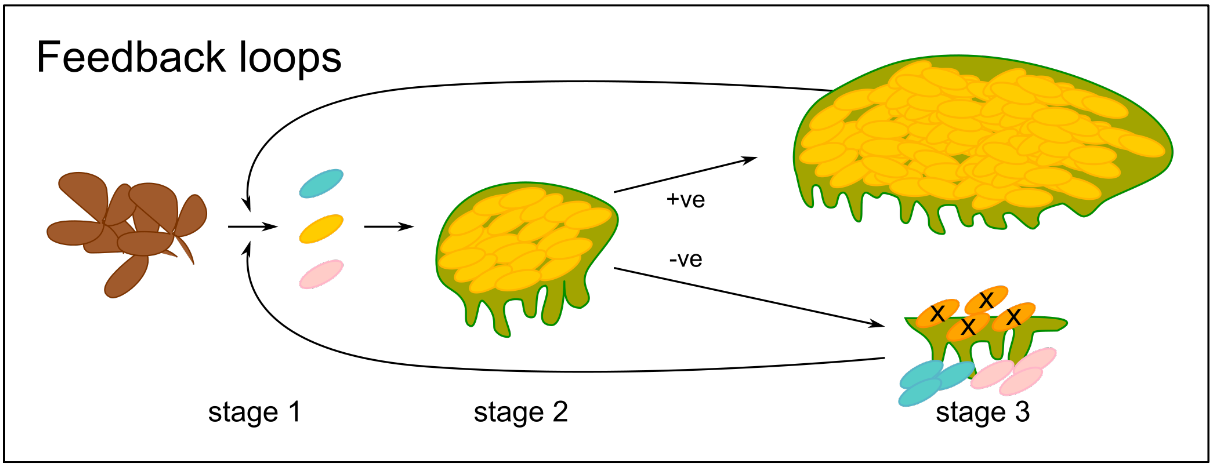

Figure 7: Feedback loop stages where: stage 1 indicates organic matter stimulating microbial growth, stage two indicates a microbial colony influencing its environment, and stage three shows two alternative states resulting from that influence. Stage 3 upper indicates a positive feedback loop (where the alteration of the microbial environment leads to expansion of the microbial colony) and stage 3 lower indicates a negative feedback loop (where the alteration leads to decline of the original colony and recolonisation by other microbes). Figure made by E Stirling using Inkscape version 0.91 (The Inkscape Team, 2018).

Positive and negative feedback loops can be found in microbial ecosystems. Sulfurisation is the process by which sulfidic materials in ASS are oxidised to sulfuric materials and is enhanced by iron oxidising microbes (Section 8.1.4). These microbes produce a strongly acid environment, which enhances their ability to outcompete non-acidiphillic microbes. Sulfurisataion is therefore a positive feedback system in this context. The development of anoxia by aerobic microbes can also be explained as a negative feedback system (Section 7.4.1). Anoxia forms when microbes deplete the oxygen in their immediate environment, which leads to the death of obligate aerobic microbes and the expansion of microbes able to exist in anoxic conditions. Depending on the conditions and communities present, feedback loops can also vary in magnitude (biomass and concentration of elements being recycled) and pace (rate of change, such as decomposition). Temperature and water availability (water level, precipitation, and flooding) can also influence the rate of decomposition and soil organic carbon accumulation (Ise et al., 2008).

4.5 Soil microbial diversity and carbon sequestration

Soil microorganisms consume and produce soil organic matter, leading to both the consumption and production of organic carbon and carbon dioxide (see Section 7.2 ‘Microbial carbon cycle’). Autotrophic microbes are able to produce organic carbon molecule *via* photosynthesis (cyanobacteria) or other metabolic pathways (such as iron oxidation); heterotrophic microbes consume organic carbon and produce their own organic carbon compounds while releasing oxidised carbon as a metabolic waste product. The form of oxidised carbon is determined by the environmental redox state and microbial respiration strategy (Section 7.1.3). In addition to microbial consumption of organic carbon, carbon can be lost from a soil system abiotically *via* leaching (dissolved organic matter) and wind or water erosion (particulate organic matter). Carbon sequestration in soils occurs when the carbon input rate is greater than the rates of microbial decomposition and abiotic losses.

4.5.1 Soil carbon types and residence time

While organic carbon can be categorised into varying levels of chemical complexity such as ‘labile’ carbon and ‘recalcitrant’ carbon, complexity does not directly correlate to time required for decomposition (residence time) (Han et al., 2016). Typically, recalcitrant carbon includes complex or nutrient poor structures such as lignin (woody structures, cell walls) and char (wood pyrolysis), while labile carbon includes simple or nutrient rich molecules such as sugars (root exudates) and amino acids (proteins). The stable pool of soil organic carbon can contain large amount of carbon compounds that are considered labile and easy to decompose (Han et al., 2016); although fungi and bacteria can rapidly consume simple organic matter when it is physically available (Fitter et al., 2005), these simple compounds in the stable pool can be relatively old (Han et al., 2016).

Chemical recalcitrance of organic matter is only relevant for matter that actually comes into contact with microbial decomposers; all other organic carbon can be protected from microbial decomposition *via* interactions with clay particles (Section 3.3.3), spatial inaccessibility, and redox barriers (Brune et al., 2000; Lützow et al., 2006). The carbon sequestration potential of wetland soils can be increased *via* improved soil structure, inundation, and by increased wetland productivity. Improving soil structure increases the amount of organic matter stabilised within aggregates and micro-aggregates (i.e. spatially inaccessible) (Trivedi et al., 2013). Flooding the wetland leads to the formation of anoxic zones in the soils where organic matter decomposition is relatively slow (Section 3). Recalcitrance of organic matter can be manipulated through management by encouraging certain assemblages of plants; wetland systems with woody plants will typically have more recalcitrant C than systems without woody plants.

4.5.2 Functional diversity and carbon cycling

Although research has been conducted into the connections between soil organism diversity and carbon cycling, it is not clear if species richness or a dominance of specific taxonomic groups are responsible for changes in carbon cycling if other environmental variables remain stable (Hättenschwiler et al., 2005). The ratio of fungi to bacteria is a diversity measure that has been used to describe differences in decomposition rates and nutrient cycling (Bardgett and Wardle, 2010). Fungi to bacteria ratios have been connected with soil nutrient status and disturbance regimes, with bacteria less tolerant of nutrient limiting conditions and more tolerant of increased disturbance regimes (Gordon et al., 2008). The ratio of fungi to bacteria can also affect the carbon sequestration potential of soils; with fungi associated with greater sequestration potential due to their lower carbon requirements relative to bacteria (Trivedi et al., 2013). However, it is not clear whether soils with high fungi biomass sequester more organic carbon or if soils with high organic carbon content favours the growth of fungi over bacteria (Trivedi et al., 2013).

High species richness of soil microbes has been associated with increased resilience to environmental perturbation, allowing soils to retain function after extreme events (Fitter et al., 2005). At any specific time, soils contain microbes in a range of activity states, from active growth or metabolic maintenance, to declining growth or spores (i.e. inactive). Microbes also have a range of growth responses to available resources: microbes that have a large number of transporter proteins in their cell membrane are able to rapidly assimilate resources and have a “feast or famine” response to resources (Trivedi et al., 2013). Microbes with few, but selective, transporter proteins can thrive in low resource situations, but are excluded by fast growing microbes when resources become available (Trivedi et al., 2013). Due to the heterogeneity of soils at the micro scale, both of these situations occur over small distances, leading to increased microbial diversity. Soils that are inundated tend to have lower microbial diversity, as populations are more able to come into contact in a liquid medium. Acid sulfate soils typically have a low level of microbial diversity compared to agricultural or forest soils due to the extreme pH (Section 8.2.1).

4.6 Concluding remarks on interactions and feedback loops of soil microbial communities and organic matter

This section reviews the literature concerned with feedback loops that may occur between organic matter inputs, vegetation communities, and soil microbial communities. Feedback loops are a relationship between resources, microbes, and their metabolic by-products (Section 4.1) that are affected by landscape scale nutrient limitations and the adaptions of individual organisms (Section 4.2). Major feedback loops include the movements of nutrients, water, sediment and gas (Section 4.3) and are affected by positive and negative reinforcement systems (Section 4.4). Feedback loops are important factors to consider in the carbon cycle and carbon sequestration (Section 4.5) as different types of carbon may have different residence times in the soil (Section 4.5.1), which can be affected by the microbial communities found in the soil (Section 4.5.2).

Feedback loops form between organic matter, soil microbes and plants; these loops are influenced by the nutrients available to primary producers, and by plant physiology and growth strategies. Both plants and microbes are able to self-regulate their nutrient requirements to a degree; for example, plants or microbes that are phosphorus limited may spend extra energy and other nutrient resources in order to locate and uptake more difficult to access forms of phosphorus. Nutrient availability can also determine carbon types and, to a degree, carbon residence time. Plants growing on nutrient poor soils tend to allocate more energy to carbon rich structures, which are then difficult to decompose, leading to nutrient limitation in the microbial biomass. In wetlands however, physical protection of organic matter within aggregates or redox barriers are more likely to affect carbon residence time. In addition, although carbon cycling has been correlated with dominant microbe taxa (such as bacteria or fungi dominated systems), soils contain a large variety of microbes that may rapidly respond to changed conditions. Changes in carbon inputs, water availability, or disturbance may lead to microbes that were otherwise inactive in the soil becoming active to take advantage of new situations. Overall, sustaining rather than suppressing riverine connectivity and heterogeneity will maintain feedback processes that support a higher level of ecological function

4.6.1 Research gaps

The questions from the project brief that are reviewed in this section were difficult to address due due to the limited number of published studies on the effect of water management on these parameters. We acknowledge this is an important yet emerging area of applied ecology, and therefore needs to be much better understood. Further research in the following areas may be useful to address these critical knowledge gaps in the literature:

- The influence of microbial and plant diversity on organic matter composition and productivity.
- The influence of chemical stability on residence time of organic matter in acid sulfate soils.
- The relationship between microbial diversity, vegetation diversity, and carbon cycling in wetlands.

4.6.2 Management priorities

This section outlines the importance of considering feedback loops during wetland management, and the possibility of positive and negative feedback loops leading to situations of stable alternative states. Although it is difficult to anticipate all feedback loops in a system, the following management priorities should be considered when considering feedback loops:

- Preventing positive feedback loops that lead to low diversity vegetation (where undesirable).
- Enabling nutrient capture and cycling in wetlands through appropriately designed inundation regimes.
- Enabling carbon sequestration, where appropriate, by encouraging the production of recalcitrant C structures and compounds.

5 Complementary management options

This section refers to Project Brief **Attachment 5** section **3.2.3 Soil Microbial Investigation - assessing microbial functioning in soils with different inundation histories/contexts** point 3: "Are complementary measures desirable to facilitate recovery of healthy/balanced soil microbial communities (e.g. soil inoculation)?".

5.1 Complementary management options to facilitate improved soil health in ASS

Soil health is defined as the capacity of soil to function as a living system, to sustain primary productivity and maintain or enhance water and air quality. Acid sulfate soils with sulfuric (pH<4) materials could be considered 'unhealthy' as plants and animals struggle to survive in them, and they actively decrease water quality *via* leaching of metals that can be toxic to most organism. In addition to liming, there are a range of complementary management options for remediating ASS. These include organic amendments (Section 9.1.2), inoculation with microorganisms (Section 5.1.1), inoculation with soil (Section 5.1.2), active planting (Section 5.1.3), or adding biochar (Section 9.1.3). However, it is important to understand that broad scale inoculation treatments to improve soil health frequently have no significant beneficial effect on the target soil (Schwartz et al., 2006); it is generally more effective to enhance resident beneficial microbial activity through improved management (Section 3).

5.1.1 Inoculation with microorganisms

Commercial microbe inoculation to improve the range of microbes present in the soil has been highly successful in leguminous agricultural systems, but this is largely uneconomical for soil health remediation (Abbott et al., 2018). For legume based crops (i.e. peas and beans), the success of inoculation is due to the highly specific relationship between legumes and their symbiotic nitrogen fixing bacteria (rhizobia) (Remigi et al., 2016). Inoculation is frequently ineffective in other soil systems as microbes with less specific plant relationships need to survive and reproduce in the soil or plant roots while competing with microbes specific to the introduction location (Abbott et al., 2018). For example, wetland microorganisms have been shown to recolonise ASS from spores remaining in the soil without the need for the addition of inoculum (Ning et al., 2011). In addition, microbes present in lab-grown inoculums are unlikely to survive in situations that they are not adapted to, such as the low pH/high metal availability environment of ASS. There is also the potential that introduced microbes do not produce the soil health results desired as microbes are generally considered to be cosmopolitan species that are present, but not active, in most environments; the activity of microbes in a setting is determined by the environment in which they exist (De Wit and Bouvier, 2006; Schwartz et al., 2006). Therefore, although re-establishing the native soil microbial community may be a limiting factor in restoration of native plant composition and diversity (Bever et al., 2010), inoculation is unlikely to be required if soil conditions are appropriate for the desired soil microbes. Rather, management should be focused on efforts that seek to favour and bolster microbial communities. For example, minimising soil disturbance and maintaining plant cover can support arbuscular mycorrhizal fungi (Bowles et al., 2017).

Inoculation with SRB is effective at increasing the rate of acid removal and metal immobilisation in environments that have a low diversity of microorganisms. This type of environment is found in bioreactors and constructed wetlands for the treatment of AMD. Bioreactors for treating AMD have shown improved efficiency at removing acidity and metals after inoculation with SRB (Morales et al., 2005; Kaksonen and Puhakka, 2007); however, the effect of inoculation decreases substantially with time. Compared to constructed environments, it is generally unnecessary to inoculate natural environments with SRB to ensure their presence; SRB are widely distributed in the environment (Muyzer and Stams, 2008). Sulfate reducing bacteria and archaea are not obligate sulfate reducers; therefore, a lack of sulfate or sulfate reducing activity does not necessarily mean that they are absent from an environment (Muyzer and Stams, 2008).

While the benefits of inoculating crops with arbuscular mycorrhizal fungi (AMF) or phosphate solubilising bacteria are well established under controlled (i.e. glasshouse) conditions, there is less evidence available on the effect of inoculation in field conditions. Inoculation may be effective in this case as spore density of AMF is generally low in ASS (Higo et al., 2011). Crop rotation with mycorrhizal plant species or AMF inoculation with liming decreased phosphorus deficiency on ASS in Thailand (Higo et al., 2009). Application of phosphorus solubilising bacteria to ASS in a laboratory system increased pH by 3 units in all treatments, regardless of plant treatment (Panhwar et al., 2014b). Although the studies discussed were successful, they were conducted under controlled conditions that may not reflect field situations. Finally, it is important to note that the scientific literature is likely to be biased towards positive results (i.e. publication bias), so it is impossible to know how many similar experiments have failed to show any improvement.

5.1.2 Inoculation with 'healthy' soil

In an attempt to displace the microbes responsible for pyrite oxidation, one might consider inoculating ASS with non-ASS soil from a nearby source. Although there is no peer reviewed literature on the effects of transferring non ASS soil to an ASS soil, the effects are likely to be similar to soil transfers in other systems. In four experimental systems where soils were transferred between locations and analysed for microbial activity and community structure, variable differences were observed between local and transplanted soils. In the first experiment discussed here, both enzyme activities and DNA analyses indicated no difference in the bacterial community between the local and transferred soil for at least 17 years after soil transfer (Bond-Lamberty et al., 2016). In the second experiment, nutrient cycling within soil cores rapidly matched the soil of their new environment (Andrianarisoa et al., 2017). In the third experiment, the abundance of fungal and bacterial species were either sensitive or resistant to soil transfer (Zumsteg et al., 2013). In the final experiment, where soil was transferred between the top soil and subsoil of the same location, transferred soils rapidly gained or lost microbial diversity depending on their direction of transfer (Preusser et al., 2017). From these examples, it seems unlikely that inoculating ASS with 'healthy' soil would be an efficient management strategy for improving soil microbial communities; however, more work is required to test this.

5.1.3 Using plants with microbes during remediation

Plants can be used to prevent acidic water leaving a site *via* leaching or percolation by intercepting water in the canopy (therefore preventing water from entering the soil) and increasing the rate of evaporation (therefore reducing the water content of the soil). However, it is difficult for plants to grow on ASS; in addition to nutrient limitations caused by strong acidity (Section 9) most plants are susceptible to acid induced aluminium and iron toxicity (Panhwar et al., 2014b). The symptoms of toxicity are seen in the root system which become severely stunted due to inhibited cell division and elongation (Panhwar et al., 2014b). Wetland plants such as *Phragmites australis*, however, are able to tolerate low pH and high metal concentrations (Guo and Cutright, 2015; Packer et al., 2017).

Inoculating soil or seeds with beneficial microbes during planting may alleviate the symptoms of metal toxicity in some plants by intercepting, immobilising, or otherwise limiting the uptake of metals into the plant. For example, the growth of pioneer plants (colonising grasses, forbs and legumes) in soils of pH 3-4 can be improved by inoculating with AMF isolated from ASS when grown experimentally in pots (Maki et al., 2008). In other experiments, plant growth promoting bacteria were shown to reduce the symptoms of Al toxicity by increasing soil pH and producing organic acids that are able to chelate Al^{3+} (Panhwar et al., 2014b; Panhwar et al., 2015). However, information about this complementary management option is limited as most tests have been conducted on rice paddies under optimal conditions (Panhwar et al., 2017); therefore, more research is needed in this area.

5.1.4 Sequestration and biochar

Biochar addition is a remediation option for ASS (Section 9.1.3), a soil improving amendment, and a carbon sequestration option. The application of biochar amendment can improve soil physical and chemical properties including: increasing pH, increasing aeration and water holding capacity, and improving the ability of soil to hold and exchange nutrients (cation exchange capacity) (Lehmann et al., 2011). For example, when incorporated into rice paddy soils, biochar decrease N losses *via* denitrification for at least four years (Zheng et al., 2016). As a carbon sequestration option, biochar is considered recalcitrant to decomposition due to its condensed structure (preventing microbial access) and lack of nutrients; however, due to variation in original materials and preparation methods, the resistance of biochar to microbial decomposition is highly variable (Han et al., 2016; Zheng et al., 2016). For example, a field study of char applied to a tropical rainforest soil indicates that up to 22% of the carbon added can be lost to decomposition in the first three years (Bird et al., 2017); temperature of pyrolysis determined decomposition in this experiment. In addition to carbon liberated from biochar, biochar addition can cause an increase in carbon mineralisation from native soil organic matter (Wang et al., 2016; Cheng et al., 2017).

5.2 Concluding remarks on complementary management options

This section discusses a range of complementary management options and whether or not they are likely to be useful additional measures to facilitate acid sulfate soil recovery during remediation efforts. These management options include inoculating with beneficial microbes (Section 5.1.1), inoculating with non-acid sulfate soil (Section 5.1.2), and combining plantings with soil inoculation (Section 5.1.3).

Inoculation is a well-established practice in some agricultural systems and is known to have beneficial effects on plant growth when grown in otherwise sterile soil. However, the situations where there are direct, measurable, benefits for plant growth after inoculation are in environments where plants which have strong symbiotic relationships with specific microbes have been inoculated as seeds or in environments that are otherwise sterile or have exceptionally low microbial diversity or inoculation potential. In experiments where soil has been translocated from local source with different soil types, there appears to be no colonisation from the translocated soil to the new location soil. The use of arbuscular mycorrhizal fungi as an inoculant for plantings on acid sulfate soil may have beneficial effects on nutrient limitation; however, this needs to be further tested. Biochar may be similarly effective as organic matter amendments, as biochar can increase pH and improve soil structure; however, it is not clear how biochar affects microbial community structure or diversity.

5.2.1 Research gaps

Detailed research on this topic is extremely limited, and the following gaps were identified:

- The effects of broad scale microbial inoculation on acid sulfate soil remediation.
- The effects of translocating non-acid sulfate soil to an acid sulfate soil.
- Sulfate reducing bacteria survival and success as soil inoculants.
- The effect of adding arbuscular mycorrhiza to wetland plantings.
- The effect of biochar amendments on acid sulfate soil remediation outcomes.
- The effect of biochar on sulfate reducing bacteria and archaea.

5.2.2 Management priorities

Complementary management options such as inoculation and biochar are largely untested and unverifiable in this context. We do not recommend these approaches be used at any scale larger than a field trial without prior rigorous scientific investigation.

6 Recommendations

The purpose of this literature review was to address the topic raise in section 3.2.3 of the project brief, which relates to assessing microbial function in soils with different inundation histories or contexts. Specifically, to investigate the relationships between pyrite rich and acid sulfate soil microbial communities and a number of environmental variables and management options. This is done in seven numbered sections, with each section explicitly relating to a topic raised in the project brief. See Section 1.2 for the plain language summary of this review.

6.1 Recommendations for management priorities

Due to limitations within the scientific literature, the recommendations for management practices to improve soils microbial outcomes herein are speculative in parts. We can make suggestions based on known levers and direction of change, however it would be premature to comment on timing or magnitude of changes. Given the potential adverse impacts of large scale management interventions, we strongly recommend any such measure be tested in a pilot field study prior to wide spread or full scale implementation. The main levers for change in wetland soil microbial ecosystems include water, organic matter, and disturbance; adjusting these factors are the easiest ways to enact change in wetland ecosystems. It is important to understand that each wetland contains different environments and experiences different external pressures, therefore, that management practices that lead to satisfactory outcomes in one wetland may not translate to the same results when applied to another wetland. Taking this into consideration, we strongly recommend that management priorities include a monitoring program that is capable of identifying poor wetland responses to management change before catastrophic tipping points are reached.

Please note though, that the following recommendations are non-specific and high level recommendations for general beneficial practices. For targeted recommendations please see the final report on WMP recommendations (in preparation) and for specific recommendations on wetlands that were assessed during the detailed field assessment, please see the report on this project's detailed field assessment (in preparation). It is also expected that the literature on this topic will develop rapidly over the next decade with the increasingly wide availability of highly detailed but economic data collection options. Thus, it will be possible (and indeed prudent) to further refine these recommendations over time.

6.1.1 Recommendation: management actions that increase wetland resilience

Wetland resilience is the ability for wetlands to 'bounce back' after a change in conditions; that change may be internal (such as an inundation event) or external (such as a heat wave) or a combination of both (such as drought). Resilience is connected to diversity – of species, of environments, of conditions – that leads to a bank of organisms that can flourish under changing conditions. Resilience is also connected with providing an environment of recovery between damaging events. Therefore, we recommend:

- Maintaining high diversity plant communities.
- Minimising soil disturbance by hard footed animals and vehicles.
- Avoid the simplification of wetland environments (such as through drains and channelling).

6.1.2 Recommendation: management actions that increase soil resilience

In addition to the recommendations above (Section 6.1.1), we recommend the following action for soil resilience specifically:

- Increasing organic matter inputs to the soil.
- Preventing surface erosion by maintaining vegetative cover.
- Preventing surface erosion by excluding hard footed animals and minimising vehicle tracks.
- Allowing a diverse set of environments to form through the actions of wetting and drying.

6.1.3 Recommendation: management actions that prevent environmental extremes

In the context of acid sulfate soils and microbial dynamics in these soils, environmental extremes are conditions of constant wet or constant dry or a sudden prolonged change from wet to dry. In soils where sulfate ions are available, constantly wet soils are at risk of accumulating pyritic materials that then pose a risk, if the soil dries, of forming sulfuric materials through pyrite oxidation (Section 8). While small amounts of pyrite oxidation can be absorbed by the environment without issue, catastrophic pyrite oxidation can breach an environmental tipping point leading to severe soil and water quality degradation that can be very costly to remediate. Constantly dry wetlands are also at risk of environmental degradation if suddenly flooded due to the formation of blackwater events. Therefore, we recommend:

- Maintaining a moisture regime that permits small accumulation and oxidation events.
- Preventing prolonged periods of pyrite accumulation.
- Withdrawing from drying events before ecological tipping points are breached.
- Preventing large quantities of dry organic matter from accumulating.
- Preventing blackwater events through slow or staged inundations.
- Prevent pH from dropping below 5 during oxidation events.

6.1.4 Recommendation: acid sulfate soil (sulfuric soils) remediation strategies

There are a number of strategies that can be used after a catastrophic pyrite oxidation event with varying amounts of evidence and experience supporting them. Enhancing environmental remediation of sulfurous soils requires the creation of soils that are anoxic, contain sulfate, contain simple organic matter, and have microsites with a pH greater than 5. These microsites are required to allow sulfate reducing bacteria to establish as they will not thrive in highly acid conditions. Therefore, remediation strategies should include:

- The provision of suitable organic matter.
- Soil anoxia through inundation or decomposition induced hypoxia/anoxia.
- Mechanisms to increase soil pH through organic matter or liming.

We do not recommend inoculation with commercial microbial inoculants or with fresh soil at this point as there is insufficient evidence to support these actions.

6.1.5 Recommendation: monitoring strategies and field requirements

As previously stated, we strongly recommend a monitoring program to prevent catastrophic change and the careful application of management decisions based on testing and observations from specific wetlands. We therefore recommend the use of field trials to test management options, and also suggest the following recommendations for future research priorities (Section 6.2).

6.2 Recommendations for future research

Very little work has been undertaken on the two broad topics covered by this report: the role of soil microbes in ASS, and the effects of management decisions on soil microbes in ASS. Therefore, most of the recommendations in this document are based on educated guesses and speculation. As far as we can determine, there is no literature that specifically investigates the following topics:

- The relationships between managed inundations and organic matter energy dynamics in naturally formed wetlands that are at risk of developing ASS (Section 2.3.1).
- The effect of biomass management decisions on ASS prevention and remediation (Section 3.4.1).
- The effect of clay content and mineralogy on management options for ASS (Section 3.4.1).
- The use of organic amendments in the field and the best practice mechanism for delivery and distribution for ASS remediation (Section 9.1).
- Basic research on how primary productivity, water quality, and soil chemistry are directly affected by soil microbial activities such as nutrient cycling, organic matter decomposition, and the anaerobic production of reduced inorganic sulfur (Section 7.5.1).
- Basic research on the biology, ecology, and relationships of microorganisms in ASS (Section 8.3.1).
- Basic research on the effects of native and added organic matter on microbial composition and function in ASS (Section 9.2.1).

The most useful future research for management outcomes is also likely to be the most time consuming and expensive research. A further challenge of managing these environments are the background issues of changing climates, environments, and policy. Discovering the effects of management decisions on acid sulfate soil development and remediation will require extensive preparatory research and field trials before scientifically proven actions of best practice can be recommended. In the absence of extensive research however, this report provides some illumination on the best way to proceed while managing for soil microbial processes in acid sulfate soils.

Soil Microbial Investigation

Assessing microbial functioning in soils with different inundation contexts

DEWNRF-00020652
DEWNR Contract #1077

Prepared for
The Minister for Environment and Water
Government of South Australia Department of Environment and Water

Part 2 of 2 – Technical information

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7 The importance of soil microbial function for water management for ecological purposes

This section refers to Project Brief **Attachment 5** section **3.2.3 Soil Microbial Investigation - assessing microbial functioning in soils with different inundation histories/contexts** and is an introduction to the importance of soil microbial functions.

7.1 Soil microbes in wetland systems

7.1.1 What are soil microbes?

Soil microorganisms (commonly referred to as microbes) are organisms too small to see without a lens or microscope. Microorganisms may be single celled (e.g. bacteria or archaea), colonies of single celled organisms (e.g. yeasts), or relatively large multicellular organisms (e.g. fungi mycelium and microfauna). Microbes have historically been classified by cellular structures and more recently by genetic similarities; when considered at the highest levels of classification (Domains), they include prokaryotes (archaea and bacteria) and eukaryotes (eukarya) (Stahl et al., 2013).

Prokaryotes and eukaryotes differ in their internal structure: eukaryotes contain a defined nuclear membrane inside which most of the cell's genetic material resides and specialised organelles (for example, chloroplasts in organisms that can photosynthesise) (Stahl et al., 2013). Prokaryote genetic material is found within the bulk contents of the cell, and they do not have specialised organelles (Killham and Prosser, 2007). The phylogeny below (Figure 8) is a simplified version of the global phylogeny of life as determined by Ciccarelli et al. (2006). It was composed from all known fully sequenced organisms. The intersection in the middle represents the last common ancestor of all life. Figure 1, and indeed all such 'Tree of Life' constructions, are biased by the history of research. Archaea (green, lower left) appear to be the smallest group in the three domains; however, this is due to a lack of complete sequences rather than a lack of diversity. There are also many theories and interpretations of diversity at all levels of biological classification; in this report, we use the "Three Domain System" (Woese et al., 1990; Zhou et al., 2018).

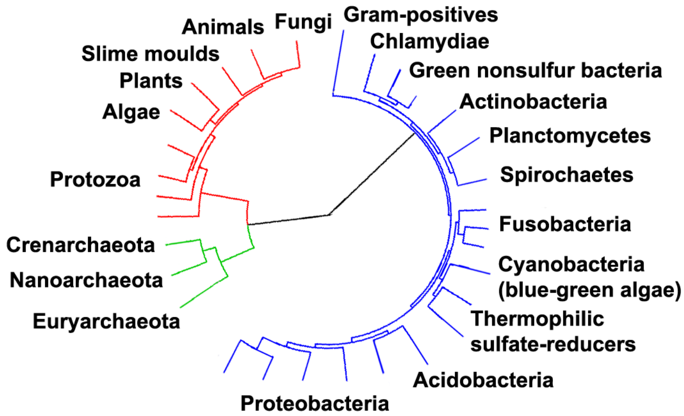

Figure 8: condensed and simplified global phylogeny of life modified from Ciccarelli et al. (2006). Image hosted in the public domain. Image depicts the three domains of life, archaea (green, lower left), eukarya (red, upper left), and bacteria (blue, right) and their relationship to the last common ancestor.

The three domains (archaea, bacteria, and eukarya) encapsulate most of known life. Non-cellular life (such as prions and viruses) are not included in the domains (Woese et al., 1990). Eukarya is the most well-known domain; microorganisms in eukarya typically include algae, fungi, and microfauna animals (Ciccarelli et al., 2006). Protists are also common microorganisms in eukarya; however, they consist of both closely related group of organisms and groups with uncertain relationships and are grouped together out of convenience (Adl et al., 2005). In addition, the common name 'blue-green algae' (*Cyanobacteria*) is a misnomer, as these organisms are not algae but are instead bacteria that obtain their energy via photosynthesis. Nevertheless, the term 'blue-green algae' has persisted; in this report, these organisms will be exclusively referred to as *Cyanobacteria* to prevent any confusion.

Microorganisms live in fluids and attached to surfaces, and they are both abundant (up to one billion cells per gram of soil) and diverse in soil (Curtis et al., 2002; Gans et al., 2005). Within soil, microbes may be motile or non-motile, may have rapid or slow growth and reproduction rates, and may live on particle surfaces or freely on soil surfaces or in soil pore water. Soil is able to have an extremely rich microbial diversity as single populations of microbes that were originally the same strain are easily separated; fast doubling times and a lack of population connectivity allows high rates of genetic drift over relatively small separation distances (Preusser et al., 2017). Mixing soils through cultivation or erosion, or increasing population connectivity *via* inundation can lead to decreases in soil microbial diversity simply by reducing the opportunities for genetic drift in spatially separated populations (Foissner, 2006).

7.1.2 What function do microbes have in soil?

Three key soil functions that influence and are affected by microbes include (i) biomass (including plant) production, (ii) elemental storage, filtering, and transformation, and (iii) habitat (Andrews et al., 2004). Microbes influence all of these functions through nutrient cycling and soil organic matter decomposition and creation. Nutrient cycling affects biomass production by capturing, storing, and transforming organic nutrients from forms that are unavailable to plants to forms that are available for plant uptake (van der Heijden et al., 2008). Autotrophic (see glossary) microbes also add to the soils primary productivity by using photosynthesis or other organic processes to capture energy in the environment and use it to transform abiotic molecules into organic matter (Ryan and Law, 2005). Microbes influence the soil environment, and therefore their habitat, through the creation of substances and structures that allow soil primary particles (i.e. mineral particles) to bind together into aggregates. The formation of aggregates increases the rate that water and oxygen can penetrate into soil while also protecting soil organic matter from decomposition within aggregates.

Many of the functions of soil microbes in soils are related to nutrient cycling. During nutrient cycling, soil microbes have a wide range of functions in carbon (C), nitrogen (N), phosphorus (P), and sulfur (S) chemistry, pools and transformations in both aerobic (oxic; containing oxygen) and anaerobic (anoxic; lacking oxygen) (Section 7.2). Soil microbes can generate soil organic matter via autotrophy and as a by-product of heterotrophy. Autotrophic organisms, such as the phylum *Cyanobacteria* or the genus *Nitrobacter*, fix C via the energy provided by sunlight (*Cyanobacteria*) or by oxidising nitrite to nitrate (*Nitrobacter*) (Jordan et al., 2001; Choi et al., 2010). Microbes that are able to photosynthesise also release oxygen to their surroundings, and may be an important source of oxygen in otherwise oxygen limited environments (such as biofilms and microbial mats; 7.1.4).

Autotrophic microbes that use iron (II) oxidation are extremely important in the development of acid sulfate soils (ASS) (Section 8.1.5). Heterotrophic microbes decompose organic matter to assimilate the C resources and nutrients for growth and reproduction. During this process, plant available nutrients are released from complex organic molecules directly as by-products of decomposition or indirectly after the microbe dies. Heterotrophic (see glossary) microbes can be obligate aerobes (require oxygen for respiration), facultative anaerobes (prefer oxygen for respiration), or obligate anaerobes (require an oxygen free environment for respiration). Organic matter decomposition can proceed in oxic or anoxic conditions (i.e. with or without oxygen), however the energy yield to microbes of anaerobic decomposition is an order of magnitude lower the equivalent aerobic reaction (Brune et al., 2000). Microbial growth rates are therefore much lower in anaerobic environments.

7.1.3 What function do microbes have in wetlands?

Soil microbes directly affect trophic energy flows and oxygen concentration in wetlands through their organic matter decomposition activities. The net effect of nutrient cycling in wetlands is determined by the organic matter source (Section 2.2), water availability (Section 3), and water quality (Section 8.1.3). In wetlands with organic matter inputs (for example, from allochthonous sources), soil microbes close the cycle between soils, primary productivity and the atmosphere by decomposing organic matter (Wolf et al., 2013). During decomposition, a portion of the energy captured and stored by plants and other autotrophic organisms is released back to the environment as the chemical bonds in organic molecules are broken. This energy is used by microbes for activity and growth, and a portion is lost as heat (Horwath, 2007).

In wetlands that are periodically or permanently inundated, soil microbes directly affect oxygen content of the soil and water. Heterotrophic microbes consume oxygen while decomposing organic matter and, in the absence of oxygen, may consume nitrate, manganese (IV) oxide, iron (III) oxide, sulfate or carbon dioxide, as determined by the redox potential (Section 7.4.2) (Muyzer and Stams, 2008). These activities lead to the development of anoxia in the soil (and potentially in the water). In anoxic sediments, facultatively anaerobic and obligate anaerobic microbes grow and thrive; these conditions and microbes use different paths in nutrient cycling that have different end products (Section 7.2). Both aerobic and anaerobic processes are important for biogeochemical cycles such as C (Section 7.2), N (Section 7.2.2), P (Section 7.2.3), and S (Section 7.2.4) cycles. Anoxic soils can lead to the development of reduced inorganic RIS minerals by sulfate reducing bacteria and archaea (SRB) if sulfate is available in the water (8.1.1). Anoxic surface water is generally undesirable, as many aquatic organisms cannot tolerant low dissolved oxygen contents.

7.1.4 Biofilms and microbial mats

Two distinct microbial features can form in wetland waters and soils: biofilms and microbial mats. Both biofilms and microbial mats are complex networks of microorganisms and organic matter: biofilms are relatively simple microscopic congregations of microorganisms that are attached to solid surfaces *via* a thin film, while microbial mats are a complex of multiple types and species of microorganisms and organic matter that exist as a visible, layered, structure (Stahl et al., 2013) (Figure 9). Microbial mats include primary producers, consumers and decomposers organised in a manner that allows interaction between communities (Stahl et al., 2013). Sulfate reducing bacteria and archaea are frequently found in association with cyanobacteria in microbial mats, where oxygen is generated by the cyanobacteria and physically excluded by the SRB to promote a reducing environment (Sigalevich et al., 2000). Microbial mats where photosynthesis and sulfate reduction is occurring result in a net precipitation of sulfide and carbonate minerals (e.g. pyrite and calcite), whereas microbial mats undergoing aerobic respiration and oxidation result in a net dissolution of these minerals.

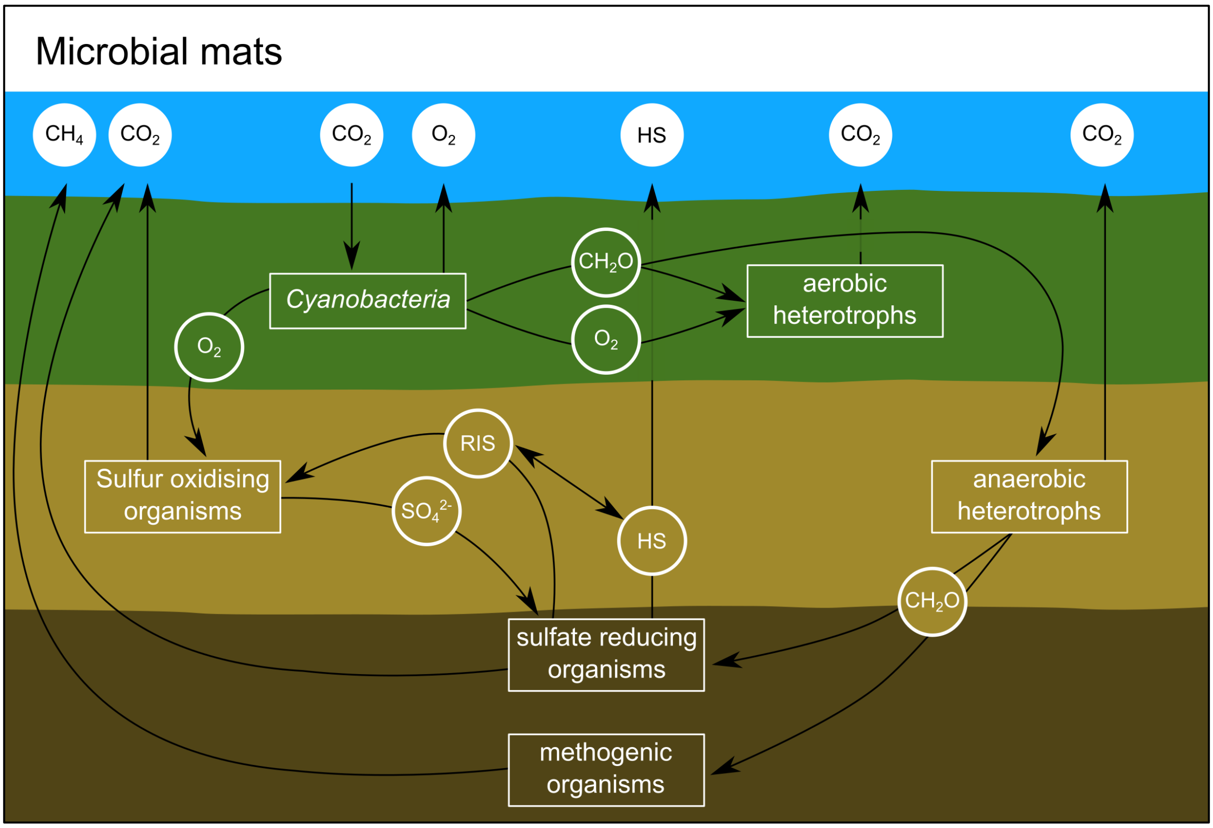

Figure 9: substrate relationships within a simplified microbial mat where colours indicate microbial layers (blue is water, green – brown is mat), CH_4 is methane, CO_2 is carbon dioxide, O_2 is oxygen, HS is bisulfide, CH_2O represents simple organic matter, SO_4^{2-} is the sulfate ion, and RIS is reduced inorganic sulfur. Image modified from Stahl et al. (2013) by E Stirling using Inkscape version 0.91 (The Inkscape Team, 2018).

In this simplified example microbial mat (Figure 9), oxygenic phototrophs (cyanobacteria) are the primary producers, using photosynthesis to fix CO_2 and generate O_2. Organic matter (‘CH_2O’ in Figure 9) is then used for respiration by aerobic and anaerobic heterotrophs in the oxic and anoxic zones of the mat, respectively. Organic matter can move through the mat to zones where SRB and methanogenic organisms are active, where it is respired to generate CO_2 and CH_4. Oxygen from cyanobacteria may also be used by aerobic heterotrophs or sulfur oxidising organisms. Sulfate produced by sulfur oxidising organisms is then available for SRB. The net effect of substrates between sulfur oxidising and sulfur reducing organisms depends on the environmental conditions of the mat (Section 8); these conditions have important implications for the development of ASS (Section 8.1).

7.2 Microbial nutrient cycles

Key microbial nutrients include elements, such as nitrogen (N; Section 7.2.2), phosphorus (P; Section 7.2.3), and sulfur (S; Section 7.2.4), and are required for organisms to metabolise and grow. Macronutrients are nutrients required in large amounts by plants for their growth and include N, P, S, potassium (K), calcium (Ca) and magnesium (Mg). Micronutrients are required in smaller amounts and include iron (Fe), boron (B), chlorine (Cl), manganese (Mn), zinc (Zn), copper (Cu), molybdenum (Mo) and nickel (Ni). Nutrient cycling is a process where these elements pass from the environment to organisms and back while undergoing chemical transformations from one chemical species to another. Although carbon (C; Section 7.2.1), hydrogen (H), and oxygen (O) are also vitally required for life, they are considered resources rather than nutrients in soil science. The effects of C on nutrient cycling are discussed further in Section 7.2.1 and Sections 5 and 3.

Nutrient cycling is an important soil function (Section 7.1.3) and an inability to capture and cycle a sufficient proportion of the nutrients passing through a soil to waterways can lead to reduced productivity or degradation of downstream water quality or both (Brinson and Malvárez, 2002; Kuwabara et al., 2012). For example, excessive amounts of nitrogen or phosphorus in waterways lead to stream eutrophication and algal blooms. Nutrients find their way into waterways *via* a variety of pathways: in agricultural systems, erosion, fertiliser runoff, livestock wastes and nutrient leaching are major pathways. In high nutrient systems, a lack of carbon resources can lead to insufficient microbial nutrient cycling to prevent stream eutrophication (de Sosa et al., 2018). Wetlands can act as a buffer against nutrient inputs into streams by capturing sediments and organic matter (Section 2.2.2).

7.2.1 Microbial carbon cycle

Soil microbes affect the carbon cycle by consuming soil organic matter for energy resources and nutrients *via* aerobic and anaerobic decomposition in oxic and anoxic conditions, respectively (Figure 10) (Horwath, 2007). Carbon is input into the soil C cycle in wetlands by plant root exudates, plant and algae organic matter, photosynthetic microbe organic matter, animal wastes and organic matter from upstream sources (allochthonous C; Section 2.2). Dust or sediments carried by wind and water may also contain substantial C inputs. Within the soil, C is cycled between soil organic matter and the microbial biomass, with a portion of C lost as CO_2 or CH_4 during respiration and a portion of the microbial necromass reverting to soil organic matter (i.e. microbial turnover) (Gougoulias et al., 2014). While a portion of the microbial biomass has obligate aerobic respiration or anaerobic respiration, there is a range of microbes that can respire in both oxic and anoxic conditions in conditions of wet and dry cycles (Section 7.1.3). The processes discussed here can proceed simultaneously within a site as both oxic and anoxic zones can exist within wet and recently dried wetland soils.

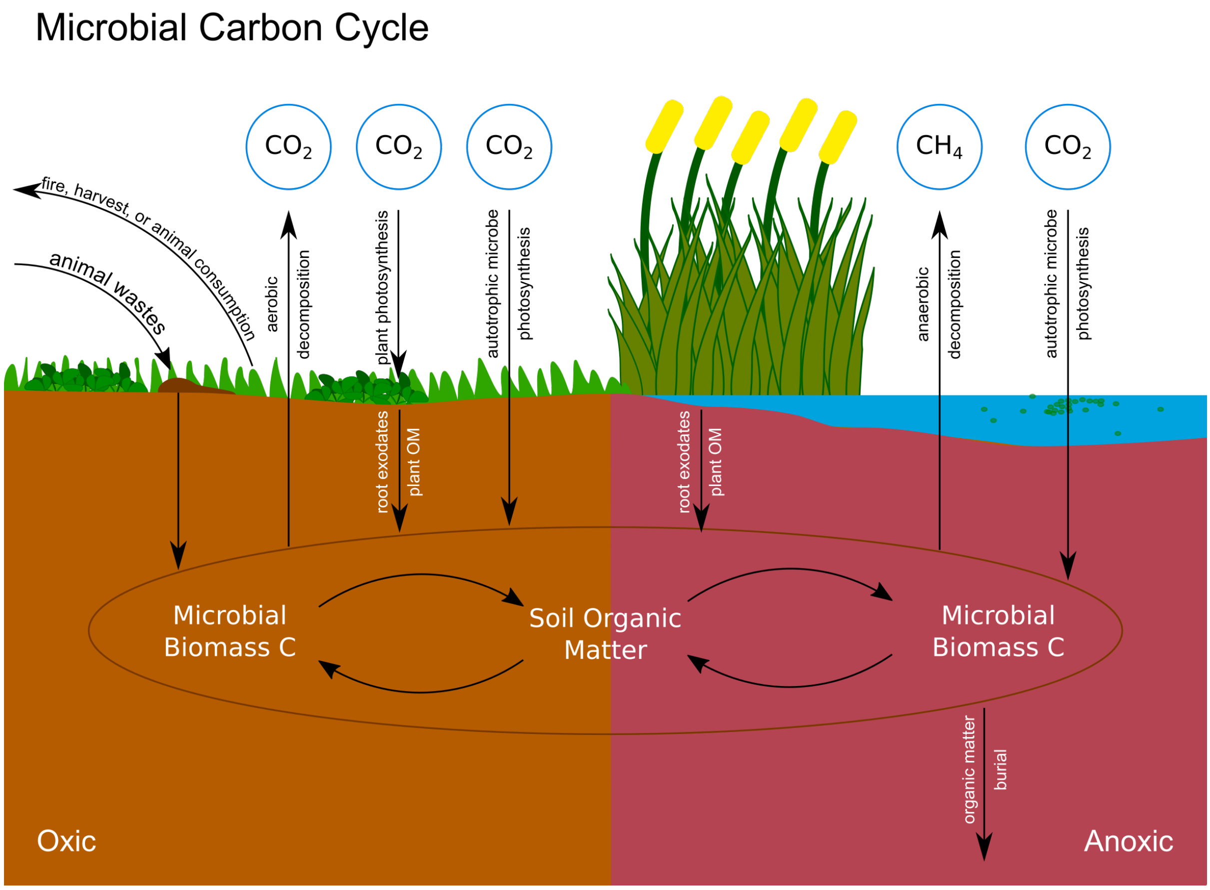

Figure 10: Microbial carbon cycle in oxic and anoxic conditions where OM is 'organic matter', CO_2 is carbon dioxide and CH_4 is methane. Figure made by E Stirling using Inkscape version 0.91 (The Inkscape Team, 2018).

7.2.2 Microbial nitrogen cycle

Before the mass production of nitrogen fertilisers, biological N fixation and lightning were the main drivers of ecological nitrogen cycling (Figure 11) (Galloway et al., 2004). Biological nitrogen inputs to the soil N cycle in wetlands include bacterial N fixation, plant and algae organic matter, microbial organic matter, animal wastes, and as organic matter from upstream sources (allochthonous N;

Section 2.2) (Roberston and Groffman, 2007). Similarly to C inputs, dust and sediments may contain high N contents. Bacterial N fixation generally occurs in association with symbiotic hosts (such as legumes or lichens), however some microbes are capable of N fixation freely (such as some species of *Cyanobacteria*) (Choi et al., 2010). Nitrogen moves between the SOM and microbial biomass *via* assimilation and reverts to SOM *via* the microbial necromass. During this process, organic N forms may be mineralised to ammonium *via* ammonification or nitrate *via* heterotrophic nitrification; both of these forms of N are available for plant uptake. After ammonium is produced, it may be taken up by plants or immobilised in the microbial biomass or further transformed to nitrate or nitrous oxide. Nitrate is water soluble and mobile in the soil and is highly susceptible to leaching. If nitrate is exposed to anoxic conditions, anaerobic microbes will use it in the denitrification process to generate energy. During this process, N is sequentially reduced and degassed as NO_x or nitrous oxide (greenhouse gasses); if denitrification is complete, N is returned to its initially inert form of N_2. In anoxic environments, nitrate may alternatively be converted back into ammonium *via* dissimilatory nitrate reduction (Silver et al., 2001), which is a process similar to sulfate reduction (Section 8.1.1 and Section 9.1.2); the ammonium formed during this process may be taken up by the microbial biomass, plants, or leached. The processes discussed here can proceed simultaneously within a site, as both oxic and anoxic zones can exist within wet and recently dried wetland soils.

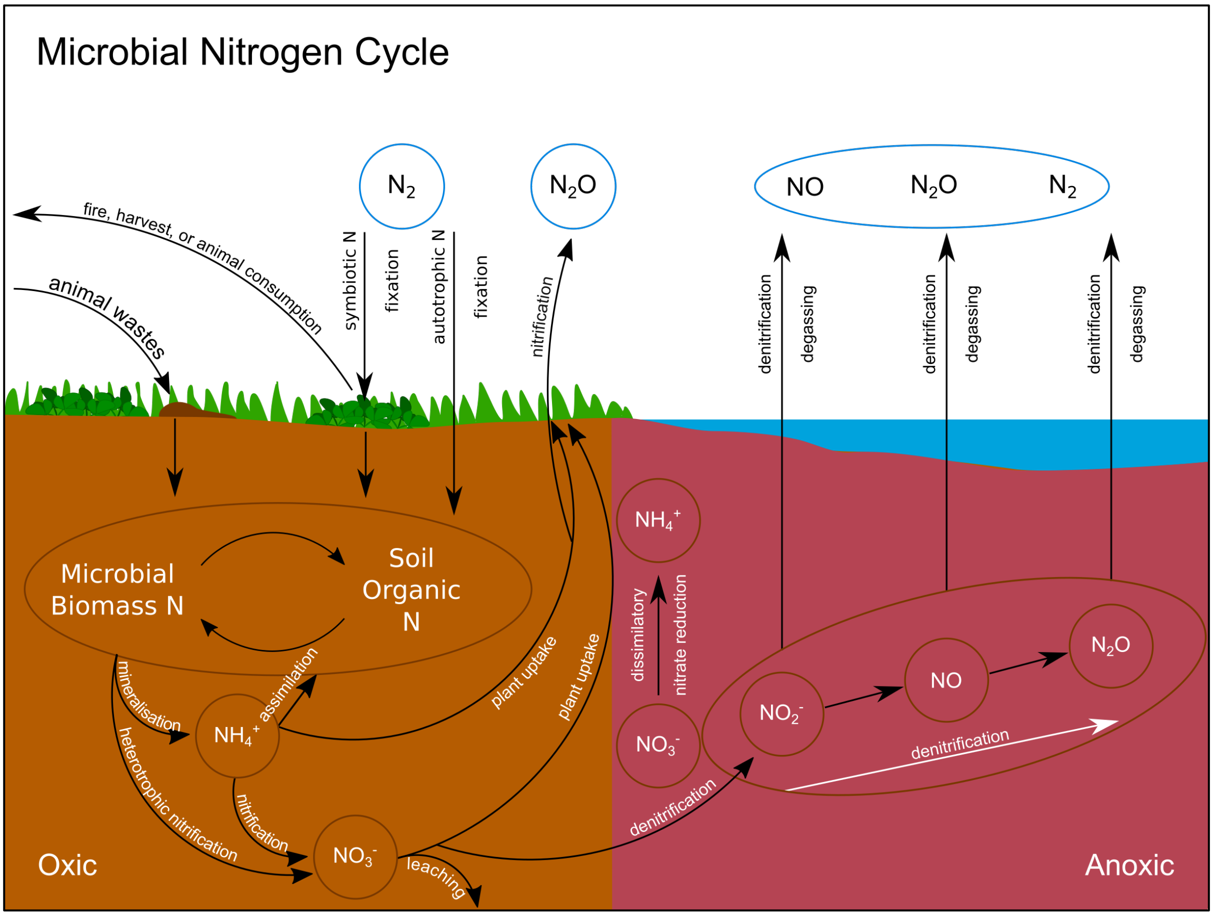

Figure 11: Microbial nitrogen cycle in oxic and anoxic conditions where N_2 is nitrogen gas, N_2O is nitrous oxide, NO is nitric oxide, NH_4^+ is the ammonium ion, NO_3^- is the nitrate ion, and NO_2^- is the nitrite ion. Note that while the microbial biomass in not indicated on the anoxic side of the figure, all reactions are microbially mediated unless otherwise indicated. Ammonium in anoxic conditions is similarly available for uptake to the microbial biomass and plants as on the oxic side. Modified from Pajares and Bohannan (2016) by E Stirling using Inkscape version 0.91 (The Inkscape Team, 2018).

7.2.3 Microbial phosphorus cycle

Geochemical cycling is equally important as plant uptake or microbial assimilation in the P cycle (Figure 12). Both plants and microbes are able to release inorganic P from soil particles or organic matter by releasing phosphatase enzymes or P chelating molecules. While soil P availability is generally low in Australian soils, wetland systems may have substantial P content *via* sediment deposition from P fertilised upstream areas, plant and algae organic matter and microbial organic matter (Section 2.2) (Newcomer Johnson et al., 2016). Microbial P uptake can be a substantial sink for inorganic P in the soil as microbes are capable of storing P over long periods in the microbial biomass (unlike C or N, which are only stored for as long as they are used) (Kong et al., 2005). Phosphorus has limited mobility in terrestrial soil, and forms insoluble minerals in strongly acid soils, leading to P deficiency for plants and microbes in ASS (Ren et al., 2004). However, there is an increase in the concentration in inundated soils of water soluble P due to: hydrolysis of iron and aluminium phosphates, release of P from anion exchange sites on clay, and reduction of iron (III) to iron (II) leading to a release of sorbed P (Ponnamperuma, 1972).

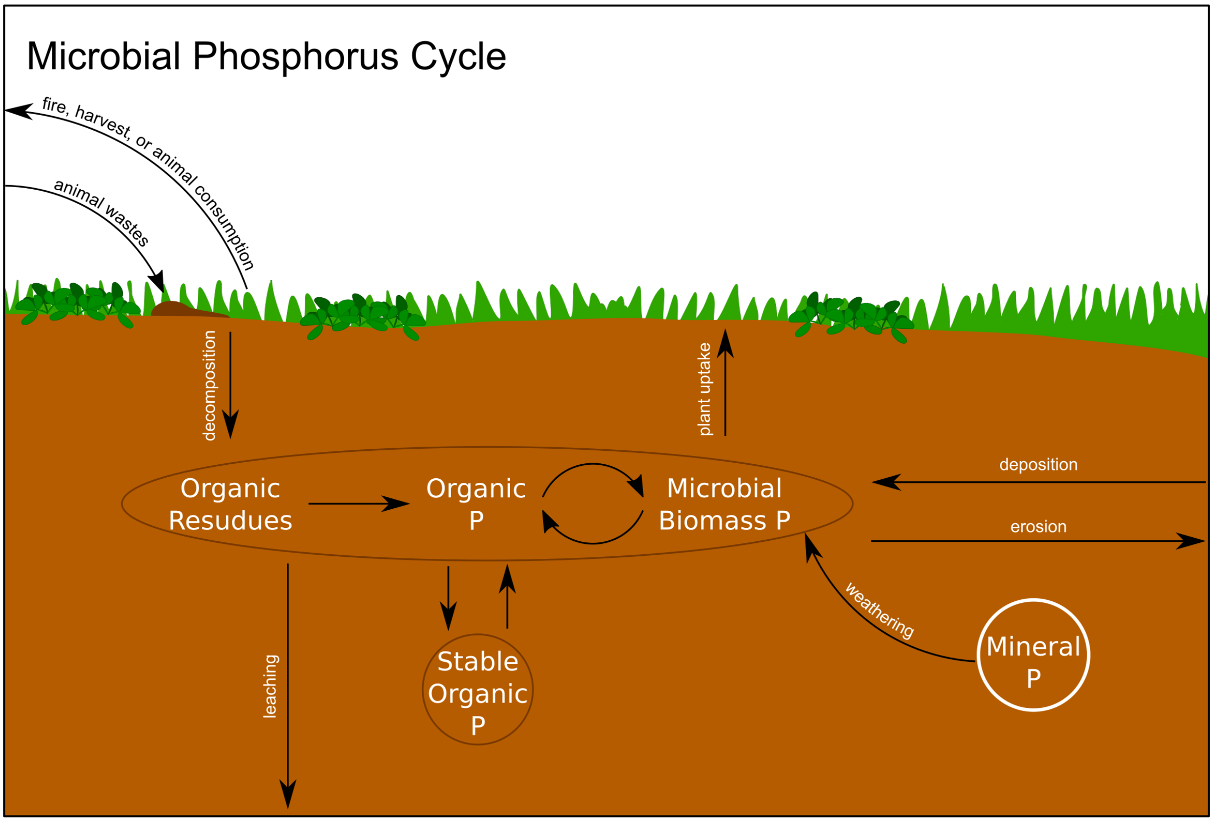

Figure 12: Microbial phosphorus cycle. Figure made by E Stirling using Inkscape version 0.91 (The Inkscape Team, 2018).

7.2.4 Microbial sulfur cycle

Similarly to the phosphorus cycle, soil microbial sulfur cycling involves substantial connections with the geochemical sulfur cycle (Figure 13) (Plante, 2007). However, unlike the phosphorus cycle, many of the transformations in the sulfur cycle involve or are controlled by microbes. Sulfur inputs to soil include atmospheric deposition, mineral weathering, and agricultural inputs through fertiliser and irrigation water; sulfur salts are also a component of saline groundwater (Section 8.1.3). Sulfate is assimilated by plants and microbes as a nutrient, or in anoxic conditions used by anaerobic microbes as an electron acceptor for respiration. Both the mineralisation of soil organic sulfur and the use of sulfur in respiration produce bisulfide (HS^-), which may then be oxidised to elemental sulfur or thiosulfate ($S_2O_3^{2-}$) in oxic conditions; these outputs may be used by chemotrophic microbes to return the S to sulfate. Under reducing conditions (i.e. anoxic conditions) and in the presence of iron, RIS forms iron (II) sulfide (FeS), pyrite (FeS_2) or other metal sulfide minerals; in oxidising conditions, sulfate may precipitate as gypsum ($CaSO_4.2H_2O$).

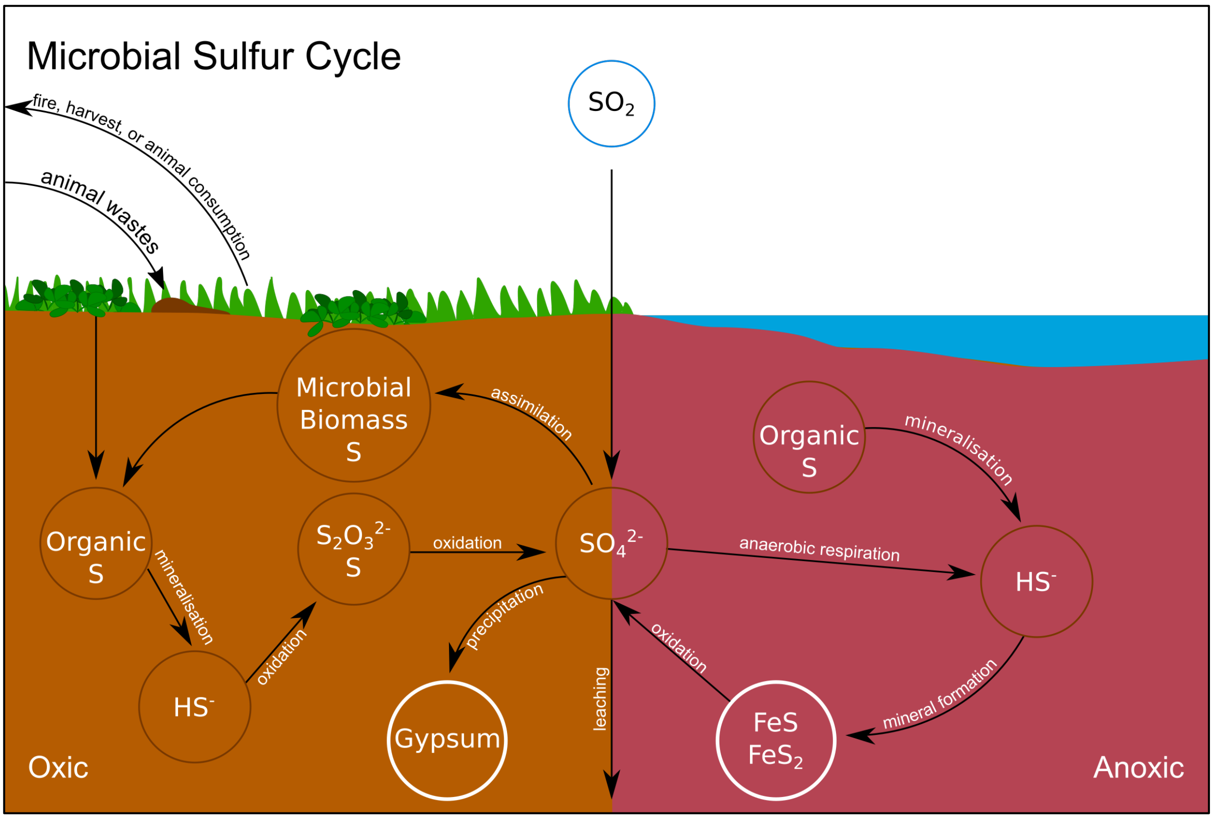

Figure 13: Microbial sulfur cycle in oxic and anoxic conditions where SO_2 is sulfur dioxide, $S_2O_3^{2-}$ is the thiosulfate ion, S is elemental sulfur, HS^- is the bisulfide ion, SO_4^{2-} is the sulfate ion, FeS is iron (II) sulfide, and FeS_2 is iron (II) disulfide. Image modified from Plante (2007) by E Stirling using Inkscape version 0.91 (The Inkscape Team, 2018).

7.3 Methods for measuring soil microbial function

The ecosystem services and functions of soil microbes are multifaceted and complex. As such, it is difficult to directly measure them in a meaningful way. Over time however, methods have been created to measure proxies of microbial function, including microbial activity, microbial biomass, microbial diversity, and net nutrient transformations (Table 2) (Kandeler, 2007; Thies, 2007). A large number of these analyses are labour intensive, imprecise, expensive or have limitations in the degree to which data can be accurately interpreted. Recently, advances in molecular methods of determining species diversity has added substantially to the literature on microbial diversity; however, the function of individual microbe species continues to be difficult due to the limited ability of researchers to isolate and grow microorganisms in a laboratory environment.

Table 2: Methods, descriptions, outcomes and limitations for a selection of methods for measuring soil microbial function.

	Method	Description	Outcomes	Limitations
Function	Organic matter (OM) decomposition	A known amount of OM is added to the field or soil samples. Field OM is later retrieved and analysed for degree of decomposition. Soil sample OM may be analysed after a period of incubation using a variety of methods to determine quantity and quality of residual OM.	Decomposition rate of OM, which is a proxy for microbial activity and microbial resource use efficiency.	Decomposition is a slow process; decomposition experiments frequently take more than 1 year to complete. Litter bag field experiments may underestimate decomposition rates due to carbon ingress during decomposition.
	Heterotrophic soil respiration	Soils are incubated in the dark and analysed for CO_2 gas evolution over a period of time.	Quantification of soil respiration, which is a proxy for microbial activity.	Strongly affected by incubation conditions; may not be appropriate for alkaline soils as CO_2 may be evolved from neutralisation.
	Potentially mineralisable N (PMN)	Soil subsamples are incubated anaerobically; the difference between soil ammonium content before and after incubation is PMN. (Figure 11, ammonification)	Quantifies soil microbial potential to convert OM to ammonium.	In some soils, ammonium may be generated or consumed by processes that are not microbially mediated.
	Net nitrification	Soil subsamples are incubated aerobically; the difference between soil nitrate content before and after incubation is net nitrification. (Figure 11, nitrification)	Quantifies soil microbial potential to convert OM to nitrate.	Only shows net movement; cannot quantify rates of nitrification.
	Chloroform fumigation extraction	Soluble C and N are extracted from soil subsamples that have undergone chloroform fumigation and compared against soil subsamples that have not been fumigated. The difference between these two values is the biomass C or N content.	Quantification of microbial biomass C and microbial biomass N.	Overestimates biomass C and N, highly sensitive to experimental errors.
	Hexanol fumigation extraction	Plant available P is extracted from soil subsamples that have undergone hexanol fumigation and compared against soil subsamples that have not been fumigated. The difference between these two values is the biomass P content.	Quantification of microbial biomass P.	May produce highly variable results, uses toxic chemicals, labour intensive.
	Chloroform fumigation incubation Substrate induced respiration	Soil samples are fumigated with chloroform and CO_2 production is measured over 7-10 days to determine biomass; or A known amount of simple organic C is added to soil samples and CO_2 production is measured over 7-10 days to determine biomass	Proxy for microbial biomass C.	Labour intensive; inaccurate; imprecise.

Diversity	Polymerase chain reaction (PCR) Quantitative real time polymerase chain reaction (qPCR)	DNA is extracted from soil, amplified for 20-40 cycles, and compared to a reference library. During qPCR, amplified products are detected after each cycle.	Generates a community fingerprint of diversity. qPCR gives an approximate quantification for relative gene expression.	Expensive and imprecise analysis that has been improved upon by advances in soil metagenomics. Overestimates microbial number due to residual DNA.
	DNA microarray (multiple methods)	In general: DNA is extracted from soil, treated with fluorescent target sequences, and attached to a microarray. The microarray is analysed using spectrophotometric methods. See “geochip” for more information.	Microbial diversity as expressed by relative gene expressions.	Assumes target sequences are specific to the desired DNA markers. Has a high rate of false positives (target sequences that match more than one protein) and false negatives (protein of similar function does not match target sequence).
	Phospholipid fatty acid (PLFA) ^{13}C PLFA with stable isotope probing (SIP)	Soil samples are extracted using organic solvents. The extracts are fractionated and the phospholipids are subjected to alkaline methanolysis to produce fatty acid methyl esters (FAMEs). These are then quantified using gas chromatography-mass spectroscopy.	Quantification of live and recently dead microbial biomass. Generates a community fingerprint of diversity from the range of FAMEs produced. ^{13}C PLFA can be used with SIP to measure microbial activity.	PLFA data is difficult to interpret and has been misused as a quantitative measure of microbial diversity (Willers et al., 2015). Not a taxonomic tool; cannot be used to calculate diversity indexes.
Diversity and Function	Soil metagenomics – marker gene survey with PICRUSt2 functional estimation	DNA is extracted from soils and analysed using high throughput genetic sequencing. For example, next generation sequencing. Marker gene analyses (e.g. 16S) are used to study community diversity and composition. When combined with the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States or PICRUSt algorithm, functions can be estimated. See Langille (2018) for more information.	Diversity and relative abundance profiles of soil microbial organisms. Can calculate diversity indexes.	Financially expensive for both data acquisition and data analyses; costs are reducing over time. Sequence databases can be limiting. Gives limited indication of function.
	Soil enzyme activities	Soil enzymes are extracted from soil and are analysed using enzyme buffers and spectrophotometric methods.	Enzyme activities can be interpreted as a proxy for resource and nutrient cycling. May provide element specific information.	Large sample sizes; highly variable results; interpretation is difficult. Enzymes may have more than one purpose, or multiple enzymes may be used for the same purpose.
	Agar cultures	An agar plate is streaked with soil water extract and incubated. The incubated plates are then analysed for microbial growth.	Diversity of culture-able microbes	Labour intensive; does not capture true diversity of soil microbes.

7.4 Soil microbial function and water management

7.4.1 Redox considerations

The effect of redox potential must be considered when discussing the effect of water management on microbial function. 'Redox' is a portmanteau of 'reduction' and 'oxidation' used to encapsulate complementary oxidation and reduction reactions. These are chemical reactions where the oxidation state (i.e. the number of electrons) of atoms is changed. Oxidation is any reaction where there is a loss of electrons (i.e. the atom becomes more positive) and reduction is any reaction where there is a gain of electrons (i.e. the atom becomes more negative). A balanced redox equation will have both an oxidation and reduction process; that is, there will be a molecule that is oxidised (the reducing agent) and a molecule that is reduced (the oxidising agent). The formation of rust is a redox reaction (Equation 1) where iron is oxidised (Equation 2) and oxygen is reduced (Equation 3). Redox potential is determined by the chemical species present, in particular, the presence of oxidising molecules. It is relevant to water management, as water inundation can lead to anoxia, which can reduce the redox potential and affect the direction of redox reactions (Figure 14).

Equation 1: simplified rust formation redox equation where Fe is iron, O_2 is oxygen, and Fe_2O_3 is iron (III) oxide. This reaction will occur spontaneously in the presence of water even though no water appears to be consumed.

$$4\mathrm{Fe} + 3\mathrm{O}_2 \rightarrow 2\mathrm{Fe}_2\mathrm{O}_3$$

Equation 2: from Equation 1, iron loses electrons, therefore it is oxidised; iron is the reducing agent in this equation. When Equation 2 and Equation 3 are combined, the 12 electrons on either side of the equation will cancel out; there is not net gain or loss of electrons.

$$4\mathrm{Fe} \rightarrow 4\mathrm{Fe}^{3+} + 12\mathrm{e}^{-}$$

Equation 3: from Equation 1, oxygen gains electrons, therefore it is reduced; oxygen is the oxidising agent in this equation. When Equation 2 and Equation 3 are combined, the 12 electrons on either side of the equation will cancel out; there is not net gain or loss of electrons.

$$3\mathrm{O}_2 + 12\mathrm{e}^{-} \rightarrow 2\mathrm{O}_3^{6-}$$

For more information on redox chemistry, consult a chemistry text such as Blackman et al. (2016).

7.4.2 Redox potential

Soil microbes are important when considering water management for ecological purposes because of the effects they have on the environment. In particular, the effect of soil oxygen levels *via* water management has a strong influence on the function and outputs of microbes (Figure 14) (Plante, 2007). During carbon cycling, photosynthesis (C reduction; O_2 oxidation) requires an electron donor (CO_2) and respiration (C oxidation; O_2 reduction) requires an electron acceptor (O_2 or others). The tendency for chemical reactions to proceed as reduction or oxidation reactions is measured by the redox potential (redox is reduction/oxidation; Eh) which is measured in millivolts (mV). Each chemical species has its own affinity for electrons, and therefore is reduced (or oxidised) at different redox potentials. Oxygen (as O_2) has a high affinity for electrons and is the primary electron acceptor in aerobic systems; the presence of free oxygen gives soils a high redox potential (Eh $\geq$ 300 mV). Once the free oxygen has been consumed, facultatively anaerobic or anaerobic microbes use NO_3^-, MnO_2, and Fe_2O_3 as electron acceptors; the reduction of these chemical species gives a moderately low redox potential (-100 < Eh < 300 mV). If nitrite (via nitrate), manganese oxide, or iron (III) oxide are not available, then anaerobic microbes will use SO_4^{2-}, and then CO_2, to produce HS^- and CH_4, respectively (Ponnamperuma, 1972); these reactions have a low redox potential (Eh < -100 mV). The hydrogen sulfide ion can then be converted to iron (II) sulfide or pyrite if exposed to iron in solution. Although the anaerobic reactions described here consume acidity (Leyden et al., 2016), anaerobic respiration also produces organic acids which may produce acidity. However, more pronounced pH changes occur if the system is returned (e.g. via wetland drying) to a high (oxidised) redox state due to the oxidation of FeS_2 to sulfuric acid (H_2SO_4) (Section 8.1.4).

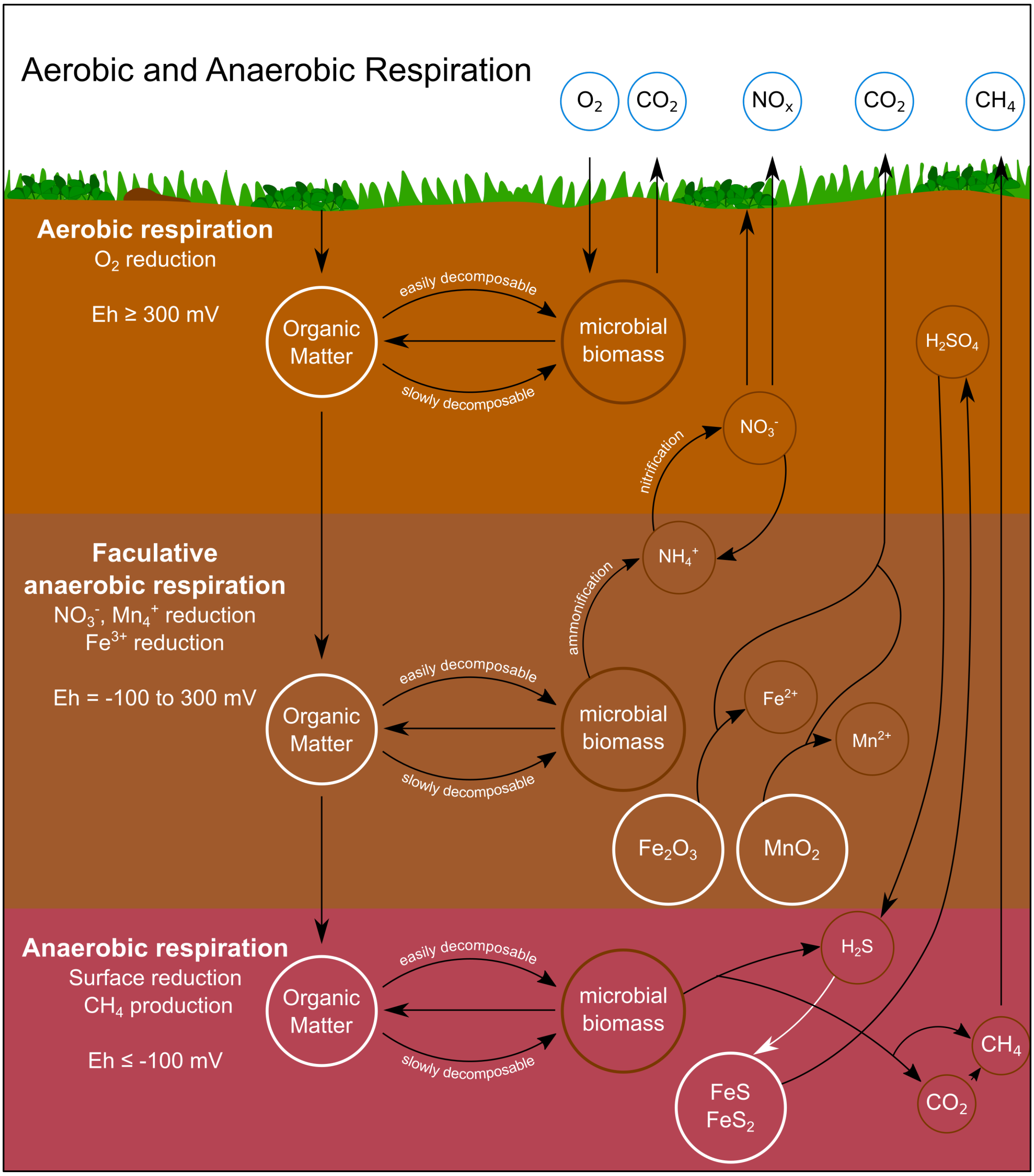

Figure 14: Respiration by aerobic, facultatively anaerobic, and anaerobic microorganisms where O_2 is oxygen, CO_2 is carbon dioxide, NOx are nitrous oxides, CH_4 is methane, H_2SO_4 is sulfuric acid, NO_3^- is the nitrate ion, NH_4^+ is the ammonium ion, Fe^{2+} is iron (II), Mn^{2+} is manganese (II), Fe_2O_3 is iron (III) oxide, MnO_2 is manganese dioxide, H_2S is hydrogen sulfide, FeS is iron (II) sulfide, and Eh represents redox potential (as measured in millivolts). Figure modified from Plante (2007) by E Stirling using Inkscape version 0.91 (The Inkscape Team, 2018).

7.4.3 Wetting and drying

Wetlands can experience wetting and drying through natural variation of water inputs and outputs, or as a result of deliberate management choices for wetland management (see Section 2 for more details). Wetting and drying wetlands affects chemical species, chemical states, and microbial activity. Adding water to a dry wetland increases microbial activity by reducing water limitation (such as desiccation) and increasing the access of microbes to organic matter (Franzluebbers et al., 2000). This increase in activity may be due to increased organic matter available for decomposition or from improved access to organic matter that has been solubilised. Inundation with water, or excessive decomposition in soils with poor aeration, can lead to the soil becoming anoxic (Figure 14). Anoxic soils may form RIS (such as pyrite) from the activities of SRB (Section 8.1). Soils that contain RIS are at risk of becoming ASS if they are then allowed to dry over long periods (Section 8.1.4). Soils that experience frequent wetting and drying cycles are less likely to develop high RIS contents and therefore less likely to become ASS during drying phases as the frequent transition from anoxic to oxic conditions prevents the accumulation of RIS (Fitzpatrick et al., 2017).

7.5 Concluding remarks on the importance of soil microbial function for water management for ecological purposes

This section addresses the need for a primer document about the importance and interaction of soil microbial function to water management for ecological purposes by providing an introduction to soil microbes, their functions, and the effect of water on those functions. This is done by introducing soil microbes (Section 7.1.1), discussing their function in soil in general (Section 7.1.2), and in wetlands in specific (7.1.3), and by introducing important microbial structures that can form in wetlands (Section 7.1.4). Microbial biogeochemical nutrient cycling is discussed for carbon (Section 7.2.1), nitrogen (Section 7.2.2), phosphorus (Section 7.2.3) and sulfur (Section 7.2.4), and methods for measuring microbial function are outlined in brief in Section 7.3. This primer includes the effects of water and the availability of oxygen on soil chemistry (Section 7.4.1), the effect of inundation or anoxia on soil chemistry (Section 7.4.2), and a brief discussion on the effect of wetting and drying on soils (Section 7.4.3).

7.5.1 Research gaps

The information provided above is only a brief introduction to the importance of soil microbes and their function in wetland soils. Primary productivity, water quality, and soil chemistry are directly affected by soil microbial activities such as nutrient cycling, organic matter decomposition, and the anaerobic production of reduced inorganic sulfur. However, this section highlights a number of gaps in the basic research literature that are relevant to the project brief, including:

- Relationships between specific microbial species or communities and nutrient cycling.
- Microbially mediated nutrient transformation fluxes in wetland soils (i.e. the rate at which nutrients change from one species to another).
- The importance of underrepresented (or those present in low abundance) microbial species on soil microbial community function.

Addressing these gaps will improve management recommendations for diagnosing potential nutrient limitations or losses, which may then affect wetland productivity and water quality.

8 Acid Sulfate Soils and soil microbial function

This section refers to Project Brief **Attachment 5** section **3.2.3 Soil Microbial Investigation - assessing microbial functioning in soils with different inundation histories/contexts** point 1: “The impact of ASS on soil microbial function”.

8.1 Development mechanisms of acid sulfate soils

8.1.1 Development of ASS: sulfate reduction/sulfidisation

Acid sulfate soils are the result of two environmental processes: sulfidisation and sulfurisation. These processes are the result of microbially enhanced redox reactions that occur in anoxic and oxic soils, respectively (Section 7.4.1). Sulfidisation is the process of reducing sulfate to RIS while sulfurisation is the process of oxidising RIS and producing sulfuric acid. In sulfidisation, elemental sulfur and metal-sulfide minerals accumulate in the soil due to a redox reaction catalysed by anaerobic sulfate reducing bacteria or archaea (SRB) (Equation 4). The organisms that catalyse this reaction (Section 8.1.2) use sulfate as the terminal electron acceptor for their cellular respiration. This reaction leads to high concentrations of RIS species including pyrite (FeS_2), iron monosulfide (FeS), greigite (Fe_3S_4), and elemental sulfur (S) (Karimian et al., 2018). The formation of iron sulfides requires a source of iron (soil minerals), sulfate (sea water or sulfur salt bearing water) and organic matter (Equation 5). If insufficient iron is available for this reaction, a build-up of sulfides may occur during sulfate reduction which may then cause a change to sulfur tolerant microbial and plant assemblages (Schoepfer et al., 2014). The effects of redox potential in relation to the production of RIS species are discussed in section 7.4 and Figure 14 (above).

Equation 4: Simplified sulfate reduction where CH_2O represents simple organic matter, SO_4^{2-} is the sulfate ion, H_2S is hydrogen sulfide, and HCO_3^- is the bicarbonate ion. This reaction is alkalising (i.e. raises the soil pH) due to the creation of bicarbonate. Sulfate reducing bacteria and archaea (SRB) are discussed in Section 8.1.2.

$$2\ \mathrm{CH_2O} + \mathrm{SO_4^{2-}} \xrightarrow{SRB} \mathrm{H_2S} + 2\ \mathrm{HCO_3^-}$$

Equation 5: Simplified iron monosufide formation where Fe_2O_3 is iron (III) oxide, SO_4^{2-} is the sulfate ion, CH_2O represents simple organic matter, O_2 is oxygen, FeS is iron monosulfide, HCO_3^- is the bicarbonate ion, and H_2O is water. This reaction is alkalising (i.e. raises the soil pH) due to the creation of bicarbonate; this reaction also generates water as a biproduct. Sulfate reducing bacteria and archaea (SRB) are discussed in Section 8.1.2. Note that this equation is an example of one pathway to iron sulfide production; other pathways are also possible.

$$9\ \mathrm{CH_2O} + 4\ \mathrm{FeOOH} + 4\ \mathrm{SO_4^{2-}} \xrightarrow{SRB} \mathrm{CO_2} + 8\ \mathrm{HCO_3^-} + 4\ \mathrm{FeS} + 7\ \mathrm{H_2O}$$

8.1.2 Sulfur reducing bacteria and archaea

Although they will only be briefly described here, SRB have been extensively studied (Rabus et al., 2013). Sulfate reducing microbes require an anoxic environment, simple organic matter, and sulfate to successfully grow and metabolise. They can use a wide range of small organic matter molecules, ranging from short chain fatty acids to aromatic hydrocarbons; SRB are reliant on other heterotrophic microbes to decompose complex organic matter such as cellulose and lignin (Muyzer and Stams, 2008). In situations where sulfate is not available as an electron acceptor for anaerobic respiration, SRB can survive by using fumarate or acetate; however, the processes is highly inefficient and provides limited energy yields (Muyzer and Stams, 2008). Although SRB compete with other anaerobic microbes for resources and electron acceptors, in the presence of sulfate SRB are highly efficient and outcompete other anaerobic microbes (Stams et al., 2003). In environments where sulfate is available but carbon resources are limiting, SRB will coexist with methanogenic bacteria, leading to the characteristic hydrogen sulfide/methane gas production of anaerobic environments (Bryant et al., 1977).

8.1.3 Sulfur sources for inland waters

The formation of sulfidic sediments *via* microbial sulfate reduction requires a source of sulfate. In coastal wetlands, sulfate enters the environment *via* seawater as oceans are a large biogeochemical pool of sulfate. While a small amount of sulfate is delivered to inland waters from precipitation and atmospheric deposition, freshwater does not typically contain high levels of sulfate (Berner, 1984). Sulfate sources for inland waters include salinisation (sulfate salts), fertiliser contamination, soil ameliorants (such as gypsum), and stream eutrophication (Lamontagne et al., 2006). Groundwater discharge is also a source of sulfate for inland wetlands (Wong et al., 2016). In Australian inland waterways, high RIS precipitation is associated with perennial saline wetlands, with less RIS in wetlands with wetting and drying cycles, or low salinity waters (Lamontagne et al., 2006).

8.1.4 Development of ASS: pyrite oxidation/sulfurisation

Reduced iron sulfide minerals can be oxidised abiotically by exposure to oxygen (Equation 6); this reaction can be enhanced by iron oxidising bacteria such as *Acidithiobacillus ferrooxidans* (Table 3). Abiotic oxidation ('sulfurisation') is rate limited by the oxidation of iron (II) to iron (III) as this oxidation step is dependent on pH and is slow under acidic conditions; the rate of oxidation can be substantially increased by acidophilic iron oxidising prokaryotes (see 8.1.5; Table 3). Once iron (III) is available, it will react with water (hydrolyse) to form iron oxides (e.g. goethite, ferrihydrite) and sulfuric acid, and with pyrite to form iron (II) and sulfuric acid. If pyrite oxidation occurs with calcium carbonate present, then sulfuric acid reacts to form gypsum, water, and carbon dioxide (Equation 7).

Equation 6: Simplified pyrite oxidation where FeS_2 is iron (II) sulfide (pyrite), O_2 is oxygen, H_2O is water, FeOOH represents goethite, H^+ is the hydrogen ion, and SO_4^{2-} is the sulfate ion; H^+ and SO_4^{2-} are the disassociated form of H_2SO_4 (sulfuric acid). The oxidation of pyrite requires both free oxygen and water, and is also described in Figure 15.

$$4\ \mathrm{FeS_2} + 15\ \mathrm{O_2} + 10\ \mathrm{H_2O} \rightarrow 4\ \mathrm{FeOOH} + 8\ \mathrm{H^+} + 4\ \mathrm{SO_4^{2-}}$$

Equation 7: Neutralisation of sulfuric acid where $CaCO_3.2H_2O$ is gypsum, H_2O is water, $CaSO_4$ is calcium sulfate, CO_2 is carbon dioxide, H^+ is the hydrogen ion, and SO_4^{2-} is the sulfate ion; H^+ and SO_4^{2-} are the disassociated form of H_2SO_4 (sulfuric acid).

$$8\ \mathrm{H^+} + 4\ \mathrm{SO_4^{2-}} + \mathrm{CaCO_3.2H_2O} \rightarrow \mathrm{CaSO_4} + 3\mathrm{H_2O} + \mathrm{CO_2}$$

An introduction of oxygen into the soil catalyses the change in microbial community from a sulfate reducing community to an iron oxidising community. This introduction of oxygen may be due to soil desiccation (from drought or drainage), soil physical disturbance (by animals, machinery, or similar), or from oxygen leaking from specialised wetland plant roots (i.e. those containing aerenchyma). Soils with a heavy texture (i.e. containing a high proportion of clay; Section 3.3) may form desiccation features such as deep cracks which permit oxygen to penetrate deep into the soil profile (Mosley et al., 2014a). Under these conditions, the transition from a circumneutral soil pH (i.e. approximately pH 7) to an extremely low soil pH (pH < 4) can be rapid and recovery times can be extremely prolonged (Mosley et al., 2014a). For example, soil pH can drop to below pH 4 during 9 weeks of aerobic incubation, and may take more than 5 years to recover after an oxidation event (Creeper et al., 2012; Mosley et al., 2017).

8.1.5 Microbial iron oxidation

The oxidation of iron from iron (II) to iron (III) is accelerated by iron oxidising prokaryotes (bacteria and archaea); At low pH, microbes can accelerate iron (II) oxidation rates by a factor of more than 10^6 (Singer and Stumm, 1970). These organisms are acidophilic microbes that may respire *via* aerobic or facultative anaerobic respiration; i.e. the microbes involved in this process can use oxygen, nitrate or iron (III) oxide during respiration (Baker and Banfield, 2003; Ilbert and Bonnefoy, 2013) (Equation 8). Iron (III) will then react with pyrite until either input is exhausted (Equation 9). Although abiotic pyrite oxidation is limited by low pH, biotic oxidation is most efficient in strongly acidic (pH 2) aerobic conditions (Singer and Stumm, 1970; Ilbert and Bonnefoy, 2013). It is for these reasons that some remediation projects using acid tolerant wetland plants have increased acidity: oxygen inputs from wetland plant roots allow more efficient microbial iron (II) oxidation (Brune et al., 2000; Michael et al., 2017).

Equation 8: Microbially catalysed iron oxidation where Fe^{2+} is iron (II), O_2 is oxygen, H^+ is the hydrogen ion (proton), Fe^{3+} is iron (III), and H_2O is water. Iron (III) can further catalyse the oxidation of pyrite via an abiotic chemical reaction.

$$2\,\mathrm{Fe}^{2+} + \mathrm{O}_2 + 4\,\mathrm{H}^+ \xrightarrow{iron\ oxidising\ prokaryotes} 2\,\mathrm{Fe}^{3+} + 2\,\mathrm{H_2O}$$

Equation 9: Oxidation of pyrite *via* the reduction of iron (III) where FeS_2 is iron (II) sulfide (pyrite), Fe^{3+} is iron (III), O_2 is oxygen, Fe^{2+} is iron (II), SO_4^{2-} is the sulfate ion, and H^+ is the hydrogen ion (proton). Note: the iron (II) produced in this reaction can then be re-oxidised per Equation 8.

$$\mathrm{FeS_2} + 14\,\mathrm{Fe}^{3+} + 8\,\mathrm{O}_2 + 2\,\mathrm{H_2O} \rightarrow 15\,\mathrm{Fe}^{2+} + 2\,\mathrm{SO}_4^{2-} + 16\,\mathrm{H}^+$$

Microbial oxidation of iron sulfide minerals is a self-reinforcing process (Figure 15); acidic conditions are generated during oxidation, which favour acidophilic iron oxidising microbes, that then increases the rate of oxidation and the degree of acidification (Baker and Banfield, 2003). Evidence for the role of microbes in accelerating iron (II) oxidation includes cell sized pits and cells in pits on pyrite surfaces (Edwards et al., 2000) and vastly increased dissolution rates when pyrite is oxidised in the presence of iron oxidising microbes (Edwards and Rutenberg, 2001), particularly when microbes are in close proximity to mineral surfaces (Larsson et al., 1993). Importantly, microbial iron (II) oxidation can occur under anoxic conditions if appropriate electron acceptors are available. This means iron (II) oxidation can occur in anaerobic conditions as the presence of nitrate (from groundwater seepage, fertilisers, or similar) can allow nitrate reducing/iron (II) oxidising facultatively anaerobic microbes to continue to oxidation of pyrite (Schaedler et al., 2018).

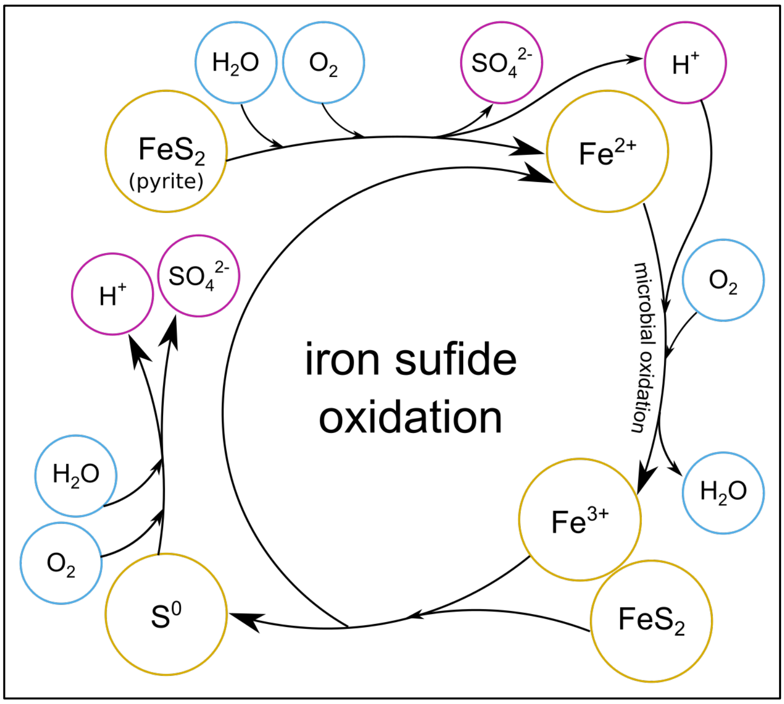

Figure 15: Iron sulfide oxidation chemical pathways where FeS_2 is iron (II) sulfide (pyrite), H_2O is water, O_2 is oxygen, SO_4^{2-} is the sulfate ion, Fe^{2+} is iron (II), H^+ is the hydrogen ion (proton), Fe^{3+} is iron (III), and S^0 is elemental sulfur. Figure made by E Stirling using Inkscape version 0.91 (The Inkscape Team, 2018).

8.2 The impact of ASS on soil microbial function

8.2.1 Acid sulfate soil microbial diversity

The development of sulfuric (pH<4) materials arising from oxidation of ASS in a wetland or agricultural system will completely change the microbial assemblages from those that thrive under circumneutral conditions to those that are tolerant to acidic conditions (i.e. acidophilic microbial communities). A change to acidophilic ASS microbes will also change the overall microbial function, discussed below in Section 8.2.2.

Acidophilic microbes are found in all three domains; however, bacteria and archaea dominate the known acidophilic microbial diversity (Quatrini and Johnson, 2018) (Table 3). Within eukarya, fungi are the dominant organisms present (Baker et al., 2009). In addition to high concentrations of acid, microbes living in ASS are also exposed to elevated concentrations of metals and metalloids, osmotic stress, and variable redox potentials. Microbial diversity in ASS is shaped by the tolerances and intolerances of microbes to these stressors. Redox potential is a strong influencer on the diversity of acidophilic microbes present as aerobic respiration will continue until all oxygen is consumed and aerobic conditions favour bacteria over archaea (Šibanc et al., 2014). Changes in pH are also a strong influencer on microbial communities; community composition changes when RIS species are oxidised (becoming more acid) and when acidic soils are neutralised (becoming more neutral) (Hedrich et al., 2011; Su et al., 2017). Community composition change initiated by pH change can lead to positive reinforcement of the pH change; for example, a decrease in pH may lead to the dominance of iron oxidising microbes that then cause a further decrease in pH (Section 8.1.5).

The limited research on microbial communities found in ASS contains microbes previously identified from acid and metal contaminated environments (Wu et al., 2015) and indicates that ASS contain more microbial diversity in the topsoil than the subsoil (Su et al., 2017). Research from acid mine drainage (AMD) shows that species richness is lower in strongly acidic environments than in environments that experience less extreme conditions (Fierer and Jackson, 2006; Denef et al., 2010). For example, a review of AMD microbial diversity found 16 genera and unique lineages, whereas agricultural soil and pasture rhizospheres had 50 and 145 genera and unique lineages, respectively (Baker and Banfield, 2003) (Table 3). Nevertheless, the organisms present in strongly acidic conditions are highly specialised and show high spatial diversity, if a range of growing conditions (such as light and resource availability) is considered. In addition, a more recent study than Baker and Banfield (2003) found up to 149 lineages present in acid mine drainage biofilms (Méndez-García et al., 2014; Goltsman et al., 2015).

Table 3: Organisms isolated from acid mine drainage (AMD) and acid sulfate soils (ASS): Their kingdom, name, energy strategy, electron strategy, and reference.

Kingdom	Organism	Energy strategy	Electron strategy	Reference
Archaea	*Acidianus brierleyi*	facultative autotroph	sulfur reduction	Konishi et al. (1995)
	Acidianus spp.	obligate autotroph	sulfur reduction	Barrie Johnson and Hallberg (2008)
	Ferroplasma acidarmanus	obligate heterotroph	iron oxidation	Jackson et al. (2007)
	Ferroplasma spp.	obligate heterotroph	iron oxidation	Barrie Johnson and Hallberg (2008)
	Metallosphaera prunae	facultative autotroph	sulfur oxidation	Liu et al. (2011)
	Metallosphaera spp.	obligate heterotroph	sulfur oxidation	Barrie Johnson and Hallberg (2008)
	Picrophilus spp.	obligate heterotroph	aerobic reparation	Fütterer et al. (2004)
	Stygiolobus azoricus	obligate autotroph	sulfur reduction	Barrie Johnson and Hallberg (2008)
	Sulfolobus metallicus	obligate autotroph	iron and sulfur oxidation	
	Sulfolobus tokodaii	obligate heterotroph	iron and sulfur oxidation	
	Sulfurisphaera ohwakuensis	facultative autotroph	sulfur reduction	
	Sulfurococcus spp.	obligate heterotroph	sulfur oxidation	
	Thermoplasma spp.	obligate heterotroph	sulfur reduction	
Bacteria	*Acidicaldus organivorans*	obligate heterotroph	sulfur oxidation	Barrie Johnson and Hallberg (2008)
	Acidimicrobium ferrooxidans	facultative autotroph	iron oxidation	Clark and Norris (1996)
	Acidiphilium spp.	facultative autotroph	sulfur oxidation	Barrie Johnson and Hallberg (2008)
	Acidithiobacillus caldus	obligate autotroph	sulfur oxidation	
	Acidithiobacillus ferrooxidans	obligate autotroph	iron and sulfur oxidation	
	Acidithiobacillus thiooxidans	obligate autotroph	sulfur oxidation	
	Acidobacterium capsulatum	obligate heterotroph	iron reduction	Kishimoto et al. (1991)
	Acidocella spp.	obligate heterotroph	iron reduction	Barrie Johnson and Hallberg (2008)
	Alicyclobacillus spp.	facultative autotroph	iron and sulfur oxidation	
	Ferrimicrobium acidiphilum	obligate heterotroph	iron oxidation	
	Ferrithrix thermotolerans	obligate heterotroph	iron oxidation	
	Ferrovum myxofaciens	obligate autotroph	iron oxidation	Moya-Beltrán et al. (2014)
	Hydrogenobaculum acidophilum	obligate autotroph	sulfur oxidation	Barrie Johnson and Hallberg (2008)
	Leptospirillum spp.	obligate autotroph	iron oxidation	Quatrini and Johnson (2018)
	Sulfobacillus spp.	facultative autotroph	iron and sulfur oxidation	Barrie Johnson and Hallberg (2008)
	Thiobacillus ferrooxidans	obligate autotroph	iron oxidation	
	Thiomonas spp.	facultative autotroph	iron and sulfur oxidation	Baker and Banfield (2003)

8.2.2 Acid sulfate soil microbial function

In addition to the effects of ASS development on iron and sulfur cycling (Section 8.1), the development of strongly acid conditions also affects community structure, carbon cycling, nitrogen cycling and phosphorus availability. In addition to the effects of pH on microbial diversity discussed above (Section 8.2.1), microbial growth can be inhibited or prevented by metal toxicities caused by low pH (Harrison et al., 2004). Although abiotic degradation of organic matter may occur due to acid driven reactions with organic molecules, microbial decomposition of complex organic molecules is inhibited by extremely low pH conditions (Jugsujinda et al., 1996). The decomposition of simple organic molecules can continue in strongly acid conditions as heterotrophic acidophilic microbes are able to use them as an energy resource for growth and metabolism (Kögel-Knabner et al., 2010). This continued consumption of simple organic matter can lead to carbon limitation in ASS, which may then affect the availability of organic matter for SRB during remediation (Mosley et al., 2017).

Carbon limitation can arise in ASS due to a lack of organic inputs into the soil, as the quality and quantity of organic matter available to microbes is largely determined by plant primary productivity (Kang and Stanley, 2005). Biomass production in ASS is directly affected by plant tolerances to acidic conditions and metal toxicities. There are a range of wetland and agricultural plants that have been shown to survive irrigation with AMD; however, they also bioaccumulate metal contaminants during this treatment. While bioaccumulation is useful for bioremediation of metal contamination, it is not desirable in agricultural systems (Lin et al., 2005; Sheoran and Sheoran, 2006), as it can be toxic to certain downstream plant species or their associated microbes.

The effect of ASS on nitrogen cycling is unclear. Nitrogen mineralisation can occur in a wide range of soil pH, including sufficient mineralisation for plant needs in low pH acid soils (Hoa et al., 2004); furthermore, pH does not have a clear effect on nitrification (Booth et al., 2005). The first reaction in nitrification (Equation 10) contributes to the production of acidity in soils, but some species of nitrifying microbes (such as *Nitrosomonas* spp.) are acid intolerant. Although N mineralisation occurs in acid conditions, it is not clear how soil microorganisms affect this process (Kowalchuk and Stephen, 2001). If ASS are returned to anoxic conditions (Figure 11) and suitable carbon resources are available, denitrification may occur and lead to N losses from the system *via* leaching or as nitrogen gases (Kögel-Knabner et al., 2010).

Equation 10: Nitrification conversion of ammonia to nitrite where NH_4^+ is the ammonium ion, O_2 is oxygen, NO_3^- is nitrate, H_2O is water, and H^+ is the hydrogen ion (proton). Note that nitrification produces 2 moles of acidity for every mole of ammonium.

$$2\mathrm{NH_4^+} + 3\mathrm{O_2} \rightarrow 2\mathrm{NO_3^-} + 2\mathrm{H_2O} + 4\mathrm{H^+}$$

In contrast, the effect of ASS on phosphorus cycling appears relatively simple. Strongly acid conditions reduce phosphorus availability in the soil as the presence of iron (and aluminium) ions in solution cause dissolved phosphate ($H_2PO_4^-$) to form insoluble phosphate precipitates. Phosphorus may be a limiting nutrient to both plants and the microbial biomass in ASS (Yampracha et al., 2005).

8.3 Concluding remarks on acid sulfate soils and soil microbial function

This section discusses the development of acid sulfate soil and its effects on soil microbial function. The two development stages of acid sulfate soil are outlined: sulfate reduction (Section 8.1.1) and pyrite oxidation (Section 8.1.4), with brief discussions on the groups of microorganisms responsible for catalysing each stage: Sections 8.1.2 and 8.1.5, respectively. The impact of the development of acid sulfate soil on microbial function is discussed in relation to both microbial diversity (Section 8.2.1) and overall function (Section 8.2.2).

This section illustrates the importance of water management, organic matter availability, soil pH on wetland soils and microbial community function. Waterlogged soil that is exposed to sulfur and has a source of simple organic carbon compounds is likely to accumulate reduced inorganic sulfur due to the activities of sulfate reducing bacteria and archaea. In Australian inland wetlands, sulfur is sourced from saline waters and agricultural inputs and soils with high pyrite contents are associated with saline waters. When exposed to oxygen, pyrite oxidation leads to a rapid decrease in soil pH, which allows the establishment of acidophilic iron and sulfur oxidising microbial communities. Once these communities are established, they increase the rate of pyrite oxidation and actively increase the soil acidity, preventing non-acidophilic microbe growth and reproduction; microbially mediated pyrite oxidation can continue even if the soil is re-saturated.

The transition from a soil that contains pyrite to an acid sulfate soil changes the microbial assemblage to one able to withstand strongly acid soils, and therefore changes the microbial community function to one that is able to withstand strongly acid soils. Due to the difficulties that plants face in strongly acid soil (nutrient limitation, nutrient toxicities and metal toxicities), carbon inputs are low and the microbial biomass can become strongly carbon limited. While nutrient cycling appears to continue to a degree it is unclear what the effect of extremely low pH is on nitrogen and phosphorus cycling.

8.3.1 Research gaps

Although there has been a moderate amount of research into the biology, ecology, and relationships of microorganisms in acid mine drainage, the literature for acid sulfate soils is relatively slim. Specific research gaps highlighted by this review include:

- The effect of strongly acid conditions on nitrogen, phosphorus, and other nutrient cycles.
- The identification of soil microorganisms responsible for iron and sulfur oxidation in soils and management techniques to reduce their activity.
- Microbial functional diversity in acid sulfate soils.
- The effect of metal toxicities on microbial activity.
- Diversity of viable non-acid sulfate soil microbes that can survive strongly acid conditions.
- Recolonisation rate and pattern of non-acid sulfate soil microbes and mechanisms to improve their success rate.
- Sources of simple organic matter in acid sulfate soils and their effect on acid production.

Addressing these research gaps will improve management recommendations for diagnosing potential nutrient limitations or losses, which may then affect wetland productivity and water quality. Further information on these topics may also help inform management decisions when faced with soils in the process of sulfurisation or soils which have become sulfuric.

9 Nutrient availability and soil microbial composition in ASS

This section refers to Project Brief **Attachment 5** section **3.2.3 Soil Microbial Investigation - assessing microbial functioning in soils with different inundation histories/contexts** point 2: “Soil microbial community composition in ASS affected soils relevant to nutrient concentration and availability”.

9.1 The influence of nutrient inputs on soil microbial community composition in ASS

Oxidation of pyritic material leads to solubilisation of calcium, iron, aluminium, magnesium, zinc, copper, manganese, and to decreased phosphorus availability (Golez and Kyuma, 1997). This can lead to both metal toxicities and deficiencies depending on whether the solutes remain in the soil or are leached from the soil. Of the solutes that remain in the soil, increased availability of aluminium, copper and manganese, which can lead to nutrient toxicity in plants (Golez and Kyuma, 1997; Panhwar et al., 2014a). Increased availability of these metals may have a similar effect on non-adapted microbes due to metal toxicities; however, soil microbes found in ASS and AMD are tolerant to high concentrations of metal ions (Baker and Banfield, 2003; Harrison et al., 2004; Wu et al., 2013). The low availability of these nutrients and micronutrients can limit plant growth if the soils are leached.

Although nutrients may be limiting in ASS, the primary variables controlling microbial community composition are pH and redox potential (Section 7.4.2). Management techniques for increasing pH in ASS include organic matter amendment or liming or both. Organic matter quality and nutrient content influence the microbial response to OM addition (Section 9.1.2). The purpose of amending ASS with organic matter is to enhance the growth of desirable microorganisms such as sulfate reducing bacteria and archaea (SRB; Section 8.1.2). In this case, it is most likely the addition of carbon resources rather than nutrients that are influencing microbial communities. Adding mineral sources of nutrients (such as N and P) may also improve soil recovery after liming (Section 9.1.4).

9.1.1 Native organic matter in ASS

Native organic matter refers to organic matter that exists within the soil without any management additions or amendments. It is one of the resources available to the microbial biomass, providing energy and nutrients for microbial activity and growth. Other sources of organic matter for ASS include allochthonous dissolved organic matter and particulate organic matter that may percolate through or be deposited on ASS during flood events (Kang and Stanley, 2005). The role of organic matter in ASS is multifaceted: under anoxic and hypoxic conditions, OM provides resources for SRB and other reducing bacteria that then actively increase pH *via* the consumption of protons; under oxic conditions, OM buffers pH against further decreases (Jayalath et al., 2016a). At sites where pH in ASS has not increased after re-inundation, it is possible that the native organic matter is not sufficient to support SRB (Creeper et al., 2015); in these cases, organic matter may be added to sites to improve remediation. When using organic matter for remediation, quality (i.e. type of carbon and nutrient contents) has a greater effect than quantity added (Jayalath et al., 2016c; Kölbl et al., 2017).

9.1.2 Microbial responses to organic matter addition

Soil pH in ASS can be increased *via* the addition of organic matter. The purpose of adding organic matter is to stimulate the growth of SRB (Section 8.1.2). Sulfur reducing bacteria and archaea are not active in ASS during the oxidising phase (Section 8.1.4) as they require anoxic conditions (Ward et al., 2004) and are not active at strongly acid pH (Creeper et al., 2015). In addition to these two limitations, SRB are less competitive than nitrate reducing bacteria in the presence of nitrate (Yuan et al., 2015b), leading to decreased sulfate reduction when nitrate is present. Sulfur reducing bacteria and archaea require organic matter that is highly biodegradable (Kölbl et al., 2017) but growth is not enhanced by simple sugars when added in isolation (Jayalath et al., 2016a).

A variety of organic matter amendments have been tested for ASS remediation in laboratory incubations and in field applications. Tested amendments included waste organic matter such as sawdust, dairy waste, chicken manure and biosolids (Fanning et al., 2004; Zhang and Wang, 2014), and also included glucose, wheat straw, pea straw, *Pennisetum clandestinum* (kikuyu grass), and *Phragmites australis* (Inubushi et al., 2005; Sheoran and Sheoran, 2006; Jayalath et al., 2016a; Yuan et al., 2016). In most cases, the addition of organic matter increased pH under anoxic conditions, and reduced the degree of acidification in oxic conditions. The ratio of C to N (C:N ratio) has been linked to microbial activity in multiple experiments, with low C:N (i.e. relatively more N) amendments stimulating microbial activity more than high C:N amendments. The inhibitory effect of nitrate was also overcome by higher application rates of organic matter (40 g organic matter kg^{-1} soil) (Yuan et al., 2015b).

Although the literature on microbial responses to organic matter is lacking information on microbial functional responses, community structure responses, and diversity responses, the literature available suggests that the effect of adding organic matter to ASS is twofold (Inubushi et al., 2005). Firstly, organic matter abiotically buffers or neutralises pH *via* binding protons to organic anions (Equation 11) or proton consumption during the decarboxylation of organic anions (Equation 12). Secondly, organic matter increases microbial activity of SRB by reducing the effects of metal toxicity, increasing pH, and generating anoxic zones (Rigby et al., 2006; Zhang and Wang, 2014; Michael et al., 2015). In this case, anoxia can be formed even in soils that are not saturated as organic matter decomposition consumes oxygen and can cause localised anoxia (Brune et al., 2000; Fitzpatrick et al., 2015).

Equation 11: pH buffering *via* proton binding to carboxylate ion where R indicates an organic molecule, COO^- is the carboxylate ion, H^+ is the hydrogen ion (proton), and COOH is carboxylic acid. This reaction is easily reversible depending on the pH of the reaction solution.

$$R\text{-COO}^- + \text{H}^+ \leftrightarrow R\text{-COOH}$$

Equation 12: pH neutralisation *via* decarboxylation of organic matter where R indicates an organic molecule, COO^- is the carboxylate ion, H^+ is the hydrogen ion (proton), H is a terminal hydrogen, and CO_2 is carbon dioxide. This reaction is not easily reversible and generally requires the carbon dioxide to be re-reduced via photosynthesis or similar.

$$R\text{-COO}^- + \text{H}^+ \rightarrow R\text{-H} + \text{CO}_2$$

Although adding organic amendments may increase the rate or degree of ASS recovery, there are further factors to consider before amendment. In several cases, adding organic matter to ASS increased acidity or was ineffective at increasing pH; plantings of wetland plant species can also lead to an increase in pyrite oxidation due to root inputs of oxygen (Michael et al., 2017). Organic matter than is rich in nitrogen (i.e. has a low C:N) can lead to increased nitrate in downstream waters and in groundwater that may become an issue for drinking water (Fanning et al., 2004). Organic amendments can also lead to increased dissolved organic carbon; DOC is considered a pollutant in freshwater systems and can lead to waterway eutrophication (Zhang and Wang, 2014).

9.1.3 Biochar

Similar to the effects of organic amendments discussed previously, biochar can also be used in the remediation of ASS. Biochar is created by the pyrolysis of organic matter under oxygen limiting conditions with the intent to make a highly carbon rich substrate. It has a high surface area, high porosity and variably charged functional groups that may immobilised metal ions, improve water holding capacity, and increase pH in soils and on mine waste rock (Anawar et al., 2015) and in acid drainage (Mosley et al., 2015). Biochar addition may alter microbial activity and diversity; however, it is not clear if this leads to 'healthier' soils (Pietikäinen et al., 2000). Similar to liming, biochar addition is most effective in nutrient limited systems when added with fertiliser (Beesley et al., 2013); however in wetland systems receiving allochthonous nutrients, additional fertiliser would not be recommended. In addition to neutralising present acidity, biochar added in high concentration is capable of preventing further acidification of Fe^{3+} oxidation by absorbing ions to negatively charged functional groups (Anawar et al., 2015). Biochar also reduces the negative effects of saline soil on growth and nutrition of some plants, which may be beneficial for ASS restoration (Drake et al., 2016). However, similarly to organic matter amendments, dissolved organic molecules may leach into adjacent water bodies and decrease water quality (Anawar et al., 2015).

9.1.4 Mineral nutrients

Of the research published, mineral nitrogen added in isolation is ineffective at treating ASS (Michael et al., 2016). Mineral nutrients such as nitrogen and phosphorus may be beneficial to plants when added during liming (Ren et al., 2004). However, it seems unlikely that mineral nutrients added in isolation would enhance microbial growth or activity, especially if sufficient organic matter is already available.

9.2 Concluding remarks on nutrient availability and soil microbial composition in acid sulfate soils

This section discusses soil microbial community composition in acid sulfate soils when considered through the lens of soil nutrient content and availability. This includes a discussion on native organic matter (Section 9.1.1), microbial responses to organic matter (Section 9.1.2) and a brief outline of the effect of adding biochar (Section 9.1.3) or mineral nutrients during remediation procedures (Section 9.1.4).

One of the primary issues in acid sulfate soils is that the extremely low soil pH leads to solubilisation of elements that are otherwise non-soluble or have low solubility at neutral pH. These elements may then be present in high enough concentrations to cause toxicity, or may be leached away leading to nutrient deficiencies. In both cases, microbes and plants struggle to survive in such stressful conditions. Waters that have passed through acid sulfate soils may also contain toxins that are harmful to terrestrial and aquatic animals. Native organic matter is organic matter that has accumulated in the soil through the normal means of primary productivity of native species, their excretions, and decomposition. In wetlands where re-inundation did not lead to the desired remediation outcomes, it is suspected that the native organic matter was either insufficient or in the wrong form for sulfate reducing bacteria and archaea to use for growth and reproduction.

Because native organic matter may be limiting the abundance of microbes responsible for converting sulfate into pyrite, research has been conducted into the effectiveness of adding a variety of organic amendments to acid sulfate soils to improve remediation outcomes. From this research, it appears organic matter can be used to initially increase soil pH, which then allows the establishment of sulfate reducing microbes. It also appears that the organic matter nutrient content is an important variable, as amendments with a greater nitrogen content appear to be more successful than amendments with low nitrogen content. While adding mineral nutrients during liming or with biochar amendments may be useful for plant growth, nutrients appear to have no beneficial effect on acid sulfate soil remediation when added in isolation.

9.2.1 Research gaps

The literature on the effects of native and added organic matter on microbial composition and function in acid sulfate soils is limited. Gaps identified in this review include:

- Best management practices for field application of organic matter.
- Minimum application rates for successful acid sulfate remediation.
- Off-site impacts of organic matter applications.
- In soils where adding organic matter has no net positive effect: investigating alternative amendment options.
- Microbial functional responses to organic matter addition in acid sulfate soils.
- The effects of adding mineral nutrients (or otherwise manipulating nutrient content) to organic matter used for remediation purposes.
- The effect of biochar on microbial community structure and diversity in acid sulfate soils.

10 Glossary

Abiotic: Not biological; not derived from living organisms.

Absorb: To hold (a substance) within an organism or other structure. *See: 'sorb'.*

Acid: A molecule which can donate a proton or accept an electron pair in chemical reactions. Solutions or soils with pH<7.

Acidophile: An organism which can tolerate or thrive in strongly acidic environments.

Adsorb: To hold (a substance) on the surface of a molecule or other structure. *See 'sorb'.*

Aerenchyma: Soft root tissue contain air spaces found in aquatic plants to assist with root respiration.

Aerobic environment: An environment that has free oxygen available for chemical reactions. *See: aerobic respiration, obligate aerobe. Also called: oxic environment or oxic conditions.*

Aerobic respiration: Cellular respiration using free oxygen undertaken in an aerobic environment. *See: aerobic environment, obligate aerobe.*

Aggregate: A self-forming soil structure composed of primary particles and organic matter. May present as a variety of shapes ranging from spheroidal to lenticular and a range of surface types ranging from angular to smooth. Aggregates may be composed or microaggregates; i.e. smaller aggregates. *See: organic matter, primary particle, soil structure.*

Alkaline: A molecule which can accept a proton or donate an electron pair in chemical reactions. Solutions or soils with pH>7.

Allochthonous organic matter: Organic matter sourced from outside of the defined space. *See: autochthonous organic matter, organic matter.*

Aluminosilicate: A silicate mineral with aluminium substituted into the crystal structure. Examples: feldspar, kaolinite.

Ammonification: Microbially mediate decomposition of organic matter leading to the production of ammonia or ammonium compounds.

Anaerobic environment: An environment without free oxygen available for chemical reactions. *See: anaerobic respiration, facultative aerobe, obligate aerobe. Also called: anoxic environment or anoxic conditions.*

Anaerobic respiration: Cellular respiration without the use of free oxygen undertaken in a anaerobic environment. *See: anaerobic environment, facultative anaerobe, obligate anaerobe.*

Anion: A negatively charge ion.

Anoxic conditions: *See: anaerobic environment.*

Arbuscular mycorrhizal fungi: Fungi with symbiotic relationships with plants capable of exchanging plant synthesised resources for nutrients extracted by the fungus.

Archaea: One of three domains of life (archaea, bacteria and eukarya). Organisms in archaea are unicellular microbes of similar size to bacteria that similarly lack a nuclear membrane. Archaea are adapted to a wide range of benign and extreme environments.

Assimilate: To absorb nutrients for the purpose of creating new organic compounds for cellular growth. Example: sulfate reduction to produce sulfur containing proteins. *See: dissimilatory sulfate reduction.*

Autochthonous organic matter: Organic matter sourced from within the defined space. *See: allochthonous organic matter, organic matter.*

Autotroph: An organism that is able to form organic substances from simple inorganic substances such as carbon dioxide. Energy for this process is captured from abiotic sources such as light, heat, or oxidation.

Available nutrient: Nutrients in a form that in a for which can be absorbed by plant roots.

Bacteria: One of three domains of life (archaea, bacteria and eukarya). Organisms in bacteria are unicellular microbes of similar size to archaea that similarly lack a nuclear membrane.

Bioaccumulation: Absorption of substances in organisms that is faster than the substance can be lost *via* excretion or catabolism.

Biochar: Charcoal made by the pyrolysis of organic matter under oxygen limited conditions.

Biofilm: A thin layer of microorganisms adhering to a surface.

Biogeochemical cycling: Transfer of elements between living systems and the environment *via* biotic or abiotic means.

Biosolids: Organic matter sourced from sewerage.

Buffering capacity (soils): The degree to which a soil can self-regulate internal chemistry against external chemical perturbations.

Carbon fixation: The incorporation of carbon into organic molecules. Example: photosynthesis.

Cation: A positively charged ion.

Chemoautotroph: An organism that is able to form organic substances from simple inorganic substances using energy derived from chemical processes.

Chemotroph: An organisms that obtains energy from the oxidation of electron donors in their environments. This process must have an available electron acceptor to proceed. *See: electron acceptor, electron donor.*

Circumneutral: pH that is approximately neutral (pH=7).

Community function: Net processes as a result of the activity of microbes. Example: nutrient cycling.

Decomposition: Decay of organic matter to its basic components.

Denitrification: Decomposition of nitrate to gaseous oxygen and nitrogen *via* nitrite, nitrogen dioxide and nitrous oxide through the activities of anaerobic microbes.

Dissimilatory sulfate reduction: Sulfate reduction by anaerobic microbes for the purpose of energy generations.

Dissolved organic matter: Organic matter smaller than particulate organic matter that is capable of dissolving in water.

Electron acceptor: A chemical that accepts electrons during a redox reaction. Electron acceptors are oxidising chemicals that become reduced during a redox reaction. Example: O_2 is an oxidising chemical that is a common electron acceptor. During respiration, organic matter is oxidised and O_2 gains 2 electrons to form CO_2 (a reduced form of oxygen).

Electron donor: A chemical that donates electrons during a redox reaction. Electron donors are reducing chemicals that become oxidised during a redox reaction. Example: $C_6H_{12}O_6$ is a reducing chemical that is a common electron donor. During respiration, organic matter is oxidised and $C_6H_{12}O_6$ donates 12 electrons to form $6CO_2$ (an oxidised form of carbon).

Enzyme: A protein which acts as a catalyst for a specific chemical reaction.

Eukarya: One of three domains of life (archaea, bacteria and eukarya). Organisms in eukarya may be unicellular or multicellular and have cells which contain a nuclear membrane. *See: eukaryote.*

Eukaryote: An organism belonging to the domain eukarya. Example: *Phragmites australis*

Extracellular enzymes: Enzymes which are produced within the cell and then released to the environment to catalyse reactions outside of the cell. *See: enzyme.*

Facultative anaerobe: A microbe that uses aerobic respiration if oxygen is available, but is capable of using anaerobic respiration if required.

Fungal mat: A complex and interconnected series of collocated microbial communities.

Fungi: An organism kingdom from the domain eukarya including mushrooms, moulds, and yeast.

Heterotroph: Microbes that capture energy through the decomposition or digestion of organic matter.

Hydraulic conductivity (soil): A measure of the speed in which water can pass through a soil.

Immobilisation: The process where nutrients are held within the microbial biomass and are therefore unavailable for other processes in the environment.

Inoculant (microbial): A substance containing microbial spores or active microbes that can be applied to soil to introduce new microbes to a system.

Inorganic nutrient: Nutrients in their elemental form (such as S^0) or held within a molecule that does not contain carbon (such as NH_4).

Labile: Simple organic matter that is decomposable up to 1-2 years after application.

Leaching: The removal of nutrients, toxins, or other substances *via* the movement of groundwater.

Methanogenic microbe: Anaerobic microorganisms from the domain archaea that produce methane as a biproduct of cellular respiration.

Microbe: *Also called: microorganism*

Microbial activity: A measure of the biproducts of microbial processes in order to compare microbial communities under different conditions. Examples: soil respiration; enzyme activity.

Microbial biomass (soil): A measure of the mass or concentration of microbes in a known mass or volume of soil.

Microbial biomass turnover: A process in which the entire microbial biomass has died and been replaced by new cells.

Microbial community: Groups of microbes living in the same general location which may or may not interact with each other.

Microbial diversity: A measure of the range of microbial species or strains present within an environment.

Microorganism: An organism which usually requires a microscope to observe. May be unicellular or multicellular. *Also called: microbe.*

Mineral precipitate: A mineral that forms from a solute due to a change in the abiotic environment. Examples: salt crystals forming as water evaporates; iron minerals forming due to pH changes.

Mineralise: The process in microbial nutrient cycling where mineral forms of nutrients are released from the microbial biomass as a biproduct of organic matter decomposition.

Necromass: The dead portion of the microbial biomass.

Neutralisation (acid): The process of removing protons (or acidity) from a solution *via* the addition of alkaline (or basic) substances.

Nitrification: The microbially mediated formation of nitrate from ammonium.

Nitrogen fixation: The incorporation of atmospheric nitrogen into compounds available to living organisms.

Nutrient (soil): An element (except C, H and O) required for growth or metabolism of living organisms.

Obligate aerobe: An organism which can only survive by using aerobic respiration. *See: aerobic environment, aerobic respiration, facultative anaerobe.*

Obligate anaerobe: An organism which can only survive by using anaerobic respiration. *See: anaerobic environment, anaerobic respiration, facultative anaerobe.*

Organic matter: Organic molecules (i.e. containing carbon) sourced from living or dead organisms. Organic matter covers all size ranges from single molecules through to entire organisms.

Osmosis: The diffusion of water across a semi permeable membrane in response to a concentration gradient.

Oxic conditions: *See: aerobic environment.*

Oxidation: A redox reaction in which electrons are donated from one chemical species to another. *See: electron acceptor*

pH: A measure of the concentration of hydrogen ions in a solution. pH=7 is neutral; pH<7 is acid; pH>7 is alkaline.

Phyllosilicate: Minerals composed of interlayered sheets of interconnected SiO_4^{-4} tetrahedral with varying levels of aluminium and other metal substitutions. 'Phyllo' refers to the overall structure of the sheets, which resembles phyllo pastry. Example: clay minerals.

Primary particles (soil): Mineral particles including clay, silt, and sand.

Prokaryote: Unicellular organisms lacking a nuclear membrane and specialised organelles.

Protist: An organism from the domain eukarya that does not belong to Animalia, Fungi, or Plantae. Although these organisms do not form a kingdom, they are often grouped together out of convenience.

Proton: A hydrogen ion (H^+).

Recalcitrant: Organic matter that is difficult to decompose either due to its inadequate nutrient content or inaccessible size. Example: cellulose.

Redox reaction: A chemical reaction where there is an exchange of electrons from one chemical species to another. Although they may be called a 'reducing reaction' or an 'oxidising reaction' both reduction and oxidation happen simultaneously within the reaction. Whether it is called a reducing or oxidising reaction is determined by the chemical species of interest.

Reduce: A redox reaction in which electrons are donated from one chemical species to another. *See: electron donor, redox reaction.*

Respiration (cellular): The process through which chemical energy is gained from resources to allow cellular metabolism to proceed.

Rhizosphere: Soil in close proximity to plant roots which is affected by root growth, respiration, nutrient exchange and exudates.

Salinity: A measure of the salt concentration of soils or water.

Sorb: To absorb or adsorb one substance into or onto another structure.

Soil structure: A description of the degree to which a soil self-organises into aggregates and peds of certain size and shape. Unstructured soils have no discernible aggregation and may be single grained or massive. *See: aggregate*

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