Plants with modified anatomical structures capable of oxygenating the rhizosphere are threats to sulfidic soils under varying soil moisture regimes

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Abstract. Michael PS. 2020. Plants with modified anatomical structures capable of oxygenating the rhizosphere are threats to sulfidic soils under varying soil moisture regimes. Asian J Agric 4: 87-94. Acid sulfate soils (ASS) are naturally occurring soils, sediments or substrates formed under waterlogged, reducing conditions. These soils either contain sulfuric acid or have the potential to form it, in an amount that can have detrimental impacts on the environment. In general, ASS with sulfuric materials and that have acidified through oxidation of pyrite are referred to as sulfuric soils. ASS with sulfidic materials that contain pyrite and have the potential to acidify when exposed to air are referred to as sulfidic soils. In an undisturbed state below the water table, the sulfidic soils are benign, unless exposed due to various natural processes or anthropogenic activities. This study examined the importance of organic matter addition, plant macrophytes and turnover of organic matter from the plant macrophytes co-existing on pH, redox and sulfate content of sulfidic soil under flooded conditions. In almost all cases, organic matter without plants induced ameliorative effects. Presence of plants led to higher Eh values, low pH and higher sulfate contents, and acidified the sulfidic soil.

Keywords: Eh, live plants, organic matter, pH, sulfate content, sulfidic soil

INTRODUCTION

Acid sulfate soils with sulfuric (pH<4) and sulfidic (pH>4) materials are widely distributed globally and commonly associated with lakes, rivers and wetlands (Fitzpatrick et al. 2009; Baldwin and Fraser 2009). The global distribution of ASS is shown in Figure 1. When submerged, these soils pose no problem because sulfides are retained in the reduced state (Michael et al. 2015). The sulfidic soil formation process is shown in (1) (Michael 2013). However, when exposed to falling water levels, e.g. during a drought event (Hanhart et al. 1997), the sulfides are oxidized and lead to generation of sulfuric acid. This process is shown in (2) (e.g. Ahern et al. 2004; Buschmann et al. 2008; Fitzpatrick et al. 2010; Michael et al. 2012).

In aerobic soils, cellular respiration of plant roots is supported by oxygen that reaches the rhizosphere through porous soils or via channels created by roots (e.g. Michael et al. 2017). Under anaerobic soil conditions (e.g. flooded), adapted plants use specialized aerenchymatous structures to transport oxygen from the shoots to support root respiration (Armstrong et al. 1996). The presence of an adequate amount of oxygen in the rhizosphere of plants, through whatever mechanism, offers various advantages in almost all soil types except ASS. In ASS, oxygen causes the oxidation of sulfidic soil materials (pH>4), which leads to the production of sulfuric acid (pH<4) (Pons 1973). Under reducing soil conditions, transportation of oxygen into the rhizosphere of plants in sulfidic soil by aerenchymatous structures would lead to oxidation of sulfidic sediments and sulfide-bearing minerals, generating sulfuric acidity (Reid and Butcher 2011).

We have reported in several recent studies that incorporation of various forms of dry organic matter such as dry leaves of common reed (Phragmites australis) (Michael et al. 2015), lucerne (Medicago sativa) hay and pea (Pisum sativum) straw (Michael et al. 2016) generates reducing soil conditions that favor reduction of sulfate and increase the pH. The magnitude of the changes in pH was also dependent on the nitrogen content of organic matter (Michael et al. 2016; Michael 2020a). Under natural conditions, plant turnover adds decaying organic matter so that both live and dead plant material co-exist in or on the soil, in varying proportions (Michael et al. 2017), influence microbial activity in the rhizosphere and alter the chemistry (Jayalath et al. 2016; Michael and Reid 2018).

In a similar study, Reid and Butcher (2011) investigated the effects of several plants with shallow rooting systems on ASS pH and found that the effects varied depending on the plant types and the depth of root penetration. However, two other important aspects of soil chemistry (redox potential and sulfate content) that are strongly associated with pH changes and influenced by plant roots were not investigated. One of these plants, Phragmites (common reed) is able to grow in highly acid soils (pH optimum 2–8) and thrive in highly reduced soil conditions. In addition to the extensive rooting systems, Phragmites has self-mulching effects due to rapid turnover of organic matter either by the leaves on the surface or by root decay.
In addition, the plant possess modified anatomical structures capable of transporting oxygen into the rhizosphere (Marks et al. 1994), oxidizing sulfidic minerals present in sulfidic soil and generating sulfuric acidity, making it an ideal plant to assess the effects on ASS chemistry.

This study investigated the importance of organic matter addition, plant macrophytes and turnover of organic matter from the plants co-existing on pH, redox and sulfate content of sulfidic soil under flooded conditions.

**MATERIALS AND METHODS**

**Soil**

The ‘sulfidic soil’ was collected from a ‘sulfuric subaqueous clayey soil’ (Fitzpatrick 2013) at a depth of approximately 1 m in the Finniss River in South Australia at Wally’s Landing (35°24′28.28″S; 138°49′54.37″E). Details on soil classification using the Australian ASS Identification Key (Fitzpatrick et al. 2008) and Soil Taxonomy (Soil Survey Staff 2014) is given in Michael et al. (2016). In addition, comprehensive lists of references containing further information on the soil morphology and geochemistry prior to rewetting (i.e. sites AA26.3 and FIN26) in Fitzpatrick et al. (2009) and after reflooding are given in Table 1 of Michael et al. (2015; 2016; 2017). The ‘sulfidic material’ (Soil Survey Staff 2014) used in this study is representative of typical global inland and coastal wetlands, in terms of sulfidic and organic matter content.

The pH of the freshly collected sulfidic material measured in water 1:5 (pH<sub>w</sub>) was 6.7. The water holding capacity based on wet and dry weight was estimated to be 49%. The estimation was made by setting soil samples at 100% field capacity after soaking in water and draining through a filter overnight. These soils were weighed to obtain the wet weight, and oven-dried for 3 hours, then microwaved for 30 seconds to ensure removal of any residual moisture and reweighed to obtain a final dry weight. The residual organic matter content estimated using the weight loss-on-ignition method (Schulte and Hopkins 1996) was 10.6%. The presence of sulfidic materials (minerals) capable of producing sulfuric acidity was measured by treating 1 g of sulfidic soil with 5 ml of peroxide (1:5 w/w) as per Ahern et al. (2004). The pH following peroxide treatment (pH<sub>ox</sub>) was 1.4, a strong indication of high amounts of oxidizable sulfides (Sullivan et al. 2009).

**Organic matter**

To use as organic matter, the first three younger and fully open leaves of *P. australis* were collected and prepared as previously described in various studies (Michael et al. 2016). All the leaves were chopped into pieces, air-dried overnight under room temperature and then oven dried at 60 °C for three days. The dry pieces were finely chopped using an electric blender to pass

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\begin{align*}
\text{Fe}_2\text{O}_3(s) + 4\text{SO}_2^{-}(aq) + 8\text{CH}_2\text{O} + \frac{1}{2}\text{O}_2(g) & \rightarrow 2\text{FeS}_2(s) + 8\text{CHO}_3^{-}(aq) + 4\text{H}_2\text{O}(aq) \quad (1) \\
\text{FeS}_2(s) + 3\frac{1}{2}\text{O}_2(g, aq) + \text{H}_2\text{O} & \rightarrow \text{Fe}^{2+}(aq) + 2\text{H}^+(aq) + 2\text{SO}_4^{2-}(aq) \quad (2)
\end{align*}
\]
through a ≈0.5 mm sieve. The nitrogen content of the organic matter analyzed by ICP-OES using 0.5 g samples (n=3) was estimated to be 3.7%. The carbon content can be approximated to be similar to grass (leaf) clippings from the data in Kamp et al. (1992).

**Plant establishment**

The Phragmites plants were initially raised as shoots (plantlets) by rooting rock stocks in a rooting medium (compost: sandy loam 2:1 w/w). The well-rooted plantlets used in setting the experiments were approximately 8–12 weeks old. In each treatment, two plantlets each was transplanted which produced multiple shoots throughout the experiment. A dibble was used to make small holes, shoots, or seedlings transplanted and the soil gently pressed to ensure the roots were in contact with the soil as would have been the case under any soil use condition. In all the experiments, the control treatments were not planted.

**Experiments and treatments**

The experiments described below were conducted in 50 cm tall (9 cm in diameter) stormwater tubes whose bottom ends were tightly capped. In all the tubes, the bottom 22 cm was filled with sand and the top 22 cm with 1300 g of sulfidic soil by weighing to add the exact amount in each tube. The treatments were replicated four times and set out in a complete randomized design under glasshouse conditions in polythene crates. The anoxia was created by keeping all the treatments under flooded conditions with adequate amount of water ponding on the surfaces by regular addition of water (once in the morning and in the evening) (Michael et al. 2016) for 6 months.  

**Experiment 1**: This experiment was conducted with P. australis plants established with organic matter incorporated in the soil (80:1, soil: organic matter w/w) by bulk mixing. Bulk mixing was done by weighing out the amount of soil or organic matter needed using a portable scale at 80:1 (w/w), and thoroughly mixed in 20 L mixing troughs using a spade. The control treatment contained the same amount of organic matter but not plants.  

**Experiment 2**: In this experiment, P. australis plants were established in the sulfidic soil under the same soil conditions but without added organic matter to compare the results of Experiment 1. The control treatments were set without plants.

**Measurements and root biomass quantification**

In all the treatments, measurements were made only from the top 22 cm of the sulfidic soil. Changes in redox potential (redox/Eh), pH and sulfate content were measured from the surface (0-20 mm), middle (50-100 mm) and deep (150-200 mm) soil profiles as previously described (Michael et al. 2015; 2016; 2017). Redox was measured using a single Ag/AgCl reference and platinum (Pt) electrode combination using an automated data logger (Michael et al. 2012; 2014). To measure the Eh, a handheld electric drill, with a drill bit head the size of the Pt electrode, was used to make holes through the tubes with care taken to avoid disturbing the soil. The Pt electrode was inserted in the holes and reference electrode was inserted into the soil from the surface. This was allowed to equilibrate for 10 minutes and then Eh measured at 1 minute intervals for the next 10 minutes and averaged (Rabenhorst et al. 2009). These values were corrected for the reference offset to be relative to the potential of a standard hydrogen electrode by adding 200 mV (Fiedler et al. 2007). The stability and accuracy of the electrodes were maintained according to Fiedler et al. (2007). The pH was measured using 2 g soil (1:5 water) with a pre-calibrated Orion pH meter (720SA model).

Sulfate was extracted according to the method of Hoeft et al. (1973) for soluble soil sulfate. Replicate samples (0.5 g each) were placed in tubes with 1.5 ml of an extraction solution (0.2 g CaH₂PO₄, 12 g glacial acidic acid and 88.5 g deionized water). After 30 minutes, soil was sedimented by centrifugation for 5 minutes, and duplicate aliquots from the three replicates were transferred into 4 ml cuvettes and diluted with 1.5 ml of the extraction solution. The samples were mixed with 0.7 ml of 0.5 M HCl, and 0.7 ml of 0.1 M barium chloride-polyethylene glycol reagent was added and mixed again. After 10 minutes, the samples were mixed again and the absorbance read at 600 nm using a spectrophotometer. The readings were compared with a standard solution of 0–2 mM Na₂SO₄. The initial sulfate content of the sulfidic soil ranged between 12–16 µmol g⁻¹ soil. The detection limit based on an absorbance reading of 0.1 of this method is 0.6 µmol g⁻¹ soil.

The root biomass was quantified as described by Michael et al. (2017) from the soil profiles from which the changes in Eh, pH and sulfate content were measured. Soil from these sections was placed in a sieve (0.05 mm) and held under gentle running tap water and the soil is carefully broken up to free the roots using the aid of forceps. The loose soil particles were allowed to drain through but roots that were trapped by the sieve and those that floated during washing were collected. These roots were taken, gently washed again to remove soil material, placed in weighing boats and oven dried for two days. The dry weights were taken by weighing, and weights of the replicates were pooled, averaged, and kept as the final data.

**Statistical analyses**

The Eh values obtained over a 10 minutes period were averaged and a treatment average obtained by taking the mean of the three replicates (Michael et al. 2012; 2014). Similarly, treatment average pH and sulfate content were obtained by taking the mean of the three replicates (Michael 2015). To compare the treatment means, significant differences (p≤ 0.05) between treatment means of each profile were determined by two-way ANOVA using statistical software JMPIN, AS Institute Inc., SAS Campus Drive, Cary, NC, USA. If an interaction between the treatments and profile depths was found, one-way ANOVA with all combinations was performed using Tukey’s HSD (honest significant difference) and pairwise comparisons. The values shown in all the figures are mean ± s.e. of three replicate measurements. The dotted line is the initial pH. An asterisk indicates significant difference (p≤0.05) between the treatments and the controls at same depths.
RESULTS AND DISCUSSION

Combined effects of organic matter and plants on pH

The main aim of the experiments was to assess the importance of organic matter addition and the effects of live plants as well as the organic matter turnover on sulfidic soil chemistry (pH, Eh and sulfate content) under falling water regimes, such as during a drought event. The expectation is that under reduced soil conditions as a result of flooding, the sulfidic soil will remain reduced. However, in the planted soils, parenchyma is expected to pump oxygen into the rhizosphere, resulting in oxidation of the oxidizable sulfides, lowering the soil pH. The second aim associated with the live plants was the distribution of the biomass (root) and the effects on sulfidic soil chemistry and whether organic matter addition had influenced the biomass distribution and whether that had an effect on the sulfidic soil chemistry.

The biomass was equally distributed throughout the soil profiles except in the middle, near 5 g, in the soil organic matter was added (Figure 2). Interestingly, presence of plants lowered the alkaline pH to 6 from an initial pH of 6.7, compared to the changes measured in the control soil where no organic matter addition was made. The decrease in soil pH measured showed that the plant with modified anatomical structures capable of pumping oxygen into the rhizosphere did exactly that and the amount was sufficient to oxidize the sulfidic soil, lowering the pH. There was no clear relationship between the biomass distribution and the changes in pH measured. Under the flooded soil condition with organic matter addition, pH was expected to remain higher than the starting pH, however, that was not the case on the surface (Figure 3). A decrease in pH of the surface soil occurred as a result of fluctuation in the amount of water that was ponding on the surface as would be expected due to loss from evaporation (Michael and Reid 2018).

Combined effects of organic matter and plants on redox potential

Under the flooded soil conditions, biomass distribution and soil redox had no clear relationship (Figure 4). In the 20 mm profile of the control, Eh was 168 mV when the lower depths were at -11 and -28 mV, respectively. In the organic matter amended, the soil was moderately reduced, Eh ranged from -18 mV within the surface to -100 mV at depth. These results showed presence of the plant led to oxidation because the soil was expected to remain reduced as a result of anoxia created by flooding as seen in the control soil. The unplanted soil was reduced as deep as expected because of flooding and reduction reactions of the organic matter. Generally, the changes in Eh measured correlated to the changes in pH, indicating that redox influences soil pH. For example, in profiles where changes in pH were the lowest, the Eh values were high and positive, compared to high pH and low and negative Eh values. In profiles where Eh values low and negative, more biomass tended to accumulate. This phenomenon could be an adaptive mechanism for the plant used to deliver more oxygen and oxygenate the rhizosphere so as to keep the roots alive under anoxic conditions. This assertion is true in many plants adapted to living in specific soil and water conditions that use such modifications to survive, e.g. modified parenchymatous tissues in plants adapted to marshlands (hydrophytes) to meet oxygen demand, extensive root systems of plants adapted to deserts (xerophytes) in search of moisture or as a survival mechanism under harsh conditions, such as in acidic soil (tolerant) or high salinity (halophytes).

Figure 2. Root biomass distribution in soil amended with organic matter

Figure 3. Effects of organic matter with or without live roots on pH
Combined effects of organic matter and plants on sulfate content

In the organic matter amended soils without plants, the sulfate content was significantly reduced to near 3 µmol g\(^{-1}\) soil throughout the profiles (Figure 5), compared to the initial sulfate contents, ranging from between 12-16 µmol g\(^{-1}\) soil. In the soils organic matter and plants were co-existing, the sulfate content was smaller within the surface and higher at deep soils, ranging from 5.7 to 13.6 µmol g\(^{-1}\) soil. As in pH and Eh, no clear relationship between biomass and sulfate content was observed. These results have strongly indicated that in the presence of plants capable of oxygenating the rhizosphere, sulfate reduction is minimal even if labile organic matter is available to soil microbes. The significant reduction in sulfate contents by 9 to 14 µmol g\(^{-1}\) soil showed organic matter is an important resource needed by facultative or anaerobic microbes. In most soils, sulfur-reducing bacteria (SRB) operate when the soil redox was reduced to values lower than or closer to -100 mV (e.g. Michael et al. 2015; 2016; 2017). The results shown in Figure 4 shows that the redox, especially in the deep profiles, was reduced to -80 to -100 mV, conducive for SRB to operate. This is a strong point that SRB was responsible for the reduction in sulfate measured. We have reported similar findings in a number of studies (e.g. Michael et al. 2015) and pointed out SRB was responsible, even under aerobic soil conditions with organic matter addition. The results further pointed out how important the slightest change in redox is to sulfate reduction. For instance, in the deep soil, Eh was -28 mV (Figure 4) and the sulfate content remained nearly unchanged at 14 µmol g\(^{-1}\) soil from a range of 12-16 µmol g\(^{-1}\) soil, regardless of organic matter availability.

Effects of common reed alone on soil pH

The results of study conducted without organic matter addition is shown in Figures 6, 7 and 8. Under the flooded soil conditions, the distribution of biomass was irregular with more roots being found in the middle profile (Figure 6). The biomass distribution was well below 3 g (Figure 6) compared to the biomass data given in Figure 2. These results indicate that organic matter is important for root biomass, or maybe just general plant growth. In both studies, however, more roots were found in the middle soil than the surface or the deep. The main probable reason for this observation is that the plants as much as possible accumulated roots near to the surface where there was sufficient oxygen than at depth where anoxia was quite pronounced. This phenomenon, again, is an adaptive mechanism (aided by the modified anatomical structures) to avoid drowning of the roots and enhance continued respiration to survive under soil condition of limited oxygen. The opposite is true for plants that are used to aerobic soil conditions. Most terrestrial plants do not possess the modified anatomical structures as such as parenchymatous tissues; therefore, do not survive long under flooded soil conditions. Roots of plants used to well aerated soils immediately suffocate as soil get flooded, and as anoxia sets in, cellular respiration stops and the root tips slowly die out, resulting in starvation and ultimate death of the whole plant.
In the unplanted, the pH remained nearly unchanged with a small decrease within the surface soil as shown in Figure 3. The similarity in the changes in pH in both experiments meant the mechanisms involved in inducing the changes in soil chemistry measured were the same. In the planted soil, the pH significantly decreased to near 5 units, with the highest decrease in the profile of bigger biomass. Unlike the previous experiment (data in Figure 3), in the absence of organic matter higher biomass resulted in significant reduction in pH (Figure 7). For example, 3 g of roots resulted in 1.3 unit decrease in pH. The opposite was true in the surface soil of 1.8 g biomass which resulted in pH decrease by 1.1 unit only. These results strongly demonstrated that in the absence of organic matter, oxygen penetration into the soil through modified anatomical structures results in severe acidification, lowering the pH. Under the continuously flooded soil conditions, the influence of plants on pH was less pronounced in the soil with organic matter, which most likely reflects the dominant role of certain microbes depleting the oxygen transported via parenchyma using the organic matter as energy source. In the absence of added organic matter, the opposite happened. The mechanism underlying the adverse effects of live plants can be related to the processes occurring when dead plant material is either incorporated (Charoenchamratcheep et al. 1987) or distributed as surface mulch (Michael et al. 2017).

**Effects of plants alone on redox potential**

Plants adapted to growing in water have developed anatomical modifications that provide channels for the exchange of oxygen and carbon dioxide between roots and shoots (Michael 2018a). This study demonstrated that *Phragmites* have the capacity to transport large amounts of oxygen into the rhizosphere and leak some of this oxygen, as evidenced by the high redox values (Michael 2018b). The oxidation phenomenon was more pronounced in the soil without added organic matter (Figure 8). Explanation for this is that the oxygen delivered via the parenchyma to the rhizosphere oxidized the soil, even under flooded soil conditions. In the unplanted soils, redox was highly reduced in the absence of added organic matter, as a result of the anoxia created by the flooding and the reduction reactions that ensured. In our previous studies, organic matter addition in sulfidic soil under various moisture regimes without live plants had similar changes in pH, Eh and sulfate concentrations (e.g. Michael et al. 2015; 2016), confirming that organic matter ameliorates sulfidic soil chemistry even under falling soil moisture regimes (Michael 2018c; 2020c).

Organic matter, especially that with large nitrogen content induces the proliferation of aerobic microbes which consume available oxygen (Michael 2015) and cause the Eh to fall into the range in which SRB can convert sulfate into sulfides (Michael et al. 2015). This phenomenon explains the changes in soil chemistry of the unplanted soils with added organic matter, in addition to a moderate level of residual organic matter (10.6%) already in the soil. The most obvious difference between the control soils and the planted soils was the large difference in Eh. The planted soils with organic matter resulted in high Eh values (168 mV) within the 20 mm profile (Figure 4) compared to the planted soil without organic matter (Figure 8) of 400 mV.
Effects of plants alone on sulfate content

The *Phragmites* plants were young with very few senescing leaves and therefore little dead organic matter turnover to the soil. In the planted soils with added organic matter, a high concentration of sulfate was measured at deep (Figure 5), compared to the planted soil without added organic matter (Figure 9). This is a strong indication that soil microbes used the added organic matter to reduce the sulfate to sulfide (Michael 2018c; 2020e). In the control soil of study shown in Figure 9, an estimated 10.6% residual organic matter was present but this was not sufficient to help microbes to reduce the sulfate content compared to the reduction shown in Figure 5 where addition was made. In mature stands of this plant, there is constant turnover of live and dead tissues (Michael 2020a; b; c); hence, the acidifying effects of live plants would be partly offset. These studies however demonstrated this plant even co-existing with organic matter causes oxidation even under flooded soil (Michael 2020d; e). The opposite is true; existence of organic matter alone would sustain sulfidic soil alkalinity (Michael 2020d; e).

The management implications revolve around the balance between live and dead plant material turnover under falling soil moisture regimes (Aipa and Michael 2019; Michael and Reid 2018). For example, growing of live *P. australis* plants in or on the edge of water bodies can be problematic, especially if the surface and groundwater levels of such wetland areas decrease during drought conditions (an anticipated event of a change in climate), which will accelerate pyrite oxidation and the formation of deep sulfuric soils (Simpson et al. 2010). If the acidified areas are adjacent to major river systems and are refflooded, metal and metalloid contaminants can be released from the sulfuric soils and can pose risks to the public and environment (Michael 2013; Reid and Butcher 2011).

Under normal soil use and management conditions, organic matter addition as mulch or as turnover from leaf litter and organic compound secretion (even under falling moisture regimes) would enable soil microbes to act on these different substrates and consume acidity generated via Eqn. 2, generating alkalinity (e.g. Michael et al. 2015; 2016; 2017). This will create microenvironments conducive to lower Eh, sulfate content and increase the pH, important for management of sulfidic soil alkalinity (pH>4), during extreme climatic events (Michael 2019a). Presence of plants will accelerate the fall in soil moisture because of water use in photosynthetic reactions (Michael 2019b), therefore, under such conditions, the plants need to be mowed to the ground and the shoot systems (aboveground biomass) removed. In the absence of the shoot systems, the remaining root systems (underground biomass) will use the culm to draw atmospheric oxygen into the soil and continue to oxidize the sulfidic materials. This management issue will most likely be addressed by reworking the land, e.g. plowing, and getting the exposed ends of the culms covered by soil to prevent oxygen penetration (Michael and Reid 2016).

In contrast to the positive effects on sulfidic soil of organic mulches derived from dead plant material, turnover of organic matter as leaf litter or secretion of organic compounds from live plants, the growth of live plants with roots capable of transporting oxygen downwards via aerenchymatous tissue induces acidity. Organic matter addition gave rise to more biomass, resulting in more oxygen in the rhizosphere. Under excess moisture regime, in other words, flooded soil condition, an important management option would be to slash but would lead to deoxygenation. The culms would continue to transport oxygen into the soil if the slashed ends remain open and were not covered. Under falling moisture regimes, decomposition of the slashed plant matter on the surface would lead to offsetting of oxidation and generation of sulfuric acidity.

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