Effects of Organic Matter and Live Plants on Sulfidic Soil pH, Redox and Sulfate Content under Flooded Conditions

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Abstract

In an undisturbed state below the water table, the sulfidic soils are benign, unless exposed due to various natural processes or anthropogenic activities and sulfuric acidity is produce, which has negative impacts on the environment. This paper examines the effects of organic matter, organic matter co-existing with live plants or live plants alone on sulfidic soil pH, redox and sulfate content under flooded conditions. In almost all cases, organic matter without plants induced ameliorative effects on sulfidic soil chemistry. In soils with or without organic matter, presence of plants led to higher Eh values, lower pH and higher sulfate contents. The reduction reactions of the added organic matter and the anoxia created by flooding were ineffective in reducing the amount of oxygen that was pumped into the rhizophore via the arenchymatous tissues in all the soils with plants. Under falling soil moisture regimes, e.g. during a drought event, presence of this type of plant species would aerate the reduced soil conditions. Aeration would then lead to oxidation of sulfides, producing sulfuric acid, which in turn would have negative impacts when released into the environment. One strategic option to reduce the amount of oxygen entering the soil would be to slash the shoots and leave them on the surface to help generate more alkalinity upon decomposition, even if the culm would continue to facilitate oxygen transport.

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

Introduction

Sulfidic soils (pH>4) of acid sulfate soils (ASS) are naturally occurring soils formed under reducing conditions (Pons, 1973; Baldwin and Fraser, 2009) and widely distributed associated with lakes, rivers and wetlands (Dent and Pons, 1995; Fitzpatrick et al., 2009; Fanning et al., 2017). Wilson (2005) and Joukainen and Yli-Halla (2003) reported these soils have been formed in the last 10 000 years after the last sea level rise. The microbially induced process through which sulfidic soils formed is shown in equation (Eqn.) 1 (Bloomfield and Coulter, 1973; Fanning and Fanning, 1989; Michael, 2013). When the sea level rose and inundated land, sulfate in the sea water mixed with iron oxide in the sediments and organic matter (OM), allowing sulfate reducing bacteria (SRB) to form iron sulfide (Canfield et al., 2006).

$$Fe_2O_{3(s)} + 4SO^{4-}_{(aq)} + 8CH_2O_{(aq)} + ½O_{(g)} \rightarrow 2FeS_{2(s)} + 8CHO^{3-}_{(aq)} + 4H_2O_{(aq)} \quad \text{Eqn. 1}$$

Under reducing conditions, sulfidic soils pose no problem unless exposed to atmospheric oxygen (Pons, 1973; Appleyard et al., 2004), e.g. by falling water levels such as during a drought event (Simpson et al., 2010), oxygen is pumped into the rhizosphere via the aerenchymatous by plants (Michael et al., 2017; Michael and Reid, 2018), land use means such as excavation, drainage or ploughing (Fitzpatrick et al., 2010) and the sulfides are oxidised, generating sulfuric acidity (Cook et al., 2000; Powell and Marten, 2005; Sullivan et al., 2009). Equation 2 shows the oxidation processes of the iron sulfide (FeS2) and production of sulfuric acidity (Bloomfield and Coulter, 1973; Charoenchamratcheep et al., 1987; Michael et al., 2015).

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

Under natural soil use and management conditions, sulfidic soils are often flooded, resulting inundation due to the presence of excess water. Under such conditions, two major things happen; drowning and dying of plants that are unable to survive under excess water and striving of plants that are able to withstand flooding. When soils with water loving plants get flooded, these plants co-exist with the dead plant matter of those that died out as a result of drowning. When the flooded soil is without water loving plants, the OM from the dead plant matter exist alone, at least for some time. What happens to the sulfidic soil pH, redox and sulfate content under such scenarios on a short- or long-term is not often reported. We have reported in a long-term study (12 months) using *Phragmities australis* that live plants oxidise sulfidic soils and generate sulfuric acidity (Michael and Reid, 2018). However, the short-term effects (<6 months) were not investigated.

Therefore, this paper examines the effects of live plants alone or co-existing with OM and compares that with OM alone on sulfidic soil pH, redox and sulfate content under flooded conditions within a period of 6 months. This study was conducted as a preliminary study of the long-term study reported by Michael and Reid (2018).

Materials and Methods

Soil

The ‘sulfidic soil’ was collected from a ‘sulfuric subaqueous clayey soil’ at a depth of approximately 1000 mm in the Finniss River in South Australia at Wally’s Landing (35°24028.28″S; 138°49054.37″E). Details on soil classification using the Australian ASS Identification key and Soil Taxonomy are 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). The ‘sulfidic material’ (Soil Survey Staff, 2014) used is representative of typical global inland and coastal wetlands, in terms of sulfide and OM content.

The pH of the freshly collected sulfidic material measured in water 1:5 (pH_w) 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 OM content, estimated using weight loss-on-ignition method (Schulte & 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_{ox}) was 1.4, a strong indication of presence of high amount of oxidisable sulfides. The sulfate content of the sulfidic soil prior to use was 12 – 16 μmol g^{-1} soil.

Organic Matter

To use as OM, the first three younger and fully open leaves of *P. australis* were collected and prepared as previously described (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 through a ≈0.5 mm sieve. The nitrogen content of the OM analysed by ICP-OES using a 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 root 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 500 mm tall (90 mm in diameter) stormwater tubes whose bottom ends were tightly capped. In all the tubes, the bottom 220 mm was filled with sand and the top 220 mm with 1300 g of moist 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. In all the treatments, measurements were made only from the top 220 mm of the sulfidic soil. The treatments were always 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) for 6 months prior to data collection.

Experiment 1: This experiment was conducted with *P. australis* plants established with OM incorporated in the soil (80:1, soil: OM w/w) by bulk mixing. Bulk mixing was done by weighing out the amount of soil or OM needed using a portable scale at 80:1 (w/w), and thoroughly mixed in a 20 L mixing trough using a spade. Only the treatment soils were planted.

Experiment 2: In this experiment, *P. australis* plants were planted in the sulfidic soil in a similar manner under anaerobic (flooded) soil conditions but without added OM to compare to the results of experiment 1.

Measurements

The procedures used in the measurements were described in several studies (e.g. Michael et al., 2015; 2016; 2017; Michael, 2018 a, b, c). In all the measurements, only three treatments out of the four were used. 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). 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 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 in water (1:5 soil: 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 CaH_2PO_4, 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 Na_2SO_4. The initial sulfate content of the sulfidic soil ranged between 12 – 16 $\mu lmolg^{-1}$ soil. The detection limit based on an absorbance reading of 0.1 of this method is 0.6 $\mu lmolg^{-1}$ 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 a gentle running tap water and the soil 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 as we reported. Similarly, treatment average pH and sulfate content were obtained by taking the mean of the three replicates. 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 combination was performed using

Turkey's HSD (honest significant difference) and pairwise comparisons. The values presented in all the figures are mean ± s.e. of three replicate measurements. An asterisk indicates significant difference ($p<0.05$) between treatment and control at same depth.

Results and Discussion

Effects of organic matter co-existing with live plants on pH

The aim of the two experiments was to assess the effects of OM co-existing with live plants under natural anaerobic soil conditions or following flood events with the common knowledge that the sulfidic soil would remain reduced.

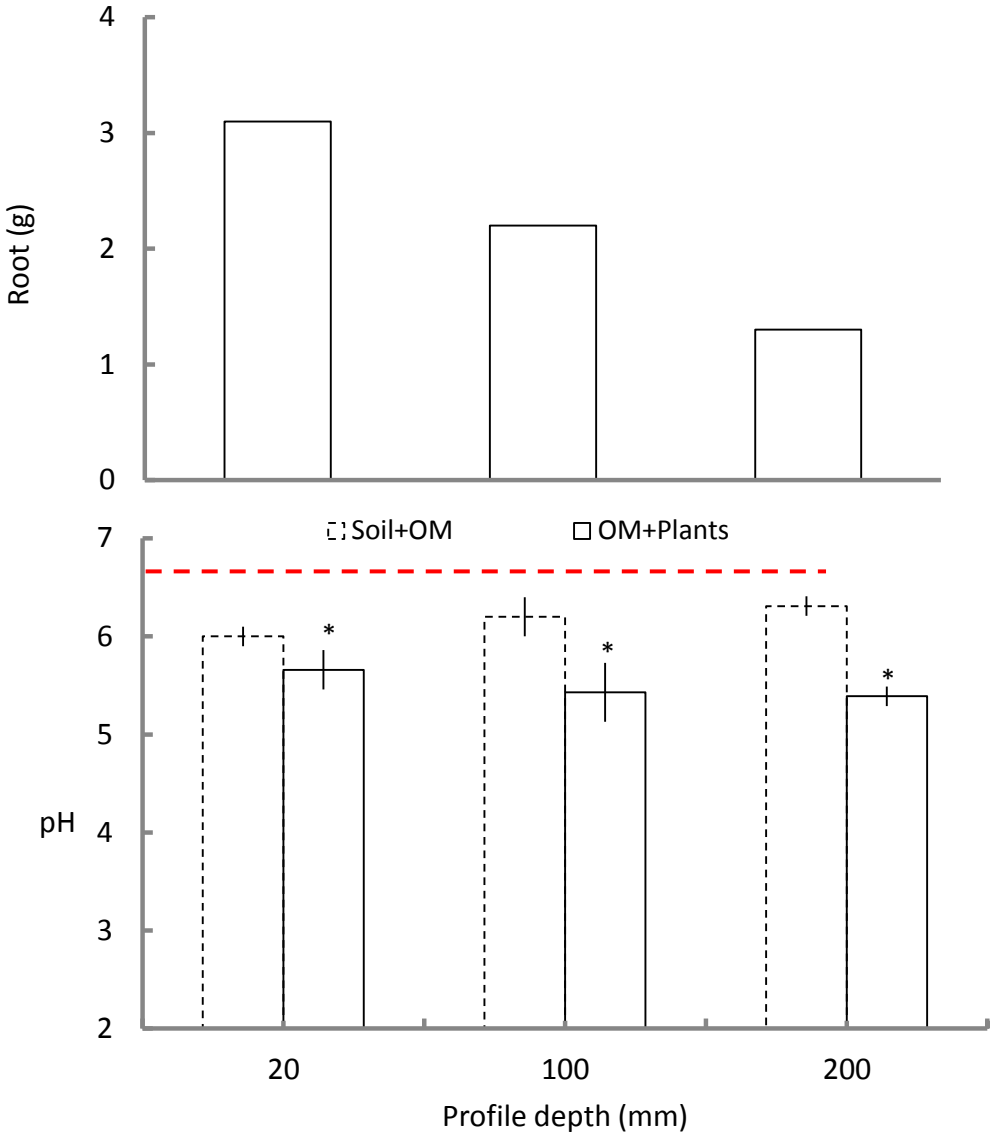

Figure 1. *Effects of organic matter with or without live roots on pH under flooded soil conditions. The dotted line is the initial pH.*

In the presence of plants capable of pumping oxygen into the rhizosphere, soils are expected to be oxidised, lowering the soil pH. In the planted soil, the change in pH was near 6 units at the surface and near 5 units at the deep, respectively (Fig. 1). In the unplanted soil, OM addition lowered the pH well below the initial pH of 6.7 units. As shown in Fig. 1,

biomass was distributed as would be in young plants, with more roots within the surface soils than at the deep. There was however no clear relationship with the changes in pH measured and the biomass distribution.

Effects of organic matter co-existing with live plants on redox potential

Under the flooded soil conditions, biomass distribution and soil redox had a clear relationship (Fig. 2). Redox was reduced where the biomass was bigger and got highly reduced in the deep soil of small biomass. The co-existence of OM and live plants did increase the pH (Fig. 1) but the reduction reaction of flooding was dominant at deep soil. Existence of OM alone sustained the reduced conditions of the sulfidic soil, Eh ranging between -10 mV to -80 mV (Fig. 2).

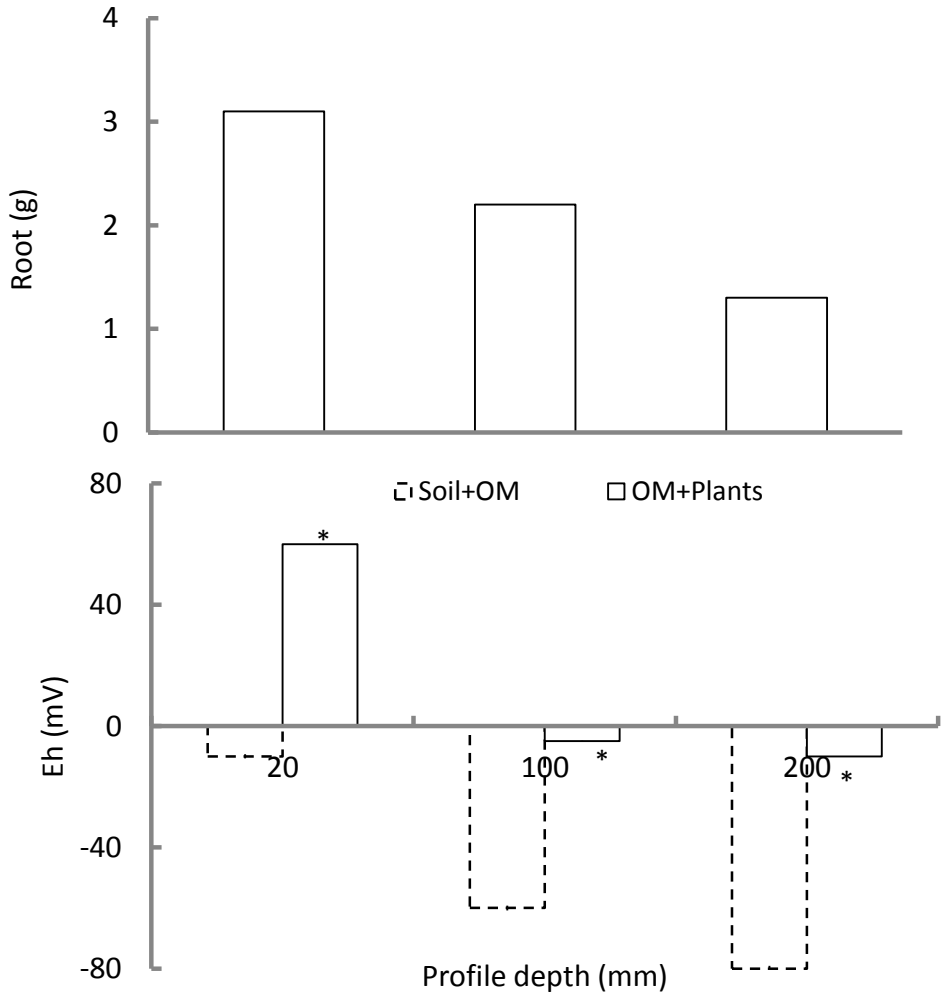

Figure 2. *Effects of organic matter with or without live roots on redox potential under flooded conditions.*

Effects of organic matter co-existing with live plants on sulfate content

In the OM amended soils without live plants, the sulfate content was near 3 μmol g^{-1} soil throughout the profiles (Fig. 3). In the planted soils with OM, the sulfate content was reduced to near 6 μmol g^{-1} soil and there was no change at deep soils. In the soil with both

OM and plants, the sulfate content was highly reduced in the presence of bigger biomass, and the opposite happened with smaller biomass, respectively (Fig. 3).

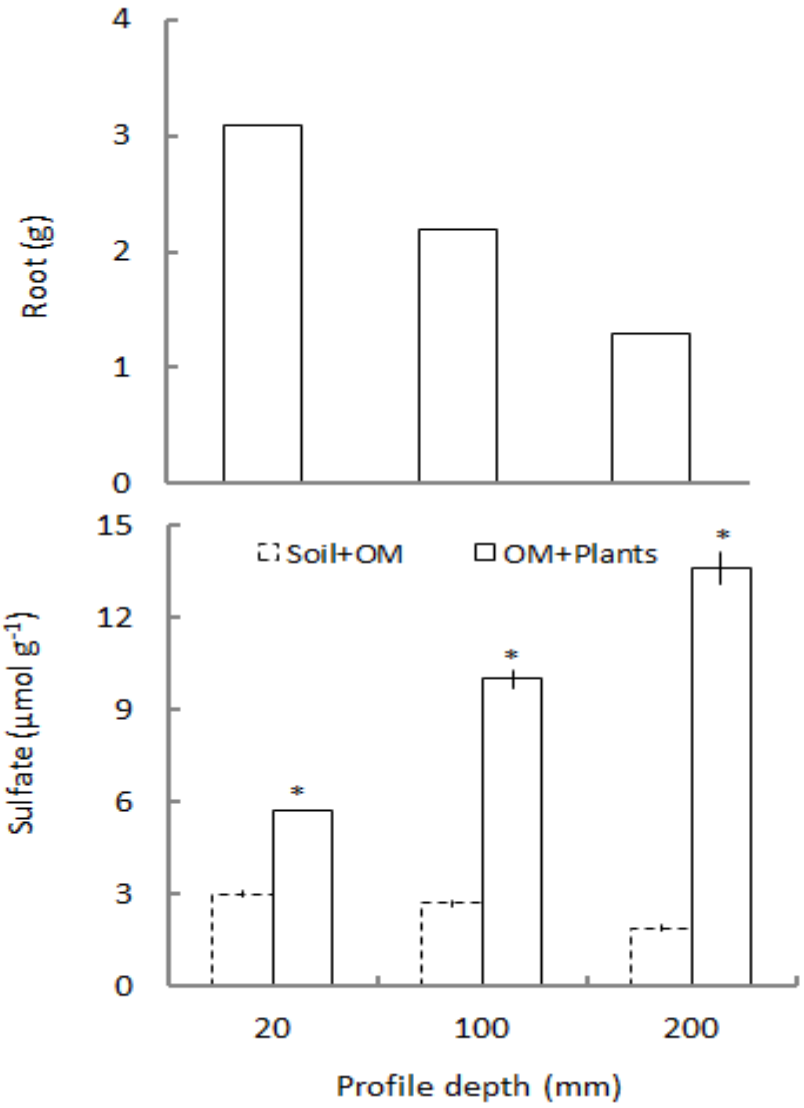

Figure 3. *Effects of organic matter with or without live roots on sulfate content under flooded soil conditions. The initial sulfate content range from 12-16 µmol* g^{-1}.

Effects of live plants alone on soil pH

Changes in soil pH measured in the absence of OM are shown in Fig. 4. The biomass distribution was fairly the same throughout the soil profiles in the absence of OM. In the unplanted soil, the pH remained nearly unchanged with a small decrease within the surface soil. In the planted soil, pH decreased, more so at the deep soil where the biomass was bigger (Fig. 4). Unlike the previous experiment (shown in Fig. 1), less biomass was produced in the absence of OM.

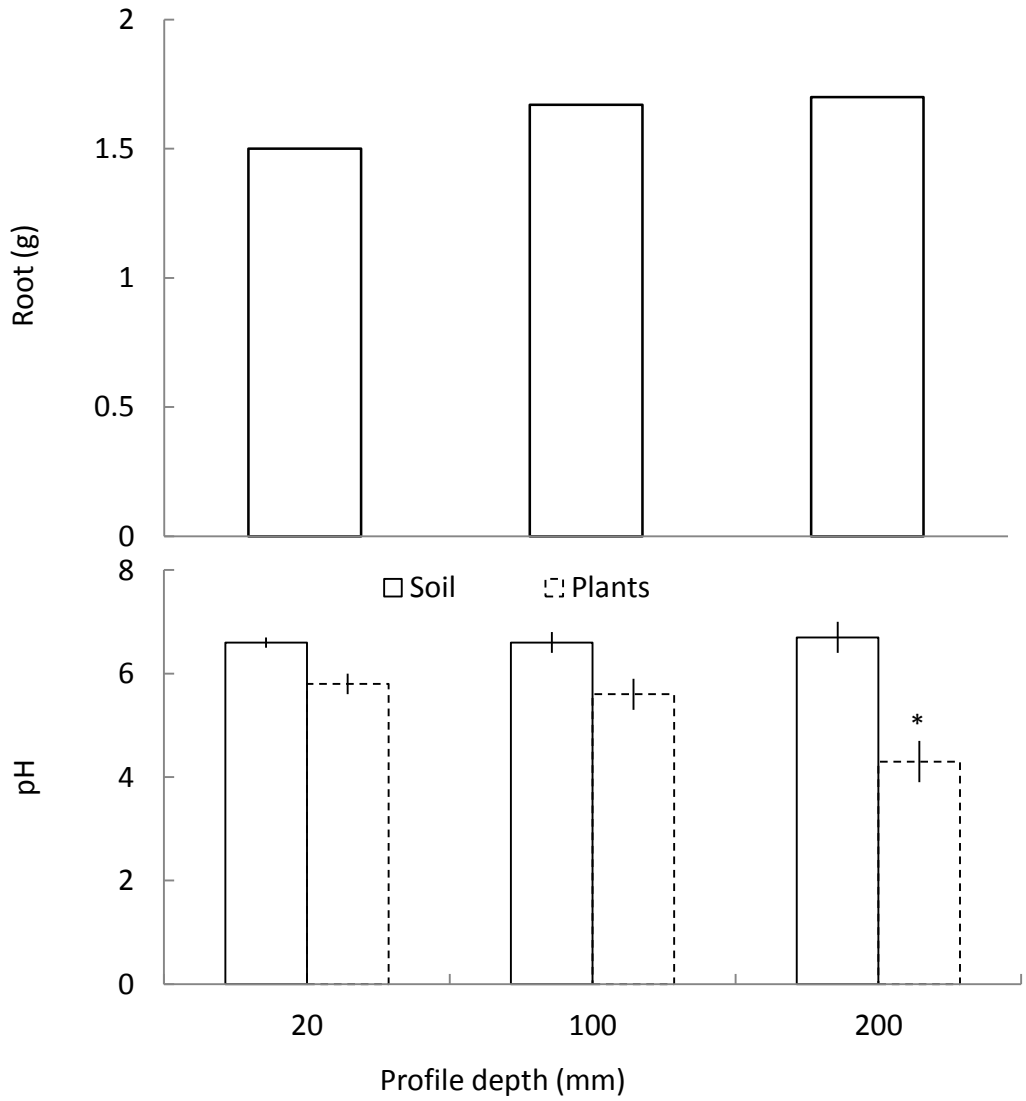

Figure 4. *Effects of live roots on pH of soil without added organic matter under flooded conditions.*

Effects of live plants alone on soil redox potential

Under the flooded soil conditions, the redox of the planted soil was oxidised within the surface to reduce at deep, Eh ranging from 60 mV to 10 mV (Fig. 5). The influence of biomass on the changes in redox was not clearly seen. In the control soil, redox was highly reduced, with the overall changes ranging from -100 mV at the surface to -70 mV depth.

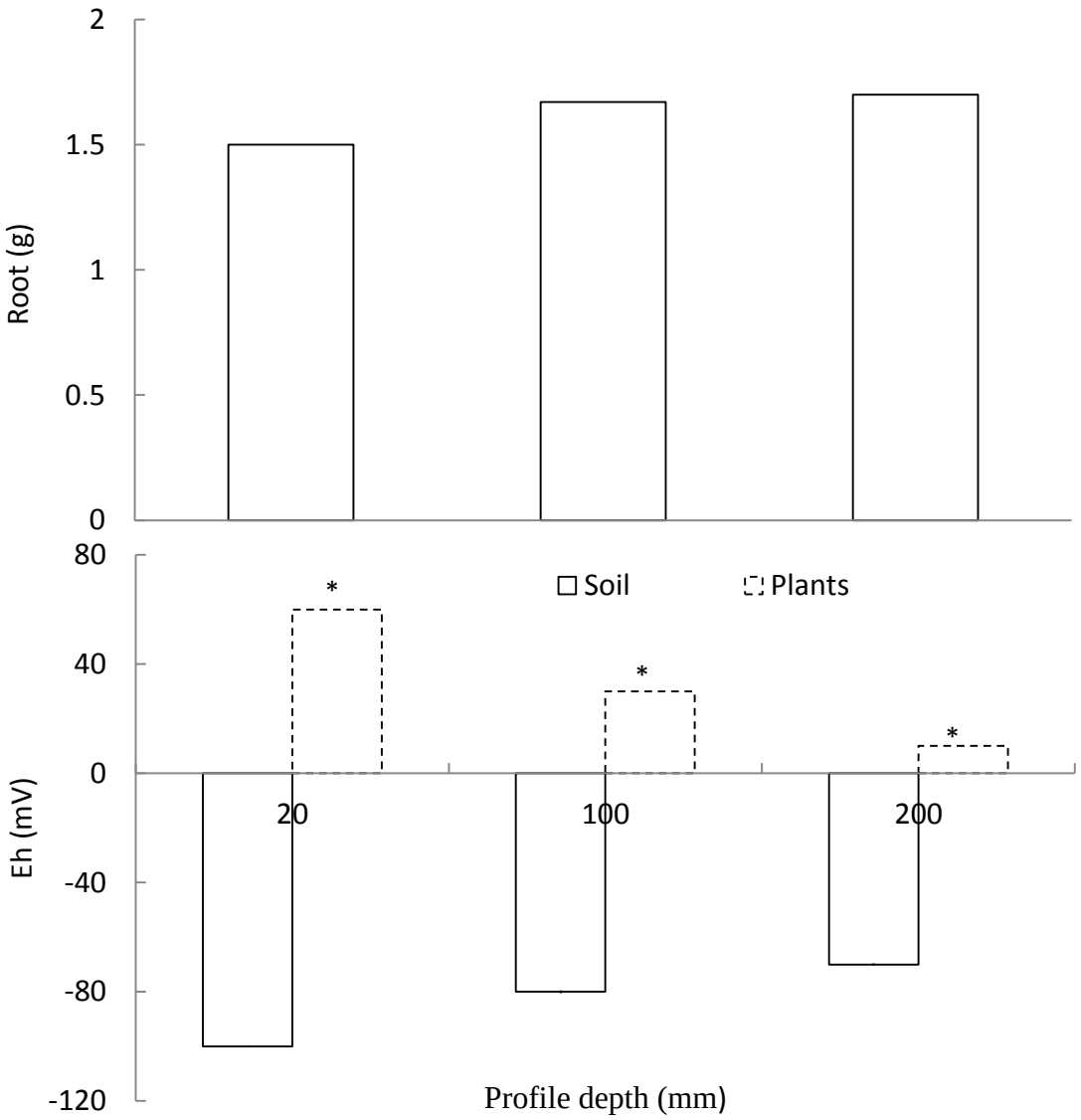

Figure 5. *Effects of live roots on redox potential of soil without added organic matter under flooded soil conditions.*

Effects of live plants alone on soil sulfate content

Under the flooded soil conditions, sulfate content was higher in the planted soil at the surface soil with smaller biomass than at deeper profiles of bigger biomass (Fig. 6). Generally, the changes in sulfate content were small in the profiles of smaller biomass, especially within the surface soil. In the unplanted soil, sulfate content was small and decreasing with soil depth.

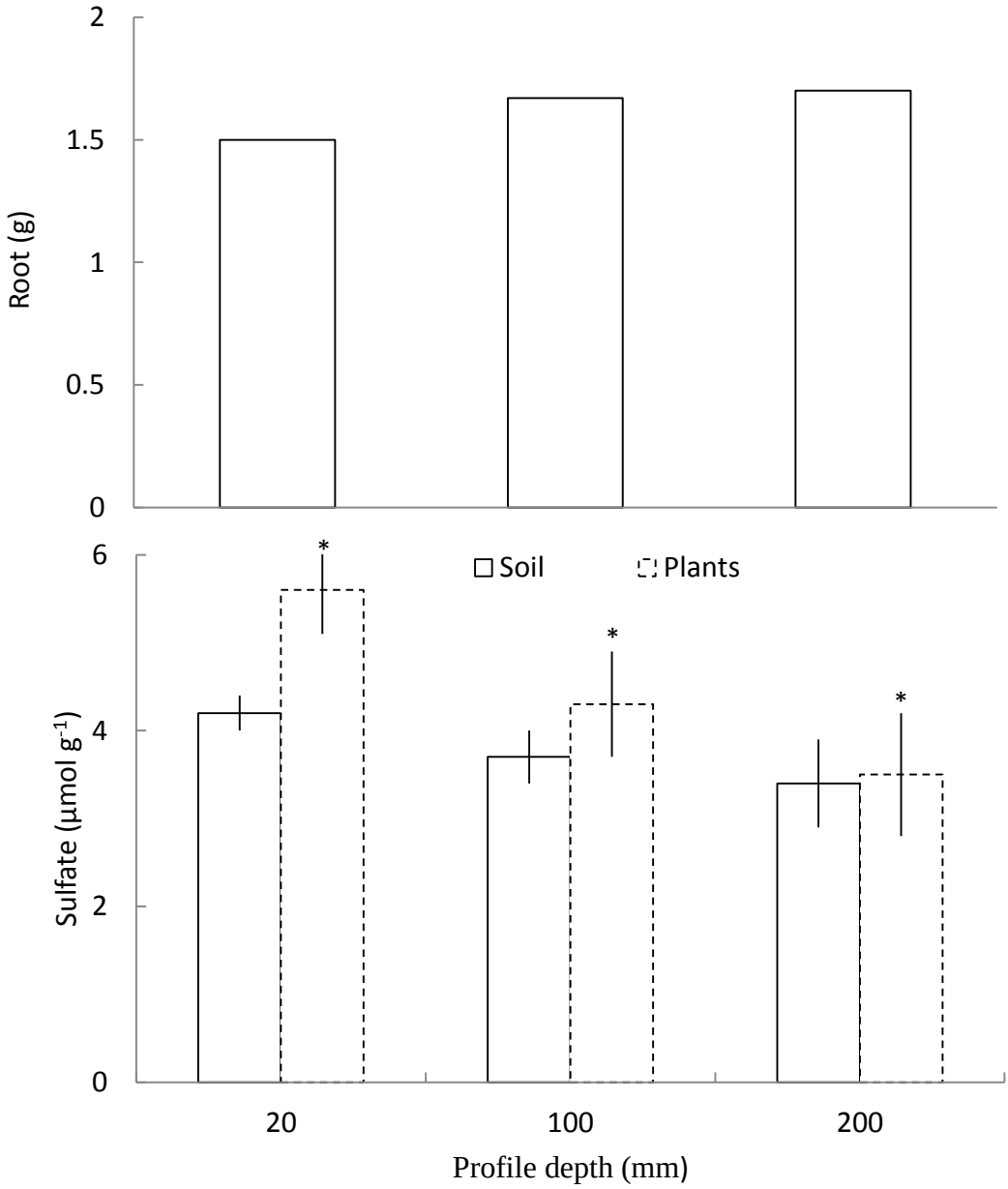

Figure 6. *Effects of live roots on sulfate content of soil without added organic matter under flooded soil conditions. The initial sulfate content range from 12-16 µmol g^{-1} soil.*

Sulfidic soil status under reduced soil conditions

Michael (2013) reviewed the negative impacts of the sulfuric acidity produced and pointed out basic management strategies that are available in managing either sulfidic soil materials or the sulfuric soil material, respectively. The former is managed by water table management (flooding) and the later by application of an alkalizing agent, e.g. such as lime (Shamshuddin et al., 2004). Under soil use conditions, flooding is not often favoured because it limits land uses and availability of refined lime is expensive to afford in poor economies, e.g. in the developing countries where ASS is found. We have pointed out in several studies that addition of OM, which is relatively cheap and readily available is an alternative management strategy to manage oxidation and acidity (e.g. Michael et al., 2015; 2016; 2017; Michael, 2015; 2018a, b, c). We demonstrated through some of these studies (e.g. Michael et al., 2015) that addition of OM followed by flooding revert sulfuric soil to sulfidic soil. The

mechanisms for these being consumption of protons (H^+) by the reduction reactions of flooding and reduction of sulfate to sulfide by sulfate reducing bacteria (SRB) using the added OM as a metabolic substrates (Michael, 2018b).

Mechanisms of changes in sulfidic soil chemistry associated with organic matter

The experiment with OM addition but without live plant sustained the pH of the sulfidic soil within the surface and even increased it at the deep soil (Fig. 1). These changes were consistent with the changes in pH measured in the experiment without OM addition (Fig. 4). As shown in Fig. 2, addition of OM reduced the Eh, the changes ranging from -10 mV within the surface to -80 mV at depth. Compared to these changes, the soil remained highly reduced without added OM, the changes ranging from between -100 mV at the surface to -70 mV at depth (Fig. 5). In both studies, the changes in Eh at deep soil are quite similar except within the surface (0 – 20 mm). The mechanisms for the differences in the surface soils seem to come from the added OM (Michael et al., 2015; Jayalath et al., 2016). OM addition increased porosity for oxygen to penetrate the surface soil and reduce it to -10 mV, whereas in the absence of added OM, there was no pore space due to the flooded nature of the soil for this to happen, making the soil to remain highly reduced, with Eh near -100 mV. Sulfate reducing bacteria are known to work when the soil pH is >5 units and the redox is <-100 mV (Michael, 2018b). When OM was added, the sulfate content was nearly depleted to well below 3 μmol g^{-1} soil (Fig. 3). In the absence of OM, the sulfate content was high but reduced to near 4 μmol g^{-1} soil (Fig. 6). The probable reason for this is the presence of the residual OM content of 10.6%, which was utilized by soil microbes to reduce the sulfate content in the absence of added OM. The changes in pH and Eh in soil with OM were within the ranges of SRB to function, making the changes in sulfate content measured to be inducted by SRB (Michael et al., 2016; 2017). In addition to the reducing reactions created by flooding, oxygen was consumed by aerobic and facultative microbes that initially acted on the OM, creating micro-niches sufficient for SRB to function (Michael, 2018b).

Mechanisms of changes in sulfidic soil chemistry associated with live plants

Under the flooded soil condition, greater root biomass was produced when OM was added at surface soil, however, smaller biomass was measured at deeper soil (Fig. 1). The type of biomass distribution measured is typical of newly established plants. In the unamended soil, biomass was statistically equal throughout the soil profiles (Fig. 4). These results seem to indicate that the OM within the surface was adequately decomposed, probably by aerobic microbes, making available nutrients to support the production of bigger biomass, compared to the deep soils where decomposition is slow, and less or low nutrient availability, resulting in smaller biomass production. Despite the flooded soil conditions, plants co-existing with OM lowered the soil pH (Fig. 1) when the opposite happened in the soil without plants (Fig. 4). The overall change was in the range of 6 units at the surface to near 5 units at the deep soil (Fig. 1). In the absence of OM, plants significantly acidified the soil, the changes in pH ranging between 5.8 units at the surface soil and 4.3 units at the deep (Fig. 4). Comparatively, even if plants acidified the soil, OM was able to offset that and sustain the pH, conducive for root growth.

Flooded soils are expected to remain reduced because of the reduction reactions and lack of oxygen diffusion. In the presence of plants capable of delivering oxygen into the

rhizosphere, at least a certain degree of oxidation is expected (Hanhart et al., 1997). In almost all cases, presence of plants oxidised the soil, more so in the absence of OM. Co-existence of OM and plants oxidised the surface soil (Fig. 2). This might have been possibly facilitated by the combine effects of oxygen that penetrated the soil via the aerencymatous tissues, porosity created by the added OM and by the roots. In the soil without added OM, the soil was reduced and the changes in Eh were high, compared to the results shown in Fig. 5. This variability demonstrates that the plants delivered oxygen, sufficient to oxidise the soil however offset by OM (Yuan et al., 2015a, b).

The changes in sulfate contents measured in the soil amended with OM and planted without OM are shown in Figs. 3 and 6, respectively. Except in the surface soil, plants had minimal effect on the sulfate content soil amended with OM, following an observation that in the profiles of bigger biomass, a smaller amount of sulfate was quantified (Fig. 3). In the unamended soil, sulfate content was high in the presence of plants and was decreasing at deep soil but higher than that measured in the control soil (Fig. 6). Michael (2018b) pointed out that sulfate reduction occurs when the condition, i.e., Eh <-100 mV and pH at least >5 is sufficient for SRB to function. The high sulfate content shown in Fig. 6 corresponds to the high redox values of Fig. 5, the major reason why SRB did not function under these soil conditions. The results of the planted soil following OM amendment being similar show the same mechanism was operating under the conditions of the experiment shown in Fig. 3.

Management of the mechanisms underlying the changes in ASS chemistry

This study shows that plants will accelerate the fall in soil moisture because of water use in photosynthetic reactions, evapotranspiration and oxygenation of the rhizosphere via the modified structures, leading to oxidation. The results further show that if oxidation happens in soil with live plants co-existing with sufficient OM, e.g. from dead plants that have drowned as a resulting of flooding, then the negative impacts of the oxidation process will be offset. On the other land, the results indicate that live plants existing alone could potentially cause oxidation and acidification of the soil. The underlying mechanism for the changes in soil chemistry measured in the presence of *P. australis* is that this plant used its modified structures (aerencymatous tissues) to pump oxygen into the rhizosphere, leading to oxidation and lowering of the pH when without OM (Armstrong et al., 1996; Mark et al., 1994). When oxygen was pumped into soil with sufficient OM, soil microbes, especially the facultative ones, used the available oxygen to oxidise the OM, reducing the redox and sulfate content, thereby decreasing the pH. Under flooded soil conditions, decomposition of OM is slow and release of nutrients from the OM to support root growth is small (Michael and Reid, 2018). The bigger biomass produced by plants co-existing with OM (Fig. 1) compared to without OM (Fig. 4) seems to suggest that sufficient nutrients were available from aerobic decomposition using oxygen that were pumped into the soil. These nutrients were utilized by the plants to produce the bigger biomass shown in Figs. 2 – 3. In the absence of OM, the reducing conditions created by flooding were not sufficient to consume the oxygen that was pumped into the soil, resulting in oxidation, which led to lowering of the pH and no effect on the sulfate content (Figs. 4 – 6). Under these conditions, nutrient limitation resulted in small biomass production, as shown in Fig. 4.

The management implications revolve around the balance between OM turnover and live plants (Michael and Reid, 2018). Under normal soil use and management conditions, OM

addition from mulch, turnover of plant matter or organic compound secretion by plant roots enable soil microbes to act and modify the soil chemistry (Reid and Butcher, 2011; Pocknee and Summer, 1997). These processes are sufficient to create micro-environments conducive to alter the pH (Yan et al., 1996), redox and sulfate content, important for management of sulfidic soil. In flooded soils, reduction reactions consume available oxygen and sustain alkalinity. This study showed flooding did not sustain sulfidic soil alkalinity when *P. australis* was present without OM. An option to manage the oxygen entry into the soil via the modified anatomical structures is to remove the shoots by cutting them off, even if the remaining culm will still pose problems (Michael and Reid, 2018). This strategy will not only reduce oxygen entry but the dead shoots will help build up the OM, which will further reduce the soil when microbes act on the resources and generate alkalinity.

Conclusion

In contrast to the positive effects on sulfidic soil of OM derived from dead plant material, turnover of OM 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 in sulfidic soil induces acidity rather than sustaining alkalinity. OM addition gave rise to more root biomass, resulting in more oxygen into the rhizosphere of the plants. Under falling water regimes such as during a change in climatic conditions, an important management option would be to slash the plants and return them as mulch, generating alkalinity through the decomposition processes. Culms would continue to transport oxygen into the soil; therefore, reworking on the soil following slashing to prevent oxygen penetration is an important management option.

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