Annals of Tropical Research 47 (2):19-31(2025)
https://doi.org/10.32945/atr4723.2025

Original Article

Mitigation of the stresses of acid sulfate soils by terrestrial and aquatic plants (*Melaleuca armillaris* and *Phragmites australis*) under varying moisture regimes

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ABSTRACT

The long-term roles of live plant roots in mitigating stresses due to acid sulfate soil remain poorly understood. Three studies, each lasting twelve months, were conducted using *Melaleuca armillaris* and *Phragmites australis*. In the first study, alkaline sandy loam soil was mixed into the sulfuric soil to increase the pH to 6.7, and *M. armillaris* seedlings were planted. In the second and third studies, *M. armillaris* and *P. australis* were planted in sulfuric and sulfidic soils and maintained at 75% water-holding capacity and flooded soil conditions. All the studies were set using 300mm stormwater tubes with sealed bottom ends. The treatments were replicated four times, set up under a glasshouse in a completely randomized design, and harvested after 12 months. The pH and root biomass were measured from the surface, middle, and deep profiles. Results showed that the neutralization obtained by mixing alkaline sandy loam soil with sulfuric soil was stable but deteriorated due to plant root penetration. In the sulfuric soil material (pH <4), *M. armillaris* produced more roots at the surface than in the deep soil under circumneutral pH and aerobic soil conditions. In sulfidic soil material (pH >4), more roots were produced in the deeper soils. In the sulfuric and sulfidic soil materials, *P. australis* produced more roots at the surface than at the deep soil under pH >4 and aerobic conditions. Under anaerobic conditions with a pH >4, root distribution was even. Our findings suggest that common terrestrial and aquatic plants maintain a characteristic distribution of roots to mitigate the stresses of acid sulfate soils.

Keywords: acid sulfate soils, mitigate, moisture, plants, roles, stresses.

Received: 19 April 2025
Revised: 2 September 2025
Accepted: 5 September 2025
Published: 12 December 2025

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INTRODUCTION

Acid sulfate soils (ASS), which contain either sulfuric acid (sulfuric soil material) or have the potential to form it (sulfidic soil material), have been described by Pons (1973) as the "nastiest soil" on earth. The description was given based on the fact that ASS produces sulfuric acid (H_2SO_4), which dissolves the soil matrix in which potentially toxic soil constituents, e.g. As and Al, are held. Production and release of sulfuric acid, mobilization followed by accumulation and transportation of the toxic soil constituents, production of monosulfidic black ooze, and deoxygenation have negative impacts on the natural and built environments (Yuan et al., 2021; Timotiwu et al., 2023). Some of the most common adverse effects of ASS are on soil and the environment (water quality, biodiversity abundance, human health, commercial and recreational fisheries, engineered and community infrastructure, scenic amenities and tourism, and agriculture) (Michael et al., 2015; 2016; 2017). Management of the negative impacts includes neutralization of acidity, control of the by-products of oxidation, and prevention of the exposure of the sulfidic materials. Under general land use and management conditions, applying alkaline material, e.g., agricultural lime, to neutralize the sulfuric soil materials and minimize the exposure of the sulfidic soil are established management strategies (Michael et al., 2015). The main concerns, however, are the need for a large quantity of the alkaline material and the practicality of applying it to large areas of sulfuric soil material. Secondly, when the economic pressure to use the land is high, the alternative of leaving sulfidic soil material unexposed or undisturbed is an unlikely option. For instance, flooding farmland with sulfidic soil to create inundation and prevent exposure is practically impossible because of the need to use the land for farming.

An alternative strategy that has begun to receive equal attention is the application of organic matter of varying nutrients in ASS (Michael et al., 2015; 2016; 2017; Dang et al., 2016; Bob & Michael, 2022; Michael, 2021a). The principle is to deplete oxygen by microbial respiration and induce an anaerobic reduced micro environment to stimulate sulfur-reducing microbes to generate alkalinity. The biogenic alkalinity created depends entirely on the organic matter type and the kind of microbial ecology established (Michael et al., 2016; 2017; Yuan et al., 2018). Compared to agricultural lime, which is expensive, organic matter is readily available, and crop stubbles are often produced on farms. Its application can potentially ameliorate sulfuric soil and prevent the oxidation of sulfidic soil (Michael et al., 2015).

Our recent studies (Michael et al., 2015; 2016; 2017; Michael, 2015a; b; 2018a; b; c; 2020a; b; c; d; Michael & Reid, 2018) and those of Jayalath et al. (2016) established the changes in soil pH, redox, and the sulfate content induced when organic matter is added to ASS. However, these studies did not consider the changes in soil chemistry caused by live plants and how roots respond. Apart from the land users (e.g., farmers), organic matter is shed as dead plant matter (leaf litter and root exudates) by live plants under natural conditions. There is a need to understand the role of live plant roots that have the adaptive advantage to grow in terrestrial and aquatic environments and the underlying mechanism to mitigate stresses, particularly low pH and sulfuric acidity, as well as high pH and inundation in ASS. Understanding the type of above- and below-ground biomass produced by adaptive plants to mitigate stresses, in either sulfuric soil (pH>4) or sulfidic soil (pH<4) material, under different environmental conditions (e.g., aerobic or anaerobic soil), is essential for land use and management planning, (e.g., vegetation establishment on acid scalded land or a farm). It is also necessary to establish clearly whether alkaline soil materials, besides mineral lime and dead plant matter, can be added, particularly to sulfuric soil material, worked into it, and then establish vegetation. The added advantage is that the land users have several options to choose from when the ASS land-use plan is made. The objectives of the three studies were to (i) establish the importance of amending sulfuric soil material with an alkaline sandy loam soil to reduce acidity stresses and (ii) assess the profile-specific distribution of root biomass as an indicator of root responses to mitigate stresses due to changes in pH under varying moisture regimes.

MATERIALS AND METHODS

Source of Soils and Seedlings

The sulfidic soil material was collected from the Finniss River (35˚24'28.28"S; 138˚49'54.37"E) in South Australia. The pH in water (pH_w 1:5 w/w) of the sulfdic soil material was 6.7, the field capacity was 49%, and the organic matter content estimated by weight loss-on-ignition was 10.6%. Soil classification using the Soil Taxonomy (Soil Survey Staff, 2022) is given by Michael et al. (2016). The sulfidic soil material was kept underwater in sealed tubs to prevent exposure and oxidation before use. To produce sulfuric soil, the sulfidic soil material was oxidized under glasshouse conditions to pH_w<3.7. To make the soil material used in the first study (hereafter referred to as "neutralized sulfuric soil", NSS), alkaline sandy loam soil (pH_w 9) was obtained from a supplier in Adelaide, South Australia. The sulfuric soil was mixed with the alkaline sandy loam soil (1: 2) using a portable cement mixture by slow addition until the NSS pH_w was 6.7, similar to the Finnis River sulfidic soil of pH_w 6.6. This study was purposely designed to assess how sulfuric soil acidity can be mitigated to allow the establishment of plants. The *M. amillaris* seedlings (less than a month old) were obtained from a local supplier in Adelaide, South Australia, and used in the studies. The *P. australis* shoots with up to 5 leaves (less than a month old) were obtained along the Adelaide River, Adelaide, South Australia, by isolating them from the parent stocks with intact roots. The entire shoots were brought to the glasshouse, and the roots were carefully washed under running tap water and acclimatized by leaving them in a tub of tap water for two days before planting. *M. amiliaris* and *P. australis* were used as common terrestrial and aquatic (wetland) plants, respectively.

Experimental Design

All three studies used 300mm stormwater tubes, the bottom ends of which were tightly sealed with screw caps, and each was filled with 1300g of the different soil materials. In the first study, NSS (pH_w 6.7) was used and planted with *M. armillaris* seedlings. In the second study, *M. armillaris* seedlings were planted in sulfuric (pH_w 3.7) and sulfidic (pH_w 6.7) soils. Similarly, *P. australis* was planted in sulfuric (pH_w3.7) and sulfidic (pH_w 6.7) soils in the third study. The first two studies maintained under aerobic soil conditions (75% water holding capacity [WHC] on a weight basis). In contrast, the third study was kept under flooded soil conditions throughout. Three seedlings or shoots were planted in each stormwater tube for each treatment. The control treatments were left unplanted with corresponding moisture levels maintained. All the treatments were replicated four times and set up under glasshouse conditions for twelve months in a completely randomized design to allow the plants to reach maturity. For each treatment, data were collected from three profiles from three stormwater tubes (replicates), and the fourth was kept frozen for security against loss.

Sampling and Measurements

All the sampling and measurements were made within the soil surface (0–20mm, 20–100mm, 100–200mm, and 200–300mm), respectively. To sample soil for pH measurement and collect the roots, a permanent marker was used to mark the profiles on the tubes that were then cut using a small handheld saw, and carefully placed in pre-labeled trays to avoid mixing them. The soil inside the cut tubes with or without roots was freed by gently pushing them out with the help of a metallic object with a diameter similar to that of the tubes. The soil and the roots were separated by gently breaking the soil up using a metal spoon. All the soils were placed in pre-labeled 250mL vials for pH measurement. The roots from each profile were collected into a 0.5mm metal sieve per treatment, gently washed under running tap water, blot-dried, and placed in an oven at 70°C overnight. The pH was measured using 2g of soil (1:5; soil: water) using a pre-calibrated Orion pH meter (720SA model) per profile per treatment. Similarly, the dry weights of all the roots from each profile per treatment were weighed and recorded.

Statistical Analyses

The treatment average pH and root weight were obtained by taking the mean of the three replicates. Significant differences ($p < 0.05$) between treatment means of a profile were determined by two-way ANOVA using statistical software JMPIN, AS Institute Inc., SAS Campus Drive, Cary, NC, USA 27513 to compare the treatment means. If an interaction between the treatments and profile depths was found, one-way ANOVA with all combinations was performed using Tukey's HSD and pairwise comparisons. In all the data figures, the values are mean ± standard error of three replicates (n=3). An asterisk indicates a significant difference ($p \leq 0.05$) between the control and the treatment at the same depth.

RESULTS

Effects of Sulfuric Soil Acidity Neutralization with Alkaline Sandy Loam Soil and Planting

The changes in pH of the control treatments (unplanted) are shown in Table 1. What is interesting to note from a general soil use point of view is that the neutralization obtained by mixing an extremely acidic soil (pH<4) with an alkaline sandy loam soil (pH_w 9) was stable and increased to 8.4 at the deeper soil level. The pH of the sulfuric soil, either at 75% or 100% field capacity, increased significantly more than 2 units throughout the profiles. The sulfidic soil pH remained nearly unchanged within the surfaces and increased as soil depths increased (Table 1). The residual organic matter content (10%) was sufficient to generate biogenic alkalinity to raise the pH of the sulfuric soil and sustain that of the sulfidic soil when exposed (e.g., Michael et al., 2015; 2016; 2017).

Table 1. Changes in soil pH of the unplanted control treatments.

Soil type	Moisture	Control	Profile-specific changes in pH at various levels (mm)				Data tables and figures
	(%)	soil pH	0-20	20-100	100-200	200-300	
NSS	75	6.7±0.2	7.8±0.2	7.2±0.2	7.0±0.4	8.4±0.4	Figure 1
Sulfuric	75	3.7±0.3	5.6±0.4	6.4±0.0	6.6±0.2	6.8±0.2	Table 2 and Figure 2
Sulfuric	100	3.7±0.2	5.9±0.3	6.3±0.4	6.5±0.3	6.9±0.3	Table 3
Sulfidic	75	6.8±0.1	6.0±0.0	6.9±0.3	7.0±0.2	7.3±0.2	Figure 3 and Table 4
Sulfidic	100	6.8±0.2	6.7±0.2	7.1±0.2	7.3±0.0	7.6±0.4	Figure 4

The changes in pH of the NSS and the root biomass of *M. armillaris* planted in it are shown in Figure 1. The neutralization obtained by mixing alkaline sandy loam soil into the sulfuric soil was lost when planted, and the soil remained acidic. Under these highly acidic soil conditions, the root biomass measured was within the range of 1–2g per profile (Figure 1), a significant amount of root accumulation, considering the small amount of soil used. In the planted soil, the surface soil pH remained near 5, and in the rest of the profiles decreased to nearly the pH of the sulfuric soil of 3.7. The overall changes in pH measured showed the pH of the control NSS was stable; however, planting resulted in a loss of the neutralizing

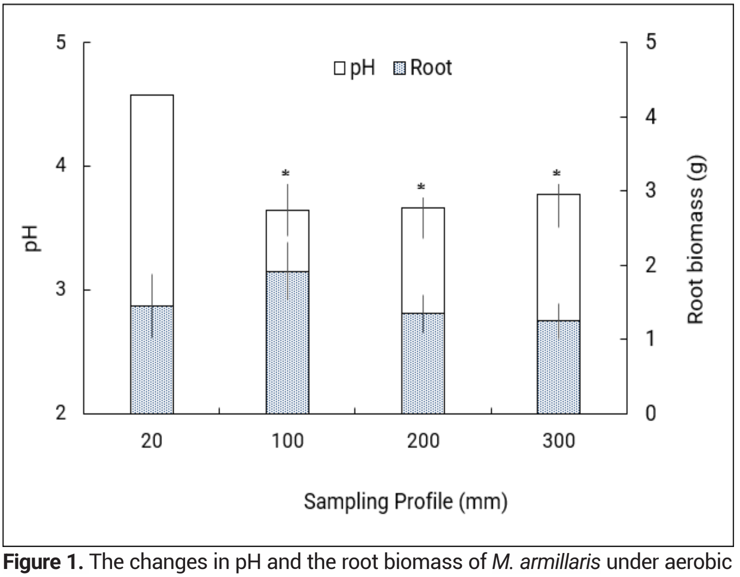

Figure 1. The changes in pH and the root biomass of *M. armillaris* under aerobic NSS soil conditions.

Soil pH-dependent responses of M. armillaris under Aerobic Sulfuric Soil Conditions

When *M. armillaris* was planted in the sulfuric soil under aerobic soil conditions, the pH increase ranged from 4.7 at 20mm to 4.6 at 300mm (Table 2), compared to the control soil pH, which ranged from 5.6 to 6.6 (Table 1). The changes in pH of the planted sulfuric soil measured remained nearly the same throughout the profiles. The root biomass, on the other hand, decreased from the surface to the deep by nearly 1.2g. There was no clear relationship between the soil pH and the distribution of root biomass measured.

Table 2. The changes in pH and the root biomass of *M. armillaris* under aerobic sulfuric soil conditions (75% moisture).

Parameters	Sampling profiles (mm)			
	20	100	200	300
Planted soil pH	4.7±0.2*	4.0±0.3*	4.4±0.4*	4.6±0.2*
Root biomass (g)	2.5±0.1	1.8±0.2	1.5±0.3	1.3±0.1

Soil pH-dependent responses of P. australis under Aerobic Sulfuric Soil Conditions

When *P. australis*, a common wetland plant (compared to the common inland shrub, *M. armillaris*), was planted in sulfuric soil and maintained under aerobic (75%) soil conditions (Figure 2), the changes in pH were similar to those induced by *M. armillaris* (Table 2). The surface soil pH increased to 5.6, and in the lower profiles, decreased to around pH 4 (Figure 2). The root biomass accumulation measured, compared to the terrestrial counterpart's root biomass shown in Figure 2, increased from the surface to the deep profiles (Figure 2). The overall results, in general, showed that as the soil pH was higher, the root biomass was smaller. For example, when the pH was 5.8 within the 20mm sub-surface, the root mass was only 0.65g. When the pH decreased to 4.43 at the deep, the root mass was almost 3g (Figure 2), an increase of over 400%.

Soil pH-dependent responses of P. australis under Anaerobic Sulfuric Soil Conditions

Under anaerobic soil conditions, the control sulfuric soil pH increased to 6.5 in the deep soil (Table 1), and when planted, decreased to 5.5 and remained relatively constant throughout the profiles (Table 3). The root biomass, on the other hand, decreased from the surface to the deep, ranging from 3.2 to 1.5g. These results are interestingly the opposite of the results of the study shown in Figure 2.

Table 3. The changes in pH and the root biomass of *P. australis* under anaerobic sulfuric soil conditions (100% moisture).

Parameters	Sampling Profiles (mm)			
	20	100	200	300
Planted soil pH	5.5±0.4*	5.5±0.3*	5.5±0.2*	5.6±0.3*
Root biomass (g)	3.2±0.0	2.3±0.1	1.7±0.2	1.5±0.1

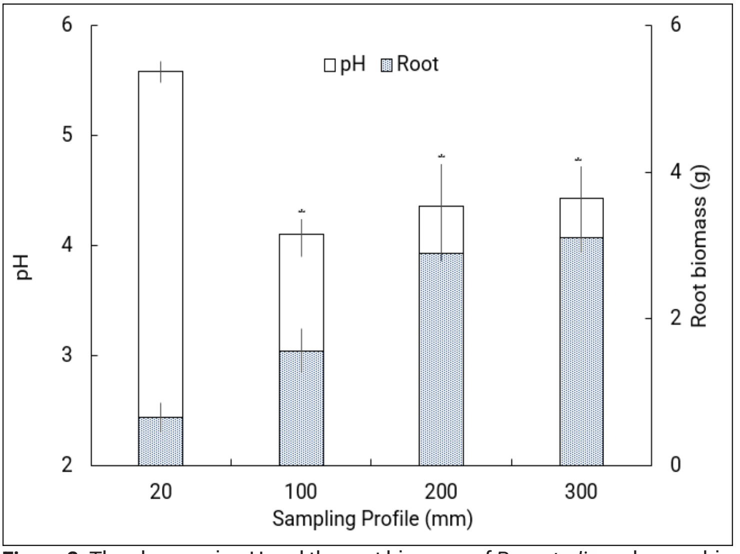

Figure 2. The changes in pH and the root biomass of *P. australis* under aerobic sulfuric soil conditions.

Soil pH-dependent responses of M. armillaris under Aerobic Sulfidic Soil Conditions

The study presented in Figure 3 showed that the sulfidic soil had acidified as expected under the aerobic (75% WHC) soil conditions, and the pH had decreased to nearly 4.6, and even lower in the deep soils. The root biomass was higher where the profile's pH was low. For instance, in the 100 to 300mm soil depth, pH was 3.7 (acidic) throughout, and the root mass was between 1.9g and 2.8g, with an increase of 0.9g of roots in the deep soil. The reason for the increase seems to be the increase in accumulation of root biomass at the sealed end of the stormwater tubes. As soil pH decreased in the lower profiles, the biomass increased accordingly (Figure 3).

Soil pH-dependent responses of P. australils under Aerobic Sulfidic Soil Conditions

The results of the study conducted using the wetland plant on sulfidic soil are shown in Table 4. Generally, the pH of the sulfidic soil decreased throughout planting except at 100mm, where it decreased to pH 4.4. The biomass generally accumulated in the deep soil where the pH was higher, and supports the finding that the plant macrophyte is sensitive to pH<4 (e.g., Tilley & John, 2012; Jones, 2022). For example, the biomass at 100mm was 1.9g, where the pH was the lowest (Table 4).

Table 4. The changes in pH and the root biomass of *P. australis* under aerobic sulfidic soil conditions.

Parameters	Sampling Profiles (mm)			
	20	100	200	300
Planted soil pH	5.4±0.1*	4.4±0.2*	5.3±0.4*	5.6±0.2*
Root biomass (g)	0.9±0.2	1.9±0.1	2.4±0.2	3.2±0.0

Soil pH-dependent responses of P. australils under Anaerobic Sulfidic Soil Conditions

Comparatively, not much change was observed in the sulfidic soil pH under flooded soil conditions (Figure 4). The pH of the planted sulfidic soil was around the mildly acidic level (pH 5.5–6). The biomass was generally the same throughout the profiles (3g) except at 100mm. The highest biomass measured was in the profile with soil pH 5.4, which was the lowest pH, very similar to the result of the common inland plant counterpart (Figure 3).

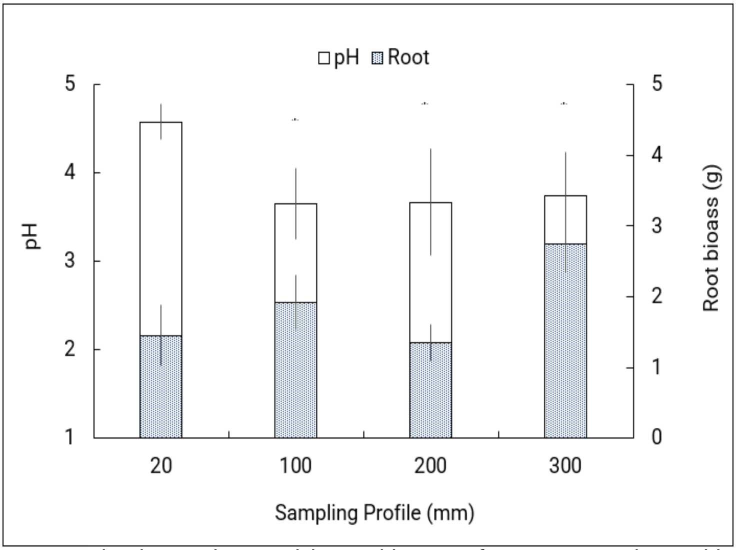

Figure 3. The changes in pH and the root biomass of *M. armillaris* under aerobic sulfidic soil conditions.

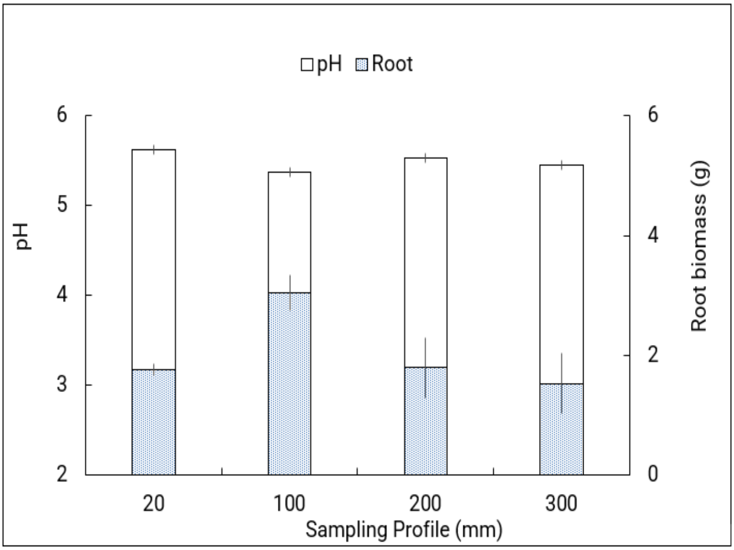

Figure 4. The changes in pH and root biomass of *P. australis* under anaerobic sulfidic soil conditions.

DISCUSSION

In the first part of this study, we explored whether sulfuric soil (pH<4) can be neutralized by adding an alkaline soil material (pH 9) and establishing a terrestrial plant (*M. armillaris*). The results showed that the neutrality of NSS obtained by mixing sulfuric soil (pH<4) with alkaline sandy loam soil (pH 9) was lost except on the surface (20mm), and the soil remained acidic (Figure 1). The probable reason for this is that the pore spaces created by root penetration facilitated oxygen to enter the soil and oxidize the rhizosphere, resulting in the production of sulfuric acidity. The lower root biomass at lower pH confirmed that plants tend to produce fewer roots in acid soils as a result of Al^{3+} and Mn^{2+} toxicity and a decrease in the availability of soil nutrients (Lu et al., 2020). When the same plant was planted in sulfuric soil without amendment under 75% WHC, the soil pH increased to near pH 5 throughout the profile. This increase in pH is understood to be caused by microbial oxidation of the residual organic matter content, as we have reported in other studies (Michael et al., 2016; 2017). The display of root biomass, however, was such that there were more roots at the surface than in the deep soil (Table 2). This, to a large extent, shows that terrestrial plants accumulate roots in the surface soil with sufficient oxygen rather than at a depth with a lesser oxygen supply. This is an adaptive mechanism of terrestrial plants to avoid suffocation and death of roots due to oxygen shortage. The various mechanisms, such as root–shoot, ethylene, and calcium signaling, and an altered reactive oxygen species dynamic, are responsible for helping plants survive under soil conditions with limited oxygen (Peláez-Vico et al., 2023; Yang et al., 2022).

When an aquatic (water-loving) plant (*P. australis*) was established in the sulfuric soil at 75% WHC to compare the results with those of its terrestrial counterpart (Table 2), the root biomass was small where pH was high and the opposite when low (Figure 3). This display of root distribution is quite different from that shown in Table 2 and demonstrates that aquatic plants produced more roots in response to stress (e.g., low pH) than their terrestrial counterparts. On the other hand, displaying more roots at the bottom soil profiles would be an adaptive mechanism displayed by such plants to avoid soil moisture (aerobic conditions) stresses. This is supported by the established knowledge that under aerobic soil conditions (75% WHC), the surface soil profiles are drier than at depth because of direct exposure to sunlight. The opposite was confirmed when the planting was done under flooded soil conditions (100% WHC). The pH increased to a circumneutral level as expected because of the reduction reactions caused by inundation (Table 3). However, the root distribution was different compared to the aerobic soil conditions (data shown in Figure 3). More roots were produced at the surface soil than at the deep even though the pH was nearly the same throughout (Table 3). Under flooded soil conditions, aquatic plants have developed adaptive mechanisms to pump oxygen to the rhizosphere through their parenchymatous tissues and oxygenate the reduced soil conditions to escape suffocation. Our results showed that aquatic plants display more roots at the surface, where there is an adequate supply of oxygen, compared to the deeper levels. Sauter (2013) showed this to be an adaptive mechanism of aquatic plants under flooded soil conditions with limited oxygen.

The study was conducted to understand how terrestrial and aquatic plants established in sulfidic soil under aerobic (75% WHC) and anaerobic (100% WHC) conditions would influence the soil pH and growth by assessing the root biomass. The pH of the sulfidic soil under aerobic soil conditions was expected to acidify due to oxidation, but, as shown in Table 4, this did not happen. This was mainly due to the oxidation of the residual organic matter content, as pointed out earlier. The plant macrophyte produced more roots (3g) at the deeper profiles compared to the surface (0.9g) under aerobic soil conditions (Table 4). In contrast, under anaerobic soil conditions, the root biomass was more equally produced (Figure 4). The display of more roots in the deep soil was evident too in the sulfuric soil (Figure 2) and seems to confirm that this is an adaptive mechanism. The root biomass produced under the anaerobic soil conditions, being almost the same at all levels, showed that the root production of aquatic plants is not directly influenced by excess moisture *per se*. There was evidence, though, that such plants produce more roots at the surface than in the deep soil. This is more evident for terrestrial plants that are used to soil conditions with adequate oxygen presence.

CONCLUSION

Root biomass distribution under common stress conditions (e.g., acidic, drought, or flooded soil conditions) is an essential indicator of roots mitigating the stresses. This study showed that mixing alkaline soil into sulfuric soil would help reduce acidity stress conducive to establishing vegetation. Under flooded soil conditions of high pH, the wetland plant studied produced more roots at the surface than in deep soil to mitigate suffocation. In aerobic sulfuric soil of mildly acidic pH, the terrestrial plant grew more roots at the surface than the wetland plants. In the sulfidic soil, both plants produced more roots in the deep soils, even if the pH was variable. The results of these studies have implications for mitigating acid sulfate soils under different soil use and management conditions.

Acknowledgment

The study was conducted at the School of Earth and Environmental Sciences, The University of Adelaide, South Australia, Australia, under an Australian Development Scholarship (AusAID) to Patrick S. Michael. The authors are grateful to everyone who has contributed to the study.

Author Contributions

PSM and RJR designed the study, conducted the experiment, collected the data, processed the data, performed a literature review, and wrote the manuscript, and RWF contributed to the final manuscript. All the authors read the final manuscript.

Funding Source

This study was financed by an Australian Development Scholarship to PSM.

Availability of Data and Materials

The data that support the findings have been included in the manuscript.

Ethical Considerations

The study did not involve humans or animals.

Competing Interest

There is no conflict of interest from the authors to declare.

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