

THE TIME COURSE OF EFFECTS OF SIMPLE CARBON AND ORGANIC MATTER ON Ph AND REDOX POTENTIAL OF ACID SULPHATE SOILS

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Abstract

It has been shown recently that addition of dead plant materials as organic matter in acid sulfate soils (ASS) creates microenvironments conducive for soil microbes to reduce the sulfate content and redox potential (Eh), thereby increasing the pH of sulfuric soil and sustaining the pH of sulfidic soil. The time course of effects of the addition of organic matter however was not clearly established. This study showed that the effect of addition of amendments on pH and Eh of ASS is immediate but treatment dependent. Organic matter as a multiple food source for soil microbe was more effective in reducing the soil pH, increasing the pH of the sulfuric soil and sustaining sulfidic alkalinity. The effects of acetate was comparatively higher than glucose, dependent on the type of microbial ecology that were engaged being simple carbon sources.

Key words ASS, time-course, simple carbon, organic matter, pH, Eh

INTRODUCTION

Acid sulfate soils (ASS) are naturally occurring soils that either contain actual sulfuric soil (pH<4) or sulfidic soil (pH>4) that has the potential to form it (Pons, 1973) in an amount that can have serious negative impacts on other soil properties (Fitzpatrick *et al.*, 2009). Under inundated soil conditions, sulfidic soils pose no threat unless exposed to atmospheric oxygen and the sulfidic minerals (e.g. pyrite, FeS₂) are oxidized, resulting in production of sulfuric soil (acidity). Release of the sulfuric acid in turn leads to solubilization of potentially acidic minerals and toxic constituents from the soil matrix in which these are held (Michael *et al.*, 2014). Generation of sulfuric acidity, mobilization of acidic minerals (e.g. Fe³⁺ and Al³⁺) and accumulation of potentially other toxic soil constituents (e.g. As) are responsible for the negative environmental and health impacts (Michael, 2013). Unless successfully managed, the negative impacts on soil and water characteristics, human health, commercial and recreational fisheries, engineered and community infrastructure, scenic amenity and tourism, and agricultural

productivity are seriously compromised (Powell and Martens, 2005). The seriousness of the negative impacts of ASS has led to development of several management strategies, focusing on two key principles. The first principle is to neutralise the sulfuric acidity by application of an alkaline material, e.g. agricultural lime. The second principle is to curtail sulfidic soil oxidation by inundation through flooding (Fitzpatrick *et al.*, 2009). In some places, however, lime availability is a problem (Powell and Marten, 2005) and under many general soil use conditions, flooding is undesirable (Michael *et al.*, 2015b).

We have shown recently that addition of plant materials in the form of complex organic matter containing varying carbon and nitrogen content creates alkalinity using microbial processes (Michael *et al.*, 2012). Addition of the organic matter created anoxic environment by microbial depletion of oxygen, conducive for microbes, such as sulfate reducing bacteria (SRB) to flourish and generate biogenic alkalinity (Michael *et al.*, 2015a). We further found that the biogenic alkalinity generated resulted in amelioration of sulfuric soil (increased pH) and prevented oxidation of the

sulfidic soil (Michael *et al.*, 2016). In comparison to lime, our findings are significant contributions towards ASS management with organic matter being relatively cheap and readily available. We have also reported the effects of live plants on acidification, redox potential and sulfate content recently (Michael *et al.*, 2017).

In our studies (e.g. Michael *et al.*, 2015), organic matter was either applied as surface mulch or incorporated in the ASS and incubated for “six months” prior to the measurements. As the changes in soil chemistry (pH, Eh and sulfate content) measured were based on “long-term studies” of six months, the short-term changes in soil chemistry induced by the organic matter applied (an important information needed for timely management of ASS negative impacts) was not clear. Therefore, this study was conducted to assess the time-dependent changes in ASS chemistry (pH and Eh) induced following addition of amendments over a shorter period (12 weeks).

MATERIALS AND METHODS

Soil: The ASS used in this study were well described in Michael *et al.* (2016). The sulfidic soil was collected from a “sulfuric subaqueous clayey soil” (Fitzpatrick, 2013) at a depth of approximately 1 m in the Finnis River in South Australia (35°24'28.28”S; 138°49'54.37”E). Figure 1 of Michael *et al.* (2015a) shows the map of the sampling site. Detailed information on soil classification using the Australian ASS Identification key (Fitzpatrick *et al.*, 2008) and Soil Taxonomy (Soil Survey Staff, 2014) are given in Table 1 of Michael *et al.* (2016). In addition, a comprehensive list of references containing additional information on soil morphology and geochemistry prior to rewetting (i.e. sites AA26.3 and FIN26 in Fitzpatrick *et al.* (2009a)) and after reflooding are given in the same table. The pH of the freshly collected sulfidic material measured in water 1:5 (pH_w) was 6.7 and the pH following peroxide treatment (pH_{ox}) was 1.4, with the water holding capacity estimated to be 49%. The residual organic matter content, estimated using the weight loss-on-ignition method (Schulte and Hopkins, 1996) was 10.6%. To manufacture “sulfuric horizon material” by oxidising the sulfides, the sulfidic soil material was spread thinly on plastic sheets and kept moist until $pH_w < 4$. The manufactured sample and freshly sampled sulfidic material are henceforth referred to as “sulfuric soil” ($pH_w < 4$) and “sulfidic soil” ($pH_w > 4$), respectively.

Carbon and organic matter sources:

Laboratory grade glucose and acetate, obtained from a chemical store within the School of Biological Sciences, The University of Adelaide, South Australia were used as simple carbon sources. To use as organic matter, bales of lucerne hay were purchased from a supplier in Adelaide, South Australia and prepared as described by Michael *et al.* (2016). The plant materials were chopped into pieces and then oven dried at 60 °C for three days. The dry pieces were finely chopped using an electric blender to ≈ 0.5 mm. The nitrogen content of the organic matter analysed by ICP-OES using a 0.5 g samples ($n=3$) was estimated to be 3.2%. The carbon content can be approximated from the data in Kamp *et al.* (1992).

Experimental treatments: Three experiments were conducted as described below with glucose and acetate as simple carbon sources and lucerne hay as organic matter incorporated in the soils by bulk mixing in proportions of 25:2, 10:1 and 80:1 (amendments: soils w/w). Set in 80 ml Falcon tubes, all the treatments were replicated three times and set out in a complete randomized design under glasshouse conditions. Although the ‘aerobic treatments’ were regularly watered, it was probable that the moisture was unevenly distributed over time, with the upper parts being aerobic and the lower parts of the profile becoming waterlogged. The anaerobic treatments were always under flooded conditions with adequate amount of water ponding on the surfaces (Michael *et al.*, 2016).

Experiment 1: This was conducted under flooded condition using sulfuric soil to assess the changes in soil chemistry induced by organic matter as a function of time over 5 weeks.

Experiment 2: Two sets of treatments with glucose, acetate or organic matter mixed in sulfuric soil each were prepared. The first sets of the treatments were maintained under aerobic soil (regular watering) conditions while the second sets were maintained under anaerobic (flooded) soil conditions, respectively.

Experiment 3: The description of this experiment is similar to experiment 2 except that sulfidic soil was used instead.

Measurements: In experiment 1, pH and Eh were logged on a daily basis from within the top 20 mm of the soils. In experiments 2 and 3, changes in Eh and pH were measured from the surface (0-10 mm), middle (10-20 mm) and deep (30-40 mm) profiles as described previously (Michael *et al.*, 2015). Redox was measured using a single Ag/AgCl reference and platinum (Pt) electrode combination using an automated data logger (Dowley *et al.*,

1998; Merry *et al.*, 2002). To measure the Eh, the frame of the redox probe was marked as per the targeted profiles from the tip (e.g. 0-10 mm etc.). During measurement, the Pt and the reference electrodes were both inserted into the soil from the surface. The reference electrode remained inserted whilst the Pt electrode was driven invariably once at a time into the soil. This was allowed to equilibrate for 10 min and then Eh logged at 1 min intervals for the next 10 min 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 as per Fiedler *et al.* (2007). pH was measured using a 2 g soil (1:5 water) with a pre-calibrated Orion pH

meter (720SA model) (Michael *et al.*, 2012).

Statistical analysis: The Eh values obtained over a 10 min period were averaged and a treatment average obtained by taking the mean of the three replicates. Similarly, treatment average pH was obtained by taking the mean of the three replicates. To compare the treatment means, significant differences ($p < 0.05$) between treatments means of each profile was determined by two-way ANOVA using statistical software JMPIN, AS Institute Inc., SAS Campus Drive, Cary, NC, USA 27513. If an interaction between the treatments and profile depths was found, one-way ANOVA with all combination was performed using Tukey's HSD (honest significant difference) and pairwise comparisons.

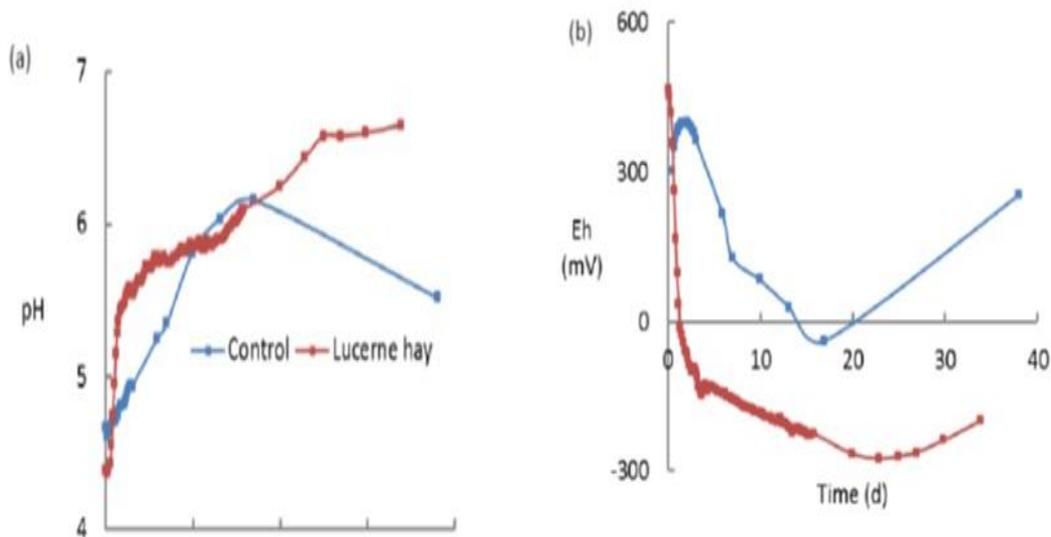


Fig. 1. Short-term changes of (a) pH and (b) redox of sulfuric soil maintained under anaerobic conditions with or without addition of organic matter (lucerne hay). The initial pH was 4.4.

RESULTS AND DISCUSSION

Effects of organic matter on sulfuric soil pH and Eh:

The changes in pH and Eh of the sulfuric soil as a function of time initially measured over 5 weeks in an 80 g of flooded soil with or without incorporation of 1 g of lucerne hay are shown in Figure 1. Within the first 3 days, an immediate increase in pH by 1.1 units from an initial pH of 4.4 and a decrease in Eh were observed in the lucerne hay treatment. Changes in the control treatment were slower. Over the next 14 days, pH continued to increase and Eh declined. Between 18 and 38 days, the pH of the control soil fell, corresponding to an increase in Eh, while the pH of the lucerne hay

treatment continued to rise and Eh was relatively stable. The time-course of changes in pH and Eh following the addition of complex organic matter showed that the values obtained by these treatments are highly dependent on treatment time. The highly oxidised sulfuric soil got reduced as expected due to the flooded condition which created anoxia in both the control and in the soil with organic matter. The changes in pH and Eh measured however were influenced by the added organic matter. The soil used contained 10.6% residual organic matter (Michael *et al.* 2015b) but the addition made significantly influenced the overall changes measured. For example, within the first 15 days, both the pH of the control and amended soils

increased, and there was a concurrent reduction in Eh but the changes in the amended soil pH were higher (Fig. 1a) and reduction in the Eh were lower (Fig. 1b). As the small amount of residual organic matter in the control soil got depleted by anaerobic microbes, the pH lowered in response to a positive increase in Eh within the next 20 days. Comparatively, the pH continued to increase and Eh decreased in the complex organic matter amended soil parallel to the slow decomposition of the complex plant materials.

It has been reported recently that the close correlation between the generation of alkalinity and the decrease in soil sulphate concentration is a strong indication that the pH changes resulted from the action of sulphate-reducing bacteria that used the organic matter as a carbon source (Michael *et al.*, 2016). The time course of effects of the changes in pH, Eh and sulfate content were not clearly established in ASS following the addition of amendments. The rapid changes in pH and Eh immediately following flooding (Fig. 1) suggested that initially aerobic microbial activity was involved and that the oxygen demand generated by these organisms drove the Eh into the range that was more suitable for anaerobic bacteria such as sulfate reducers. The pH of the control soil also responded to flooding and increased over the first 18 days but then began to decline. The changes in Eh mirrored the changes in pH. The differences between the control soil and the lucerne treatment were in the speed and magnitude of the changes, being much slower in the control and reversing after approximately 3 weeks (Fig. 1), possibly due to the exhaustion of the residual carbon in the soil. Apart from the alkalising effects found, organic matter contains other essential metabolic substrates in addition to carbon and nitrogen (Jarvis and Robson, 1983; Jarvis *et al.*, 1996; Marschner and Noble, 2000) that are beneficial to the sulfuric soil and the microbes in holding water and creating soil microenvironments.

Effects of organic matter and simple carbon on sulfuric soil pH and Eh: The results presented in Figure 1 showed that the daily changes in pH and Eh as a function of time following addition of organic matter were highly dependent on treatment time. Therefore, we monitored the effects of simple carbon compounds (as a major component of the complex organic matter) after 3, 6 and 12 weeks, for comparison. Throughout the 12 weeks, the control soil pH remained strongly acidic under aerobic conditions (Figure 2a). Among the

amended treatments, the order of increase in pH was lucerne hay > acetate > glucose and the changes elicited were rapid and essentially complete by 3 weeks, although lucerne hay continued to increase it beyond the 3 weeks (Fig. 2c). The increase in pH strongly correlated with reductions in Eh, with large changes observed in the lucerne and acetate treatments.

Under anaerobic conditions, the effect of acetate was immediate and lucerne hay with high nitrogen content was time-dependent (Fig. 3). Acetate increased the pH to 6.3 units throughout the profiles within the first 6 weeks (Fig. 3a), with lesser changes by lucerne hay. No responses in the control and glucose treatments were evident during the 6 weeks (Fig. 3b). After 12 weeks, the effects on soil pH of all the treatments remained similar to 6 weeks, except that lucerne hay increased it to units higher than 7 at the surface and 6.5 throughout the profile (Fig. 3c). pH of the soil amended with glucose remained lower than 5, close to the initial pH. The overall effects of the amendments on pH after 12 weeks were: lucerne hay > acetate > control > glucose.

A major difference between the aerobic and anaerobic treatments was in the response of Eh. As shown in Figure 2d-f, the unamended control soils, and the soil amended with glucose remained highly oxidised at the end of three weeks, while the soils amended with acetate and lucerne hay were moderately reduced (150–300 mV) throughout under aerobic conditions. Under anaerobic conditions, the control and the glucose amended soils remained highly oxidised throughout the 12 weeks (Fig. 3d, e, f) but the Eh of the lucerne hay and acetate amended soils were 200 to 400 mV lower, and remained below 0 mV throughout. Under aerobic conditions, acetate and lucerne hay increased the pH of the sulfuric soil to near 7 within the first 3 weeks (Fig. 2a). Within the next 6 weeks, the effects of acetate remained stable but lucerne hay increased to 8 (Fig. 2b-c), a strong indication that decomposition of the organic matter was slow. In the control and glucose treatments, the pH gradually decreased over 12 weeks (Fig. 2a-c). The changes in pH were generally correlated with changes in Eh, with lucerne hay and acetate maintaining an Eh in the range of 100 to 300 mV at the surface within the first 3 weeks then stabilised within the range of 200 to 300 mV in the next 6 weeks (Fig. 2d-f). The Eh of the control and glucose treatments were generally high throughout.

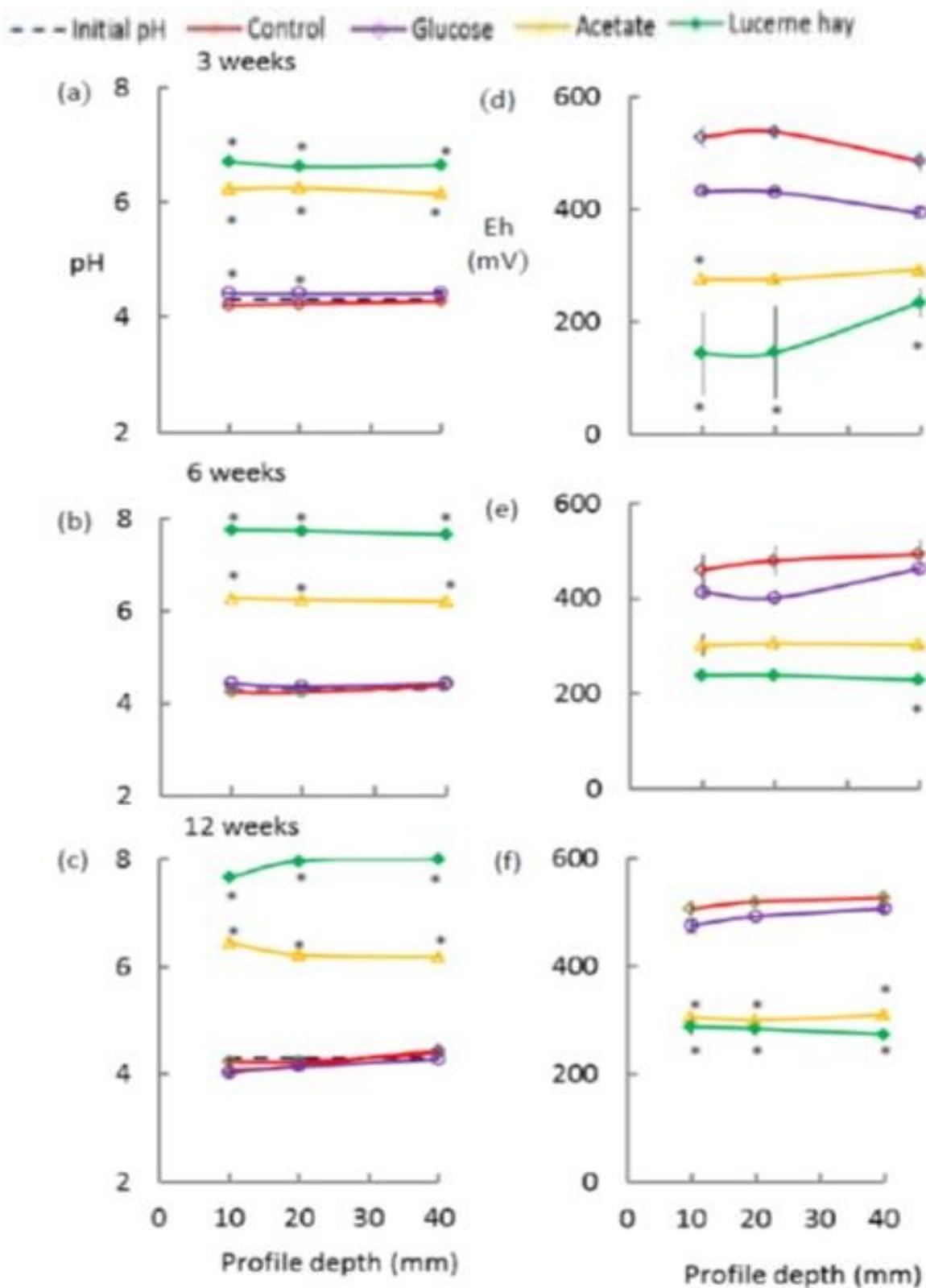


Fig. 2. Time-course of the effects of organic matter and simple carbon sources on (a-c) pH and (d-f) redox of sulfuric soil maintained under aerobic conditions for 3, 6 and 12 weeks. The values are means \pm s.e. of three measurements ($n=3$). Asterisks indicate significant differences ($p < 0.05$) between treatment and control at the same depth.

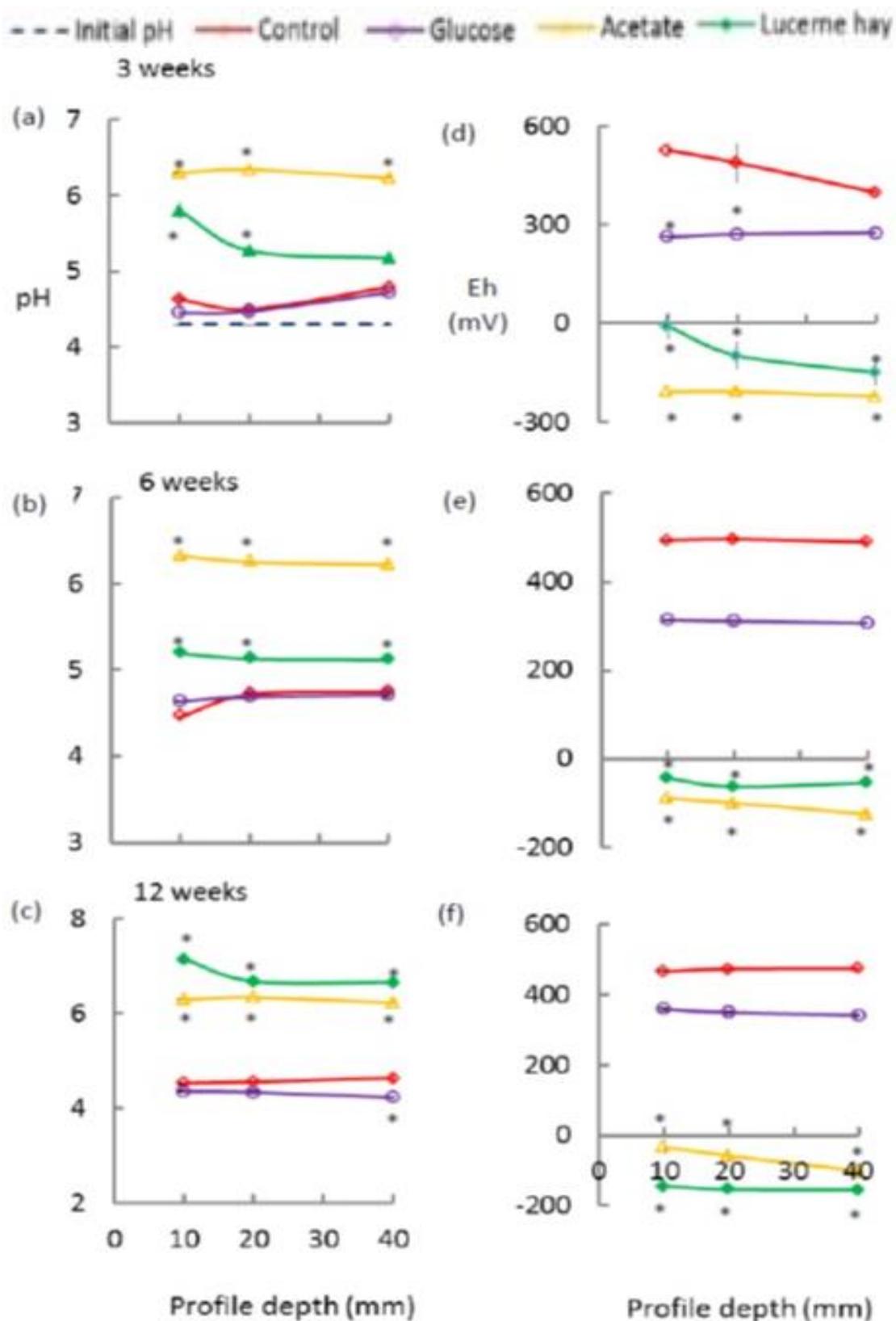


Fig.3. Time-course of the effects of organic matter and simple carbon sources on (a-c) pH and (d-f) redox of sulfuric soil maintained under anaerobic conditions for 3, 6 and 12 weeks. The values are means \pm s.e. of three measurements ($n=3$). Asterisks indicate significant differences ($p<0.05$) between treatment and control at the same depth.

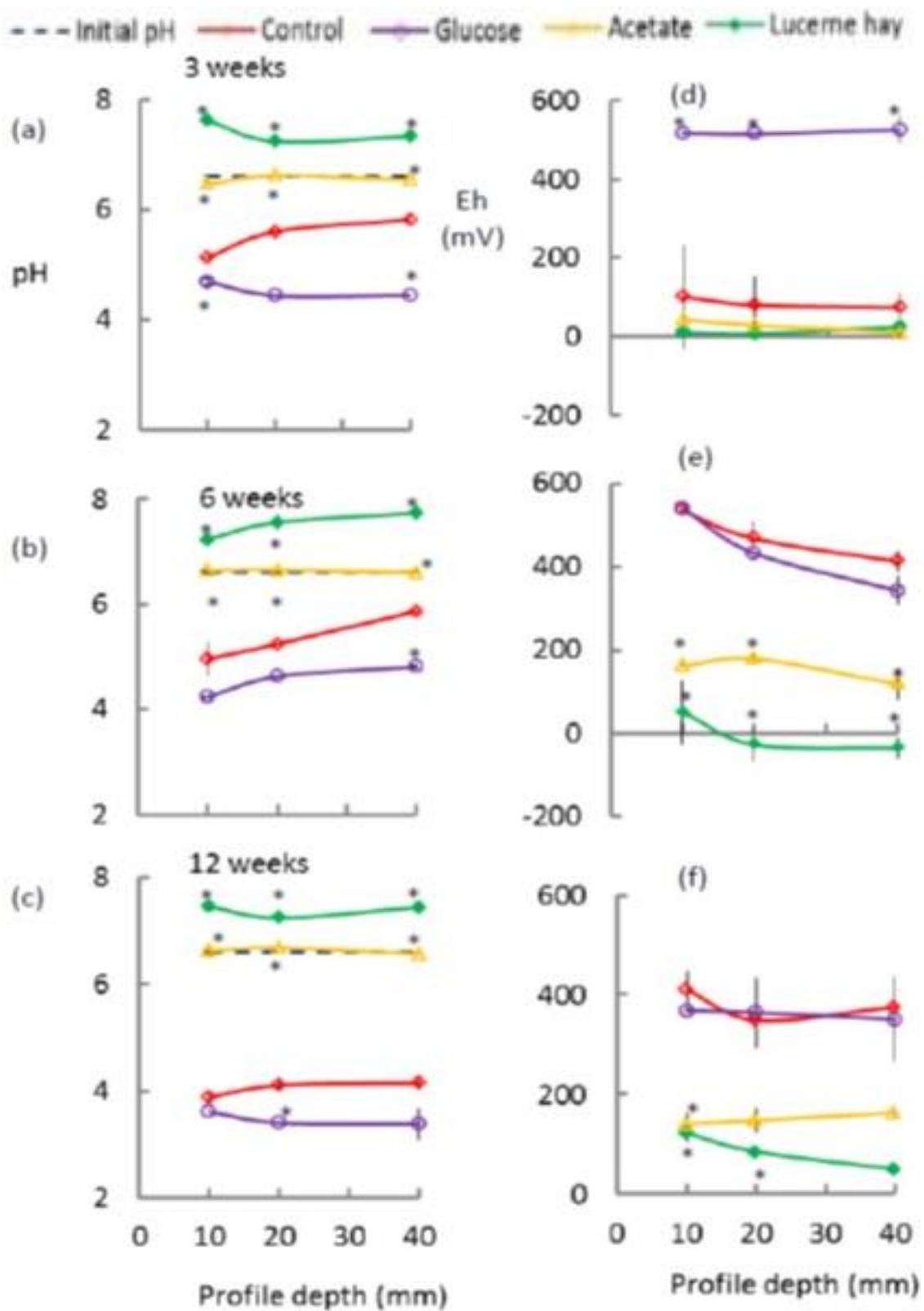


Fig. 4. Time-course of the effects of simple carbon compounds and complex organic matter on (a-c) pH and (d-f) redox of sulfidic soil maintained under aerobic conditions for 3, 6 and 12 weeks. The values are means \pm s.e. of three measurements ($n=3$). Asterisks indicate significant differences ($p<0.05$) between treatment and control at the same depth.

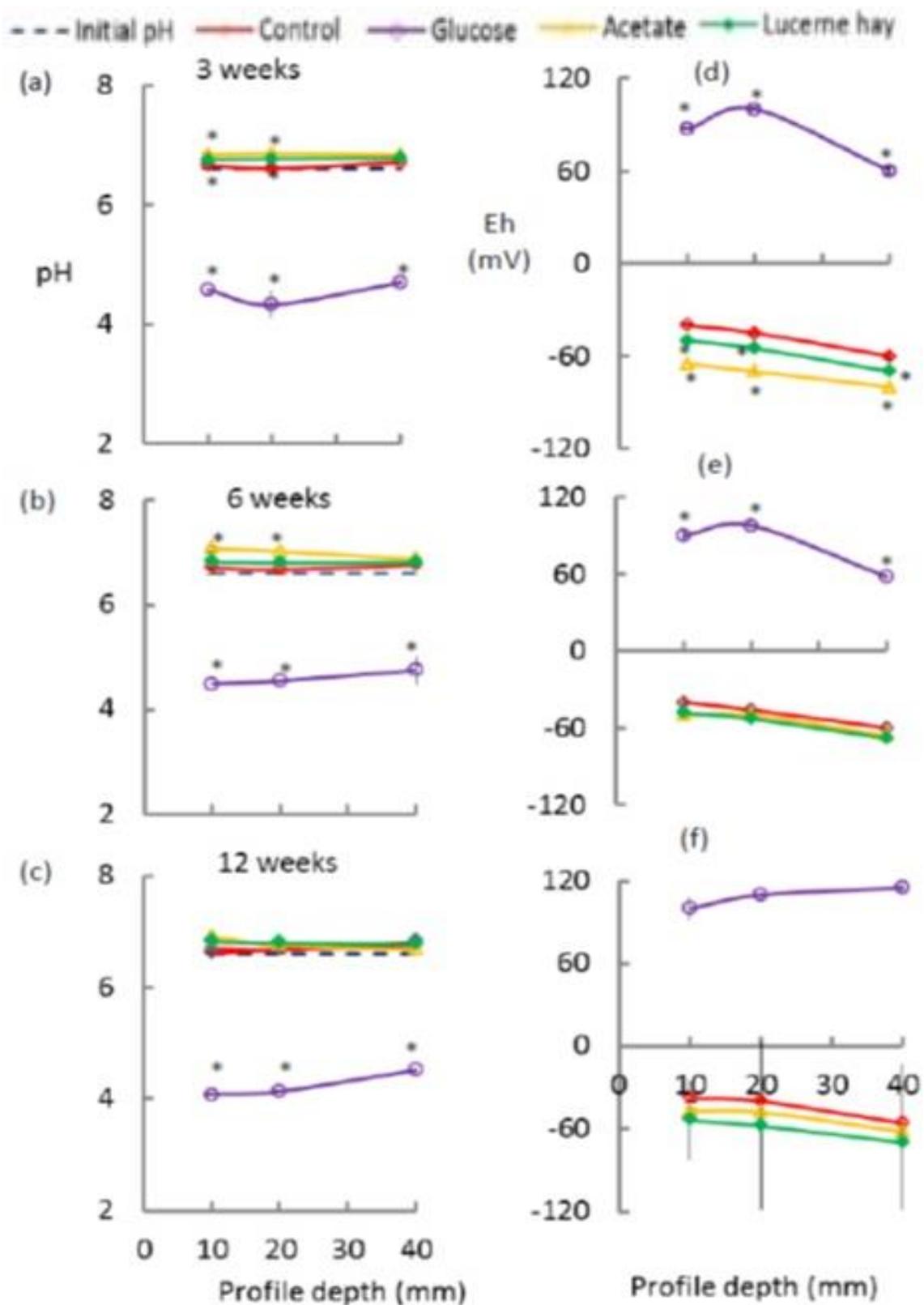


Fig. 5. Time-course of the effects of organic matter and simple carbon compounds on (a-c) pH and (d-f) redox of sulfidic soil maintained under anaerobic conditions for 3, 6 and 12 weeks. Asterisks indicate significant differences ($p < 0.05$) between treatment and control at the same depth.

Effects of organic matter and simple carbon on sulfidic soil pH and Eh:

A more detailed investigation of the effects of simple carbon compounds on sulfidic soil (pH >4) was undertaken by conducting a time course study over 12 weeks under both aerobic and anaerobic conditions, and the changes were compared with those induced by lucerne hay as complex organic matter. As shown in Figure 4a-c, acetate stabilised the pH while lucerne hay increased it under aerobic conditions within the first 3 weeks then remained stable. The pH of the control and glucose treatments acidified after 12 weeks.

The changes in pH generally correlated with changes in Eh under aerobic conditions, with lucerne hay and acetate maintaining an Eh in the range of 0 to 200 mV at the surface (Fig. 4d-f). Within the first 3 weeks, Eh of the control treatment fell to around 100 mV, but then became quite aerobic, which corresponded to more rapid decrease in pH. The Eh remained high throughout in the glucose treatment, corresponding to the low pH. Under anaerobic conditions (Fig. 5), pH of the control sulfidic soil was stable as expected, and there was no significant effect of lucerne hay and acetate on pH of the amended treatments. Glucose however acidified the soil in the first 3 weeks but between the 6 and 12 week the changes in pH remained relatively stable, between 4 and 5 units (Fig. 5b-c). The Eh in all treatments except glucose was around -60 mV at all sampling times (Fig. 5d-f). In the glucose treatment, Eh was also reduced but was mostly in the range of 60 to 100 mV.

Under anaerobic conditions, reduction reactions increased the pH of the control sulfuric soil to near 4.5, lucerne hay to near 6 and acetate to 6.5 within the first 3 weeks (Fig. 3a). However, glucose induced a very small change in pH in the same period and remained relatively near 4 in the next 6 weeks (Fig. 3b-c). The Eh in all treatments except the control and glucose was around -100 mV at all sampling times. In the control and glucose treatments, Eh were mostly in the range of 300 to 500 mV. As shown in Figure 1 and based on our other recent related studies (e.g. Michael *et al.*, 2015), flooding induces reduction reactions and results in lower Eh. The control treatment of this study was not reduced under the flooded soil condition as expected and remained aerobic. This was attributed to probable uneven distribution of residual organic matter content or poor microbial ecology of the sulfuric soil to begin the reduction processes. In addition, a strong smell of butyric acid was there during measurement in the soils amended with glucose, which may have been the end product of a specific bacterial metabolism.

Soil microbes use a range of carbon compound (e.g. glucose and acetate) for cellular respiration and the main limitation for microbial activities to use them as a carbon source and alter the soil chemistry is the supply (Kuz'yakov *et al.*, 2000; Neff *et al.*, 2002).

However, there was a clear difference in the effects of glucose and acetate on soil pH, under both aerobic and anaerobic conditions (Figs. 4 and 5). Under aerobic conditions, acetate was able to stabilise the pH of sulfidic soil (Fig. 4a) and had minimal effect under anaerobic conditions (Fig. 5a), and rapidly induce moderate reductions in Eh (Figs. 4d and 5d).

Comparatively, glucose maintained a high Eh throughout and caused strong acidification under all the moisture conditions (Figs. 4 and 5). The obvious odour of butyric acid suggests that the acidification with glucose even in the sulfuric soil was mediated by fermentative microbes producing acidic metabolic end products. The effects of acetate were interesting in relation to the apparent requirement for nitrogen when complex organic matter is added. The changes in pH and Eh following addition of acetate under aerobic conditions were almost as great as those of lucerne hay and equally rapid (Fig. 4). Since acetate can be utilized as an energy source by some groups of bacteria under aerobic conditions and by sulfate and iron reducing bacteria under anaerobic conditions (Kamura *et al.*, 1963; Thauer *et al.*, 1989), the observed ameliorative effects may have resulted from the biogenic alkalinity generated by a range of microbial ecology.

CONCLUSION

We have demonstrated through various recent long-term studies that addition of a range of nutrient sources under either aerobic or anaerobic soil conditions engages a range of soil microbial ecology that alters the chemistry of ASS. The time course of effects of the additions however was not clearly established by our studies. This study demonstrated that the effects are time course and treatment dependent. The effects of addition of complex organic matter were immediate however as the food sources got exhausted, the effects measured reversed over time. The effects of addition of glucose and acetate were variable, a strong indication that a range of soil microbes is engaged in altering the chemistry of ASS, dependent on amendments. This study also showed that addition of amendments that are multiple source of food (e.g. organic matter) engages a range of soil microbes to alter the chemistry of ASS more effectively than a lone food source (e.g. glucose), which would only attract a handful of microbes. In addition, we found that the effects of the amendments on pH and Eh measured were stable up to six months but the protection was lost after 12 months.

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