Variable impact of rice (*Oryza sativa*) on soil metal reduction and availability of pore water Fe$^{2+}$ and Mn$^{2+}$ throughout the growth period

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**ABSTRACT**

Flooding of wetland or agricultural soils can result in substantial alteration of the pore water trace metal profiles and potentially also influence the bioavailability of other trace elements adsorbed to the insoluble oxides. Experimental microcosms were used to quantify the impact of rice (*Oryza sativa*) plants across an entire growing cycle on the concentrations of Mn$^{2+}$ and Fe$^{2+}$ in two soil types (red sodosol and grey vertosol). Two water management treatments were included: a standard flooded treatment and a saturated treatment ($\sim$3 kPa). Soil pore water profiles were established from samples collected at four sampling depths (2.5, 7.5, 15 and 25 cm) on 50 occasions. Fe$^{2+}$ and Mn$^{2+}$ concentrations were higher in flooded soil than in saturated soil and greatest at a depth of 7.5 cm. The presence of rice plants increased Mn$^{2+}$ concentrations in flooded soils, but tended to decrease Mn$^{2+}$ concentrations in saturated soils. The influence of rice plants on Fe$^{2+}$ concentrations was greatest at a depth of 7.5 cm. Changes in soil pore water Fe$^{2+}$ and Mn$^{2+}$ concentrations due to the presence of rice plants were correlated with flowering and reproduction.

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1. Introduction

Current global estimates indicate the amount of land under rice cultivation is 163 million ha [1]; of this 70% (120 Mha) is produced as flooded rice.[2] Rice is not only the staple food source for nearly half of the world’s population, but also represents the largest anthropogenic wetland on earth [3] and accounts for one-third of all freshwater consumed globally. [4] A unique aspect of this is that after inundation, the soil profile of the flooded rice crop rapidly becomes anaerobic, as any soluble oxygen is rapidly consumed by respiring soil microbes, changing the soil chemistry [5] and leading to conditions that, after rice harvest, may be unfavourable to the establishment of aerobic plants, including post-rice crops.
One problem associated with flooded rice production is the increase that occurs in the concentration of soluble metal species, which may reach toxic concentrations under anaerobic conditions.[2] Of particular interest is soluble manganese ($\text{Mn}^{2+}$), which has been shown to accumulate in flooded soils [6] and iron ($\text{Fe}^{2+}$) which has also been shown to increase in concentration.[7–9] The magnitude of this increase in availability varies with soil type and environmental conditions, and reflects the redox state of the soil. The redox potential of paddy soils is governed by numerous interacting processes, and frequent changes in redox potential are commonly observed.[3] After flooding, as oxygen becomes deficient due to its consumption through microbial respiration, dissolved $\text{NO}_3^-$ may subsequently be lost via reduction to dinitrogen gas (denitrification), and a number of other redox dependent conversions can follow, including the reduction of: $\text{Mn}^{4+}$ to $\text{Mn}^{2+}$; $\text{Fe}^{3+}$ to $\text{Fe}^{2+}$; organic acids to methane, and under extreme anaerobic conditions, $\text{SO}_4^{2-}$ reduction to $\text{H}_2\text{S}$.[10,11] It is difficult to measure all related reduced species and chemical properties simultaneously but measurements of indicator metals can provide useful estimates of the reduction state of soils, particularly when oxygen becomes depleted. Indeed, a strong positive relationship has been found between soil redox potential and concentrations of $\text{Fe}^{2+}$ or $\text{Mn}^{2+}$, while a poor correlation between redox potential and oxygen level in flooded soils has been reported.[11] Oxygen depletion followed by microbially mediated iron reduction has been shown to occur just 4 mm below the surface of a flooded paddy soil leading to both adsorbed Fe(II) on the surface of soil particles and increased Fe$^{2+}$ in pore waters.[9]

Further, the presence of plants can also influence soil redox potential and change trace metal cycling.[9,12,13] In this regard, the roots of rice plants in flooded soils display two important activities that can influence soil chemistry; they oxidise Fe$^{2+}$ by diffusing atmospheric sourced oxygen through roots into the rhizosphere and also excrete H$^+$ to balance surplus absorption of cations over anions.[14] The transition to anoxic conditions after inundation has three main influences on rice cultivation; causing plant physiological changes, alterations in soil chemistry that may increase or decrease nutrient availability, and metabolic activities of microbial flora of the rhizosphere can be shifted.[15]

Mn and Fe solubility and their potential toxicity to plants and soil organisms are associated with their oxidation state in soil.[6–8] Under aerobic conditions, manganese is present mainly as insoluble $\text{Mn}^{4+}$ and $\text{Mn}^{3+}$ oxides and oxyhydroxides,[16] while in an anaerobic environment the dominant form present is soluble $\text{Mn}^{2+}$. Iron in aerobic soils is mostly found as $\text{Fe}^{3+}$, and is generally reduced to $\text{Fe}^{2+}$ under anaerobic conditions. High concentrations of $\text{Mn}^{2+}$ in soil can interfere with the growth of post-rice crops.[17] High shoot concentrations of Fe and Mn in wheat have been reported in waterlogged plants [18] and it has been shown that there is a very close relationship between the element composition of aquatic macrophyte tissue and the sediment composition during growth.[19] The pH of the rice rhizosphere also has been shown to change the availability of $\text{Mn}^{2+}$.[20]

After flooding, the oxygen in the bulk soil may be exhausted within a few hours,[20] but the introduction of oxygen to the rhizosphere through leakage of O$_2$ from the roots of rice plants may maintain an oxidised zone in the soil adjacent to plant root.[21,22] In this zone, oxygen leakage from the roots of rice plants may enable the oxidation of Fe$^{2+}$ present in the rhizosphere, thereby limiting or minimising the toxic impacts of Fe on this plant.[23] The oxidising power of rice roots is due to a combination of oxygen release and enzymatic oxidation. Rates of these processes vary with the atmospheric oxygen concentration,
presence of light and ambient temperature.[21] Estimates have predicted that the rate of oxygen diffusing into the soil is enough to result in Fe$^{2+}$ oxidation.[24] The root oxidation ability and nutrient (N, P and K) uptake have been shown to be greater in alternatively wetting and drying soil than continuously flooded soil.[25] Plant growth stage also influences nutrient uptake, with the highest rate of uptake occurring directly before flowering.[26]

The effect of flooding in agricultural soils has been the subject of numerous studies over several decades [2,10,27–29] including the influence on plant growth.[30] Fe$^{2+}$ and Mn$^{2+}$ in drained soil water were analysed from the lowest layer of a finely ground suspended soil mixture and larger quantities of these metals were associated with the soil organic matter fraction.[31] Incubated rice soil up to a depth of 15 cm was used to monitor the redox potential [20] while the relationship between metals (Fe$^{2+}$ and Mn$^{2+}$) with different flooding durations was also examined.[30,32] These studies focused on the rice-growing soils after inundation; however, most of these studies failed to include rice plants as a factor influencing the redox conditions in the soil, and particularly missed nutrient transformation occurring in the rhizosphere. A detailed study of iron cycling in the surface 10 mm of a paddy soil has been conducted.[9] In addition, changes in the concentrations of Mn$^{2+}$ and Fe$^{2+}$ to a depth of 12.5 cm after inundation of rice-growing soils both in the presence and absence of a growing rice crop for a period of 100 days has been monitored.[33] However, a typical rice plant takes 180 days to mature in Australia[34] and the full impact of rice plants on soil biogeochemistry has not been adequately studied. Rice roots can grow deeper than 12.5 cm, e.g. 90% of rice roots occur in the surface 20 cm, [35] which suggested that sampling to 12.5 cm might not represent the total root zone in flooded rice soils.

A detailed understanding of soil dynamics and nutrient reduction over time will assist in the better management of these soils, and help to avoid soil conditions persisting beyond the rice crop that may be inhibitory for the establishment of aerobic plants including rotational crops. Therefore, the aims of the present study were to examine how the concentrations of Mn$^{2+}$ and Fe$^{2+}$ change over the entire course of a rice crop as affected by stages of growth of the rice crop, and to determine the influence of the rice plant, on these changes. In this study, microcosms were used to simulate rice-growing soils and facilitate sampling at depth, enabling metal ion concentrations to be regularly measured at different soil depths in two different soil types, under two different water regimes (flooded and saturated soils).

2. Materials and methods

2.1. Soil collection and analysis

Representative rice soils were collected from Yanco Agricultural Research Station, Yanco NSW, Australia (34°36′37″S, 146°24′39″E) and Leeton Agricultural Research Station Leeton NSW, Australia (34°35′52″S, 146°21′49″E): the two soils collected were classified as red sodosol and grey vertosol (Isbell 1996) and had a prior history of rice cultivation. The excavated samples (at three depths of soil, i.e. 0–10 cm, 10–20 cm and 20–30 cm) were sieved (<2 mm) and air-dried before use. Soil chemical properties were analysed in the Environmental Analytical Laboratory, Lismore, Australia, and presented in
Supplementary Table S1; however, a more detailed report can be obtained from Haque et al.[36]

2.2. Microcosm construction

Microcosms were constructed as described in Haque et al.[36] Aquarium air stones (internal capacity of 1 mL) were used to create the sample collection frit with the modification of the method described by Doran et al.[33] The surface of each air stone was sealed with silicon sealant leaving the middle half unsealed and valspar scrim tape (0.025 m) was wrapped surrounding the air stone’s surface. Teflon tube (l = 0.10 m, o.d. 0.0016 m, i.d. 0.0006 m) was inserted through the opening of the air stone and the junction between air stone opening and teflon tube insertion area was sealed with silicon sealant. Four collecting frits were placed inside of a polyvinyl chloride cylinder (i.d. 0.15 m, l 0.40 m) through four holes previously made on the wall with a distance of 0.125, 0.150, 0.250 and 0.350 m from the top of the pipe (Figure S2 in Supplementary Information). Soils were packed into the tubes with sampling frits placed on top of each prefixed soil layer. A flangeless ferrule (length 0.0055 m and i.d. 0.0045 m) was aligned to the outer surface of the PVC wall and made flush with the end of the tubing. The lower groove of the nut was sealed by placing an ‘O’ ring followed by a hexagon-domed cap to prevent leakage. A tensiometer was installed at a depth of 0.075 m on the soil surface. Tensiometers were constructed by attaching a ceramic cap in one side and a clear PVC tube on the other side of a PVC pipe. The opening of the pipe top was sealed with a rubber stopper. The needle of the tensiometer gauge (Model: Soilspec Standard SST101G, from TK System, Australia) was injected through the rubber stopper and the vacuum suction gradient was measured (kPa). The room thermostat was set at 30°C in the rice-growing season. Two data loggers (Model TinyTag Ultra 2; range –25 to 85°C) were used to record the temperature at the plant height and recorded temperature is presented in Figure 1. A temperature-regulated glasshouse was used and average temperature conditions per day of 20.4°C were maintained. Relative humidity ranged from 17% to 96%, and light conditions varied between 10 and 14 hours light per day with an intensity of ∼550 lux at midday.

2.3. Crop and water management

Rice (Oryza sativa, variety Amaroo) seeds were dry sown in each microcosm at an approximate crop density of 300 plants m⁻². Light irrigation was applied to the freshly planted microcosms to soak the seed. Seedlings were thinned to six per microcosm at 3 weeks after sowing. Urea was applied as a source of nitrogen at a rate of 150 kg N ha⁻¹ during the active tillering stage and additional 50 kg N ha⁻¹ was applied at the panicle initiation stage. Crop management was carried out following the guidelines indicated in Ricecheck [37] and crop harvesting was done at the time of maturity (at 33 weeks after flooding). Plant dry weights (leaf and stem) were measured after drying at 70°C to constant weight.

In the flooded treatment, standing water at a depth of 3 cm was maintained once the plants reached the height of ∼5cm, about 3 weeks after sowing. For all figures in this study, this time was set as day 0 for all the treatments in both the flooded and saturated conditions. At the tillering stage, the water level was raised to 7 cm. Saturated treatments
maintained soil moisture at a tensiometer reading of −3 kPa with required water additions calculated using the equation; 
\[ y = 25.363x - 78.008; \quad R^2 = 0.9998, \]
where \( y \) is the quantity of water necessary in millilitres and \( x \) is the reading of the tensiometer. The amount of deionised water required to maintain the desired water level was monitored and adjusted every two days. The water was surface drained immediately before crop harvest. Column construction and operation are presented in the ‘Supplementary Information’ section (Figure S2).

**2.4. Data collection protocols**

Soil pore water samples were collected from the initiation of flooding up to 34 weeks after flooding. During the initial 4 weeks of flooding, samples were collected at 2-day intervals, and the sampling interval was increased to every four days up to 20th week and then on a weekly basis. An aliquot of ~1 ml was drawn with a syringe fitted with a nylon tube (i.d. 0.015 m and length 0.05 m) and discarded; approximately 2 mL of soil pore water was then collected. Samples were collected sequentially from the top (2.5 cm) to the bottom port (25 cm) in each microcosm. Samples were then filtered (0.45 μm × 25 mm and 0.22 μm × 13 mm) into a plastic microfuge tube (1.5 mL) which was pre-loaded with concentrated hydrochloric acid (50 μL). The tubes were sealed and stored until \([\text{Mn}^{2+}]\) and \([\text{Fe}^{2+}]\) could be determined. Nutrient analyses were

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**Figure 1.** Temperature recorded in the glasshouse during the duration of experiment.
performed within 24 hours from the time of collection as a preliminary study showed that the concentration of metal ions decreased if samples were stored beyond that time (Supplementary Information, Table S3). It was estimated that a total of 12 mL of soil pore water was withdrawn from each microcosm at each sampling; and to balance the total water withdrawn, a similar amount of deionised water was added (on the soil surface) to the soil microcosm.

Mn$^{2+}$ concentrations were measured using an Atomic Absorption Spectrometer (Model: Varian Spectra AA20) at a wavelength of 279.5 nm with an acetylene flame. Potassium permanganate (0–12 mg Mn/L) in sulphuric acid (2% v/v) was used to make calibration standards, and Mn$^{2+}$ was calculated from the calibration curve. Fe$^{2+}$ in the samples was determined using the Ferrozine method. Standards were prepared before each day of analysis with ferrous ammonium sulphate (0–100 mg L$^{-1}$). Soil pore water (0.5 mL) was extracted with 0.5 N hydrochloric acid for 2 h at room temperature and the pH was adjusted to 7. Ferrozine was then added to the solution and the absorbance measured at 562 nm in a spectrometer (Model UNICAM 8625). For all solution preparations and dilutions, water purified through a Milli-Q (Millipore) water system (ISO 9001) was used.

2.5. Experimental design and statistical analysis

A randomised, complete block design with three replications was used to study chemical changes in microcosm soils. Data were analysed using Genstat 16 (GenStat for Windows, VSN International Limited). Values for available Fe$^{2+}$ and Mn$^{2+}$ at four sampling depths were subjected to an analysis of variance. Least significant differences ($p < .05$) were determined using Tukey’s test. Changes in Mn$^{2+}$ ($\Delta$[Mn$^{2+}$]) and Fe$^{2+}$ ($\Delta$[Fe$^{2+}$]) between treatments (rice and no rice) were calculated by using the following equations:

$$\Delta$$[Mn$^{2+}$] = [Mn$^{2+}$]$_R$ – [Mn$^{2+}$]$_{NR}$,
$$\Delta$$[Fe$^{2+}$] = [Fe$^{2+}$]$_R$ – [Fe$^{2+}$]$_{NR}$.

Here, $R$ and $NR$ represent the concentration of metals (Mn$^{2+}$ or Fe$^{2+}$) analysed in rice and no-rice soils, respectively.

3. Results

3.1. Effect of water regimes on the growth of rice plants

Rice grown in flooded soils produced significantly ($p < .05$) higher amounts of dry matter (64%) than plants grown in the same soils under saturated conditions with less water availability than flooded soil treatments (Figure 2). Partitioning of dry matter into various plant parts over time showed that the leaf dry weight of plants from flooded soil was double that of plants from the corresponding saturated soil. Similarly, panicle dry weight was 60–90% more (depending upon soil) in plants from flooded soil than from saturated soil. Flowering was delayed by approximately 3 weeks in plants grown in saturated soils compared to plants in the flooded soil (Figures 3 and 4).
3.2. Changes in Fe$^{2+}$ in no-rice treatments

Flooded soil produced significantly higher concentrations of Fe$^{2+}$ than the saturated soil ($p < .05$) in most instances (Figures 3 and 4). Regardless of water management, Fe$^{2+}$ concentrations were greater in the red sodosol than in the grey vertosol. In both soils, the Fe$^{2+}$ concentration was greatest at the depth of 7.5 cm. After an initial increase in concentration of Fe$^{2+}$, a further rise in Fe$^{2+}$ content was observed at the later stage of the incubation phase (~25 weeks after flooding) in the top layer of the sodosol and in the second layer of the flooded vertosol. Among the four layers, the top layer (depth of 2.5 cm) showed the lowest concentrations of Fe$^{2+}$ in both the soils and both the watering treatments at majority of sampling times.

In flooded red sodosol, a limited increase in Fe$^{2+}$ was observed from 0 to 7 days at all four soil depths (Figure 3). Fe$^{2+}$ levels increased during the second week after flooding (within the range from 35 to 60 mg L$^{-1}$) at all soil depths. Following this, the concentration of Fe$^{2+}$ decreased slightly at all depths over the next 4–6 weeks. Of particular note, the concentration of Fe$^{2+}$ increased dramatically (118 mg L$^{-1}$) at the depth 7.5 cm at 2 weeks after flooding but again tapered off to 40 mg L$^{-1}$ about 8 weeks after flooding. The top two depths (2.5 and 7.5 cm; Figure 3) showed a similar pattern of increases in Fe$^{2+}$ up to 3 weeks after flooding, while at 7.5 cm, higher concentrations of Fe$^{2+}$ were maintained until drainage.

In the flooded grey vertosol, Fe$^{2+}$ concentrations varied between 1 and 1.5 mg L$^{-1}$ at all four depths at the beginning of the experiment (Figure 4). One week after inundation, the concentration of Fe$^{2+}$ increased markedly to 60 mg L$^{-1}$ at 7.5 cm depth. The surface sampling depth (2.5 cm) showed a significant but less substantial increase (15 mg L$^{-1}$)
Figure 3. Dynamics of Fe$^{2+}$ in red sodosol with and without rice plants. Line graphs represent dynamics of Fe$^{2+}$ in no rice treatments and bar graphs represent changes in Fe$^{2+}$ in the presence of rice in flooded (red colour) and saturated (yellow coloured) red sodosol. The primary y axis represents the concentration of Fe$^{2+}$ (mg L$^{-1}$) and secondary Y axis represents changes (positive and negative) in the concentration of Fe$^{2+}$ (mg L$^{-1}$). The four depths (2.5, 7.5, 15 and 25 cm) are stacked from the top over a period of 33 weeks after flooding. The vertical error bars present on data points indicate standard deviation of means ($n = 6$). The unfilled and filled arrows at the top of the graph represent active tillering and maximum tillering stage for both flooded and saturated soils, respectively; in addition, the red and yellow arrows represent flowering times for flooded and saturated soils, respectively. The least significant difference values are presented in the Supplementary Information, Figure S4.
Figure 4. Dynamics of Fe$^{2+}$ in grey vertosol with and without rice plants. Line graphs represent dynamics of Fe$^{2+}$ in no-rice treatments and bar graphs represent changes in Fe$^{2+}$ in the presence of rice in flooded (red colour) and saturated (yellow coloured) grey vertosol. The primary $y$ axis represents the concentration of Fe$^{2+}$ (mg L$^{-1}$) and secondary $y$ axis represents changes (positive and negative) in the concentration of Fe$^{2+}$ (mg L$^{-1}$). The four depths (2.5, 7.5, 15 and 25 cm) are stacked from the top over a period of 33 weeks after flooding. The vertical error bars present on data point indicate standard deviation of means ($n=6$). The unfilled and filled arrows at the top of the graph represent active tillering and maximum tillering stage, for both flooded and saturated soils, respectively; in addition, the red and yellow arrows represent flowering time for flooded and saturated soils, respectively. The least significant difference values are presented in the Supplementary Information, Figure S4.
at 2 weeks after flooding. In contrast, the deepest layer only reached values of 8 mg L\(^{-1}\) by 4 weeks after flooding, and then Fe\(^{2+}\) decreased until 23 weeks after flooding. In saturated grey vertosol (Figure 4), depths of 7.5 and 15 cm showed elevated concentrations of Fe\(^{2+}\) (~55 mg Fe\(^{2+}\)/L) by 2 and 4 weeks after flooding, and the top (2.5 cm) and bottom (25 cm) depths showed steady increases in Fe\(^{2+}\) throughout the experiment. The maximum concentration of Fe\(^{2+}\) (70 mg L\(^{-1}\)) was observed at a depth of 7.5 cm shortly after flooding, while the 15 cm depth showed a similar peak (58 mg L\(^{-1}\)) at 4 weeks after flooding. At 13 weeks after flooding, the concentration of Fe\(^{2+}\) at the lowest depth started to fall, reaching a concentration of < 1 mg L\(^{-1}\) by 28 weeks after flooding.

3.3. Influence of rice plants on the concentration of Fe\(^{2+}\)

The influence of living rice plants on Fe\(^{2+}\) dynamics in both soils is presented in the bar charts of Figures 3 and 4. The positively valued bars indicate cases where Fe\(^{2+}\) concentrations were higher in soils with rice compared to the corresponding soils without rice. Similarly, negatively valued bars represent cases where Fe\(^{2+}\) concentrations were lower in soils with rice compared to without rice. The concentration of Fe\(^{2+}\) generally increased with the presence of rice plants at depths of 7.5, 15 and 25 cm in the red sodosol to the corresponding no-rice treatment. The presence of rice plants resulted in increased availability of Fe\(^{2+}\) in flooded soils at the depth of 7.5 cm (an average range of 15–40 mg Fe\(^{2+}\)/L among two soils); however, at other depths, increases were smaller. The deepest soil depths (25 cm) in sodosol showed decreases in Fe\(^{2+}\) concentration during the initial phase of rice growth (up to 13 weeks after flooding) but levels then increased over time. Similarly, flooded vertosol soils exhibited increased Fe\(^{3+}\) content over time. In saturated conditions, the presence of rice plants resulted in decreased concentration (up to 25 mg L\(^{-1}\)) of Fe\(^{2+}\) (except initial days in sodosol, at a depth of 7.5 cm).

3.4. Changes in Mn\(^{2+}\) in no-rice treatments

Mn\(^{2+}\) concentrations in flooded and saturated red sodosols and grey vertosols are presented in Figures 5 and 6. Initially, little difference was recorded among the four depths of soil, but over time, deeper layers accumulated more Mn\(^{2+}\) than the upper layers. Flooded soil produced significantly higher concentrations of Mn\(^{2+}\) than the saturated soil over most of the study period (\(p < .05\)), but this was dependent on soil type; for instance, red sodosol contained more Mn\(^{2+}\) than grey vertosol on most of the sampling days. Mn\(^{2+}\) concentrations at 7.5 cm were higher than the other depths in most cases. The rate of increase of Mn\(^{2+}\) in flooded red sodosol was significantly higher (\(p < .05\)) at 7.5 cm compared to other depths of soil until 2 weeks after flooding (Figure 5). Compared to the initial concentration, Mn\(^{2+}\) was increased at 14 weeks after flooding, i.e. three-fold higher at depths of 2.4 and 7.5 cm, 4.5-fold higher at depths of 15 cm, and two-fold at 25 cm. The saturated sodosol and vertosol behaved differently at the deepest soil depth (25 cm). Elevated concentrations of Mn\(^{2+}\) were recorded in saturated soil during the period of 7–20 weeks after flooding, and thereafter the flooded soil showed a higher concentration of Mn\(^{2+}\) than the other soil depths on most occasions. Flooded grey vertosol showed a similar pattern to that of the sodosol (Figure 6); however, two peaks of Mn\(^{2+}\) were prominent in grey vertosol at the depth of 7.5 cm (at 3 weeks after flooding and 17 weeks after...
flooding) compared to red sodosol. In saturated grey soil, all the four depths showed two periods of elevated concentrations of Mn$^{2+}$. The first peak occurred close to 5 weeks after flooding at the depth of 2.5 cm (2.7 mg L$^{-1}$), 7.5 cm (3.6 mg L$^{-1}$), 15 cm (3.6 mg L$^{-1}$) and 25 cm (4.1 mg L$^{-1}$). The second elevated level was noted at all depths from 16 to 20 weeks after flooding. In addition, the depth of 7.5 cm maintained elevated concentrations of Mn$^{2+}$ throughout the study period.

3.5. Influence of rice plants on the concentration of Mn$^{2+}$

The influence of rice plants on the availability of Mn$^{2+}$ is presented in Figures 5 and 6. The vertical bars in these figures represent differential changes in Mn$^{2+}$ related to the presence of rice, both in flooded soil (top panel) and saturated soil (lower panel). The greatest increase in Mn$^{2+}$ (9.75 mg L$^{-1}$) occurred in the flooded sodosol (at 25 cm), and largest decrease (7.7 mg L$^{-1}$) in the saturated sodosol at a depth of 15 cm, indicating that the influence of the rice plant differed markedly between the two soil watering regimes. The influence of rice plants began earlier in the upper part of the soil profile and progressed to the lowermost depths, and the effects were weaker at the upper depth than at the lower three depths. In flooded sodosols, Mn$^{2+}$ values were increased at all depths (except at 7.5 cm), but were decreased in saturated soils (in most of the cases). The impacts of the rice plants were most prominent during 23 weeks after flooding which was close to the flowering time of the flooded rice plants. The influence of rice on the change in Mn$^{2+}$ in vertosol showed a similar pattern to sodosol, but the magnitude of the changes was less (Figure 6). In the sodosol, at 25 cm depth, the change in manganese with rice in the flooded soil was inverse to that observed for saturated soil, but this pattern was not replicated in the vertosol.

4. Discussion

Vigorous growth of rice was observed in flooded treatments for both the soils. Dry matter production is related to leaf photosynthesis, and it has previously been reported that leaf area and yield are reduced in aerobically grown rice compared to flooded rice culture.[40] The optimum growth duration for rice plants in Australia is ~26 weeks,[34] but in the current study, rice required about 33 weeks to reach maturity in the saturated treatment. The longer growth period in the present study could be associated with shorter day length in conjunction with temperature variation during the experimental period (Figure 1).

The speciation of iron and manganese was shown to vary rapidly after the onset of soil wetting and was dependent on soil type, water management and the depth within the soil profile. The rapid rise to peak concentrations occurred earlier for Fe$^{2+}$ than the Mn$^{2+}$ in both the soils. However, with the progression of time, in most instances, Mn$^{2+}$ remained higher than Fe$^{2+}$ [2] compiled the basic sequence of redox couples and reported that Mn reduction (reduced between +280 and +220 mv) occurs before Fe reduction (reduced between +180 and +150 mv) and this has been seen in the progression of layers in aquatic sediments.[41] The possible cause of the fluctuation in dissolved Fe$^{2+}$ and Mn$^{2+}$ concentration includes mass flow and diffusion of oxygen, activity of microorganisms, redox potential of soil, dissolved organic carbon and plant growth stages and biomass production and differential rates of precipitation as carbonate or sulphide mineral phases or
Figure 5. Dynamics of Mn$^{2+}$ in red sodosol with and without rice plants. Line graphs represent dynamics of Mn$^{2+}$ in no-rice treatments and bar graphs represent changes in Mn$^{2+}$ in presence of rice in flooded (red colour) and saturated (yellow coloured) red sodosol. The primary y axis represents the concentration of Mn$^{2+}$ (mg L$^{-1}$) and secondary Y axis represents changes (positive and negative) in the concentration of Mn$^{2+}$ (mg L$^{-1}$). The four depths (2.5, 7.5, 15 and 25 cm) are stacked from the top over a period of 33 weeks after flooding. The vertical error bars present on data point indicate standard deviation of means ($n = 6$). The unfilled and filled arrows at the top of the graph represent active tillering and maximum tillering stage for both flooded and saturated soils, respectively; in addition, the red and yellow arrows represent flowering time for flooded and saturated soils, respectively. The least significant difference values are presented in the Supplementary Information, Figure S4.
Figure 6. Dynamics of Mn\textsuperscript{2+} in grey vertosol with and without rice plants. Line graphs represent Mn\textsuperscript{2+} levels in no rice treatments and bar graphs represent changes in Mn\textsuperscript{2+} in the presence of rice in flooded (red colour) and saturated (yellow coloured) grey vertosol. The primary y axis represents the concentration of Mn\textsuperscript{2+} (mg L\textsuperscript{-1}) and secondary Y axis represents changes (positive and negative) in the concentration of Mn\textsuperscript{2+} (mg L\textsuperscript{-1}). The four depths (2.5, 7.5, 15 and 25 cm) are stacked from the top over a period of 33 weeks after flooding. The vertical error bars present on data point indicate standard deviation of means (n = 6). The unfilled and filled arrows at the top of the graph represent active tillering and maximum tillering stage for both flooded and saturated soils, respectively; in addition, the red and yellow arrows represent flowering time for flooded and saturated soils, respectively. The least significant difference values are presented in the Supplementary Information, Figure S4.
adsorption to particle surfaces.[8,41–43] Microorganisms are mostly responsible for governing the metal partitioning in flooded rice soil [44] and the process is dependent on temperature, with rise and fall in Fe$^{2+}$ and Mn$^{2+}$ due to the temperature variation in the system having been observed in other systems.[45] Microorganisms can oxidise organic matter by means of Fe$^{3+}$ or Mn$^{4+}$ as the electron acceptor; however, dissimilatory reduction of these metals might provide the energy for microbial development.[46] Dissolved organic carbon is, however, affected by the presence of rice roots in flooded soils.[47] Newly assimilated carbon is also affected by the management of water in a rice crop system.[48] Fluctuation in Fe$^{2+}$ content was found in soil pore water and indicated that measurement of redox potential is not enough to decide the reduction status of the system.[49] The ratio of Fe$^{2+}$/Fe$^{3+}$, which is mostly controlled by microorganisms, significantly controls the redox behaviour in a system.[50] In addition to microbial respiration, lower-chain organic acids [9,51] and humic acids [52] are responsible for the increasing Fe concentration in flooded rice systems.

The presence of rice roots in the system influences the redox balance of the rice rhizosphere. The analysis of the decrease and increase in both Fe$^{2+}$ and Mn$^{2+}$ due to the presence of rice revealed that the rice plant increases the Fe$^{2+}$ and Mn$^{2+}$ concentrations in most of the flooded treatments, with only one exception being the red sodosol at a depth of 15 cm, and in relation to Fe$^{2+}$. Why this may have occurred in this particular soil at this depth is unknown. Flooded soil also showed clear evidence of increased Fe$^{2+}$ and Mn$^{2+}$ after flowering. However, the effect of rice was not prominent in saturated soils. The increase in iron and manganese solubility at the flowering stage in flooded soil may also be an important marker for the availability of other nutrients or trace metals (e.g. P or As) that are frequently associated with the insoluble mineral phases and may indicate that the uptake of these elements at this critical time is worthy of further study. The influence of plant roots on the metal fractions of cadmium and zinc has been previously described in metal-contaminated soil.[53]

The active absorption area is an important consideration to study the activity of rice roots. This active area remained unchanged during 90–120 days after transplanting in a depth up to 30 cm.[25] In the present study, soil pore water was collected to a depth of 25 cm, which was considered a representative area of plant root growth. Root elongation rate depends on the soil properties, mostly on soil biophysical conditions. In aerobic rice, the total root number has been reported to be reduced by 28–68% relative to flooded rice.[25] In the present study, dry matter production remained higher in flooded soil, reflecting poorer root growth in saturated soil compared to the flooded soil. In addition, the growth stage of the rice plant also influences the oxygen transportability through the root. The second peak in Mn$^{2+}$ observed in this study was mostly concentrated in the upper three depths and started slightly earlier than flowering. The activity of roots is decreased at the time of flowering and onwards, resulting in two situations: secreting less root exudates,[54] and liberating less oxygen to the rhizosphere.[55] Lower flux of oxygen to the rhizosphere could leave more Mn$^{2+}$ in the soil pore water. This might again be explained by the dense root system in the upper three depths of the core. Oxidation of Fe$^{2+}$ by oxygen supplied through rice root [22] is active up to 1 mm in a highly reduced soil and up to 4 mm in a weakly reduced soil. The release of oxygen through the root has the potential to reduce the pH value by two units [22] which could be further responsible for the reduction of soluble manganese.[56]
The microcosms developed in this study contained soil profiles of 30 cm in depth, which is considered representative of the root zone of flooded rice crops. The upper layer included a 10 cm layer of standing water (for flooded treatments) and the other sets of microcosms were maintained at almost saturation (−3 kPa). Oxic and partly oxic zones, a reduced layer and a redox difference between oxic and anaerobic layers, are found in rice rhizospheres. This complex environment is further influenced by the role of soil microorganisms on the development of redox condition and subsequent reduction of Fe and Mn; however, the key microbial players which govern the process still need investigation. Poor drainage conditions might develop ferrolysis in rice soil and make it more acidic and could change the reduction behaviour of Mn. In general, the rice soil in Australia has a thick clay layer below the root zone, while in other parts of the world the soil layer beneath the root zone is deliberately puddled to minimise drainage of water and subsequently enhances the deposition of Mn.

The presence of rice plants in flooded soil increased the availability of both metals. The changes in Mn\(^ {2+}\) and Fe\(^ {2+}\) are most likely related to the presence of oxygen in the system, and while the uppermost layer can still provide a transition phase between oxic and anoxic layers of soil, oxygen was totally excluded from the lower three depths (7.5, 15 and 25 cm). In addition, rice plants can develop a vigorous root system; around 4 ton/ha that produces significant quantities of root exudates which play important roles in the metabolic activity and abundance of microorganisms. It is well documented that microorganisms are responsible for the reduction of metals in the rhizosphere. In saturated soils, some oxygen can penetrate the surface soil zone; however, the increase in Fe\(^ {2+}\) and Mn\(^ {2+}\) seen in the 2.5 cm layer of the saturated red sodsol indicates that O\(_2\) exchange was poor at best. Plant growth was significantly lower in saturated soil (Figure 2), suggesting reduced root growth. Poor root growth generally releases fewer root exudates and can reduce the activity of exudate-linked microorganisms. The abundance and activity of soil microorganism are highly influenced by the organic carbon present in soil, including root exudates. In addition, rhizosphere microbes can significantly influence root exudation, root development and nutrient availability. The root zones responsible for exudate release include the sub-apical zone and root-hair zone. Root exudates were reportedly found at lower concentrations at flowering compared to the active tillering stage of rice. The presence of rice plants increases Mn\(^ {2+}\) and Fe\(^ {2+}\) more in the flooded than in the saturated soil, suggesting that the influence of plants grown in saturated conditions is less substantive than plants grown in flooded conditions.

The similar increase in Mn\(^ {2+}\) and Fe\(^ {2+}\) at 32 weeks after flooding in both soils and at most depths could be associated with increased air temperature recorded during that period (Figure 1) which may have increased the availability of reduced metals at that point. At higher temperature, it has been shown that root respiration requires more oxygen and eventually releases less oxygen through the root and Mn\(^ {2+}\) may accumulate in the soil. The activity of soil microorganisms is also increased with high temperature, which may alter the rate of Mn\(^ {4+}\) reduction. However, care needs to be taken with interpretation of the data as the present study was not designed to isolate the effect of temperature on metal reduction.
5. Conclusion

The presence of rice plants showed clear and dynamic effects on the concentrations of Mn$^{2+}$ and Fe$^{2+}$ in flooded soils, while in saturated soils they tended to decrease the concentrations of these ions; this phenomena may be an indicative redox behaviour applicable to flooded agricultural land and saturated wetlands with emergent macrophytes. In addition, the growth stage of rice, particularly the flowering stage, can significantly alter the redox behaviour of soil in the presence of rice roots. This study has clearly demonstrated that the plateau in iron and manganese concentrations observed after 10–12 weeks of flooding does not represent the formation of a stable state and is subject to influence by the growth and development phase of the plants growing in the system. The influence of the plants on the soil chemistry penetrates to a depth of at least 25 cm.

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