



# Tree-soil Interactions Through Water Release to a Floodplain Ecosystem: a Case Study of Black Box (*Eucalyptus largiflorens*) on Loamy Sands

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## Abstract

Australia's Murray-Darling Basin (MDB) is an agriculturally and ecologically valuable region impacted by water abstraction and climate change. Black Box (*Eucalyptus largiflorens* (Myrtaceae)), an important floodplain tree largely endemic to the Basin is of concern because its overall condition is sub-optimal. Knowledge about key aspects of Black Box tree function such as mineral nutrition remains limited. This field study examined the latter by measuring essential plant nutrients, aluminium, and soil chemistry over 12 months spanning an environmental water release event. Samples were collected 4–8 weekly from mature trees on loamy sands of the Hattah Lakes system in north-western Victoria. This commenced prior to water release, continuing through peak levels at the study sites, to recession. Paired leaf and soil samples were obtained from/beneath mature trees at 8 time-points in 2017–2018. Flooding induced mostly temporary soil chemical changes in the surface horizon, which enhanced trace-nutrient access to trees. Results suggest that the short-term flooding of Black Box on drained loamy sands likely provides nutritional advantage by generating soil chemical fluxes. They also raise questions about flooding-induced movement of nutrients through the soil profile, and about the combined effects of pedology and duration of flooding on the nutritional health of Black Box.

**Keywords** Lake Konardin · Lake Woterap · Lake Mournpall · Hattah Kulkynne · Environmental watering · Nutritional dynamics · Murray-darling Basin

## Introduction

The importance of mineral nutrients for gauging the health and condition of agricultural plants and soils has long been known (Hall 1905; Gilbert et al. 1927; Ulrich 1952; Marschner 2002). Terrestrial plant mineral nutrients have variously been defined as those with specific roles in at least one plant function and/or are acquired from soil via the roots,

notwithstanding less typical means of acquisition (Marschner 2002; Miller 2014). Discovery of the individual functional roles of macro- and micro-nutrients has greatly benefitted cultivation, with fundamental knowledge drawn primarily from studying crop plants (Marschner 2002). While these principles can also be applied to plants in natural systems, similar basic nutritional studies in the ecological context have remained comparatively scarce, likely due in part at least to the complexities of inherent heterogeneity, and prioritisation of agriculture. The nutritional dynamics of large trees with respect to external changes in their natural environments for instance would be far more complex to assess, than for example, plants in uniform cultivation.

It is known that solar radiation, temperature and water supply can have direct effects, either individually or interactively, on plant processes (Marschner 2002; Taiz and Zeiger 2002; Lynch and StClair 2004; StClair and Lynch 2004); whereas soil changes induced by wetting and drying cycles can indirectly affect plant health by influencing the bioavailabilities of mineral nutrients including trace metal nutrients

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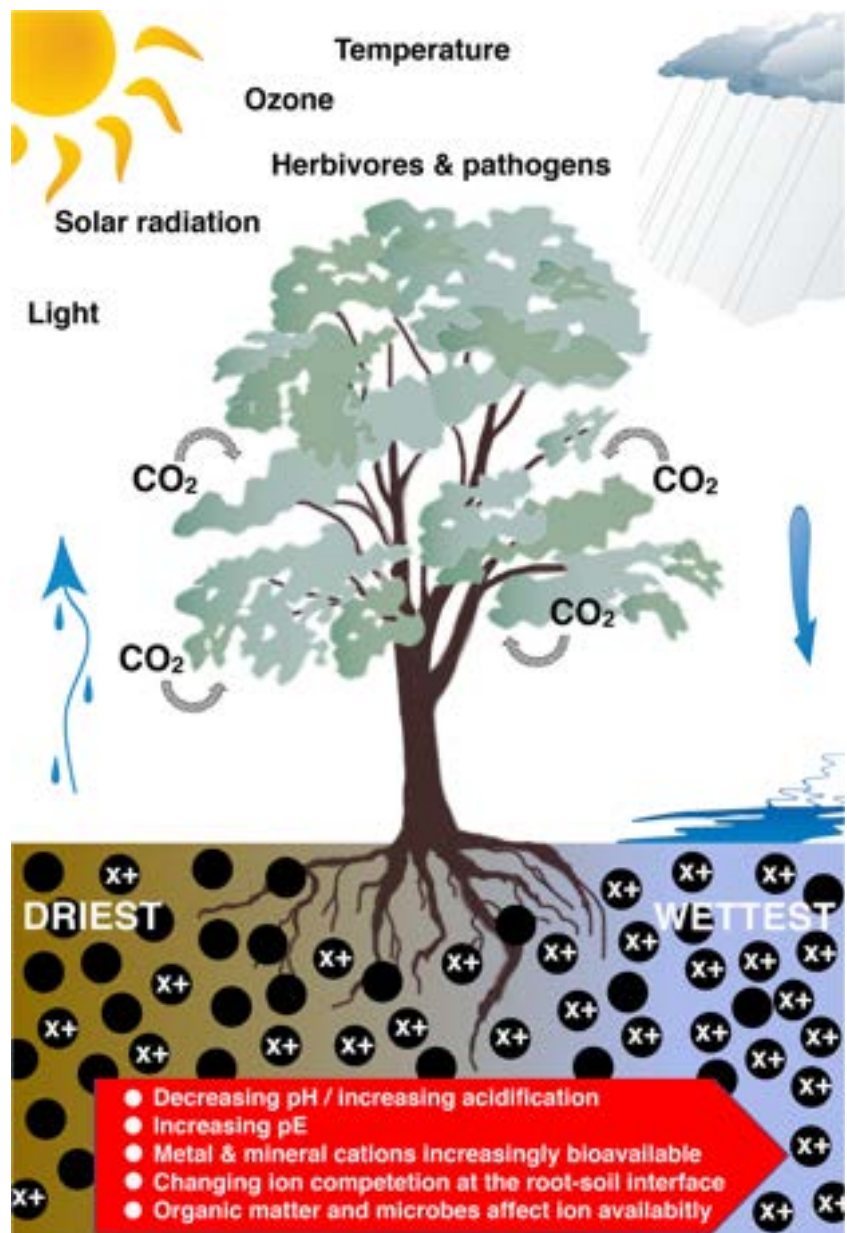
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and non-nutrient metals (Fig. 1) (White 1997; Lynch and StClair 2004; Fernando and Lynch 2015). Soil chemical changes originating from anthropogenic activities have been proven to significantly impact tree health. For example, in North America and Europe, acidification induced by nitrogen and sulphur oxides and CO<sub>2</sub> emissions have contributed to native forest decline (Kitao et al. 2001; StClair et al. 2005; McNaulty et al. 2014). It is known that waterlogging can enhance the bioavailability of certain soil metals such as manganese (Mn) and aluminium (Al) via increased soil acidity and/or reducing conditions (Bromfield et al. 1983; Singleton et al. 1987; Graham et al. 1988). When in over-supply, these metals can have multiple adverse effects including impaired root growth due to Al-toxicity in many

agricultural plant species (Rout et al. 2001; Navascus et al. 2011). For Mn, these include, (a) disrupting normal enzyme activities, (b) driving damage to photosynthetic apparatus via photobleaching, and (c) outcompeting plant uptake of the essential nutrients magnesium (Mg) and calcium (Ca) in particular, ultimately driving deficiencies of these nutrients (Marschner 2002; StClair and Lynch 2004; 2005; Fernando and Lynch 2015). In Australia, intrinsic soil chemical traits such as elevated Mn and Al can impact the health of cultivated plants (Siman et al. 1974; Foy 1984; Graham et al. 1988), while induced soil salinisation is well documented as highly detrimental to plants in both cultivated and natural systems (Kozłowski 1997; Rengasamy 2006; Munns and Gilliam 2015).

**Fig. 1** Conceptual framework of a plant-soil-environment system showing key dynamic variables and interactions. (Figure constructed with artwork sourced from IAN Image Library (ian.umces/imagelibrary/))



Native plants in healthy balanced ecosystems benefit from intrinsic environmental conditions, yet ecological equilibria are vulnerable to detrimental anthropogenic alterations via relatively short- to mid-term effects such as water removal and/or pollution, or, longer term effects such as climate change (Lynch and StClair 2004; Fernando and Lynch 2015). Mutually associated stress factors can precipitate a gradual ‘cascade’ of decline, say through heightened vulnerability to biotic stress, drought, excess sunlight etc. Predicted extended periods of high intensity solar radiation, for example, increases the risk of oxidative stress in nutritionally challenged plants, thereby exacerbating other detrimental processes such as susceptibility to insect attack (Lynch and StClair 2004; Dean 2007; StClair and Lynch 2010; Fernando et al. 2016; Fernando et al. 2016). Therefore, a healthy tree-soil system comprising an inherently complex, yet balanced framework of interrelated variables is vulnerable to direct and indirect anthropogenic effects that may undermine that balance.

Australia’s Murray-Darling Basin (MDB) (Fig. 2) is crucial to food production (LeBlanc et al. 2012; Holland et al. 2015; Swirepik et al. 2016) while encompassing important riverine ecosystems increasingly impacted by water removal and climate change (Kingsford et al. 2011; Pittock and Finlayson 2011; Capon et al. 2013; Colloff et al. 2015). Furthermore, the allocation of limited freshwater resources is an increasingly pressing issue under climatic changes that are predominantly warming and drying (Grafton et al. 2018). Efficient and effective water allocation to support environmental assets demands sound theoretical understanding around asset-water relationships. Among key assets are native trees, where a clearer understanding of the dynamics around their nutrient acquisition from soil with respect to natural water flows stand to strengthen management decisions (Overton et al. 2009; Doody et al. 2014). A deeper theoretical understanding of possible associations between water flows/availability and tree-soil nutritional interactions stands to benefit MDB ecosystems by value-adding to decision making around environmental water allocations.

*Eucalyptus*, an important genus of the southern hemisphere Myrtaceae family, is iconic to Australia. Of the three key riverine tree eucalypts of the MDB, River Red Gum (*E. camaldulensis*), Coolibah (*E. coolabah*) and Black Box (*E. largiflorens*), only the latter is restricted to the MDB. The reasons for decline in *E. largiflorens*, an ecologically important tree widely distributed across MDB floodplains and also occurring on wetlands, remains unclear (Colloff et al. 2015; Capon et al. 2016; Fernando et al. 2018; Overton et al. 2018). These habitats are predominantly in semi-arid to arid parts of Australia. It is difficult to observe initial stress in mature trees across large areas of the MDB as there is often a lag time of months (Doody et al. 2014) before visual decline can be seen via tree canopies. In some instances,

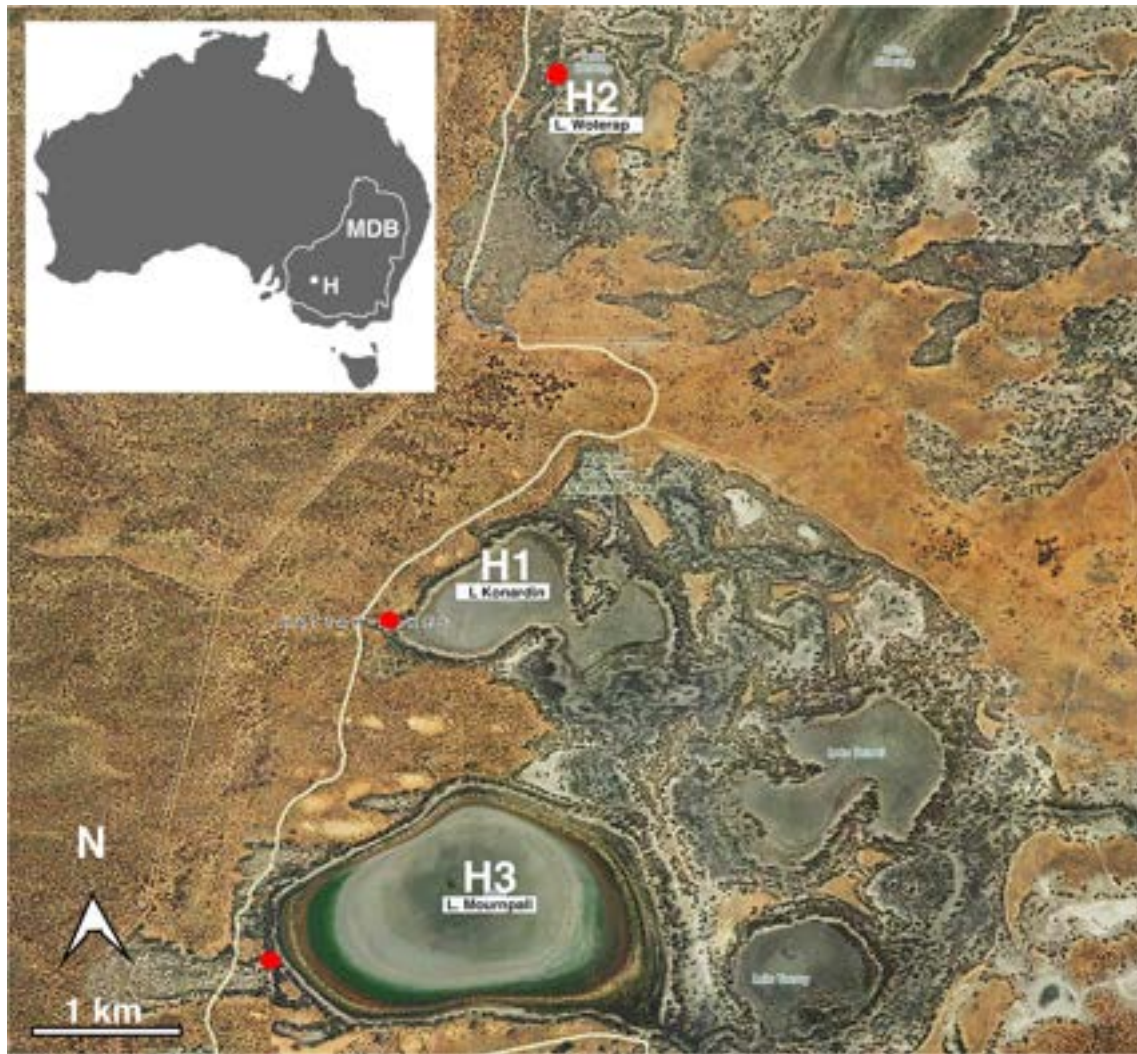
tree decline might become evident beyond the scope of potential mitigation strategies such as environmental water release, for example. Predicting tree response on the basis of empirical data ultimately stands to inform water management strategies. Benchmark field data do not currently exist in relation to mineral nutrition of MDB riverine eucalypts. Knowledge about visual canopy time-lag phases associated with responses in mature *Eucalyptus* trees to environmental fluctuations such as water supply is very limited. A recent pilot study into Black Box inundation detected early shifts in soil chemistry capable of altering nutrient availability (Fernando et al. 2018). Current knowledge about Black Box ecophysiology has largely been drawn from field studies in the lower Murray (Chowilla, Bookpurnong, Calperum and Markaranka floodplains; South Australia) and Hattah Lakes system to provide valuable insight into growth responses to flooding (Slavich et al. 1999; Bramley et al. 2003; Miller et al. 2003; Doody et al. 2009; Moxham et al. 2018; Doody et al. 2019; Wallace et al. 2019). Aside from the study by Moxham et al. on Black Box growth response to flooding (2018), these aforementioned studies variously investigated tree water use and balance with respect to groundwater renewal and flooding regimes, response to saline soils on Chowilla floodplain, and floodwater infiltration through root channels.

Base knowledge about tree-soil mineral nutritional dynamics across MDB ecosystems is far from complete. An improved understanding of these dynamics will aid interpretation of tree response to environmental fluctuation, ultimately benefitting management practice. The aim of this study was to collect foundational information on Black Box nutrition and soil traits over a 12-month period in response to flooding provided by an environmental flow, to examine patterns in tree-soil nutritional dynamics. Knowledge attained from this study is fundamental in the continual quest to integrate data and understand floodplain tree responses to floods and drought in arid and semi-arid floodplains globally. Furthermore, assimilation of longer-term field data from varied riverine soils and habitats across the MDB will bolster understanding of tree nutritional response patterns.

## Methods

### Field Site

The Hattah-Kulkyne National Park includes a system of lakes hydrologically connected to the River Murray by an anabranch, Chalka Creek. These lakes and surrounding areas are within the bed of a large freshwater paleo-lake (Hattah Mega-lake) from the Upper Pleistocene (Kotsonis et al. 1999), evident as grey soil (Fig. 2). Immediately to the west is the Sunset Country dune field, a system of longitudinal



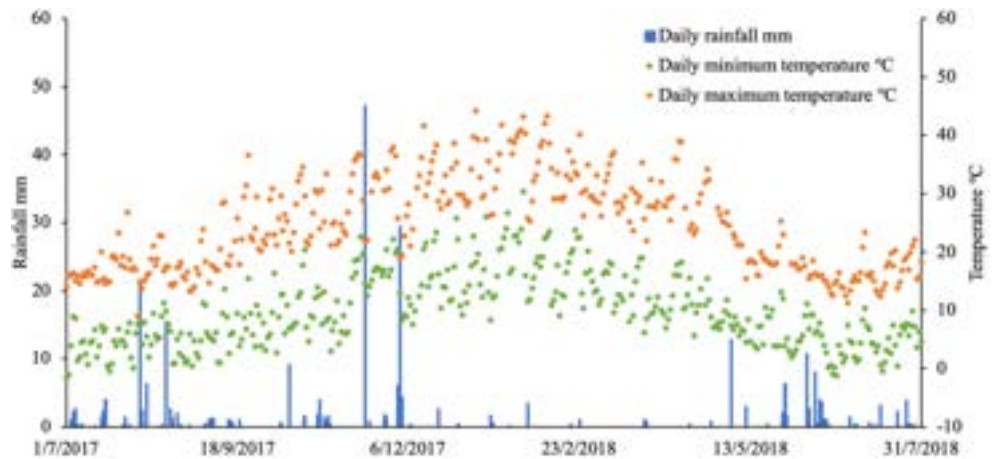
**Fig. 2** Inset: The Murray Darling Basin (MDB) Australia, and Hattah Kulkyn National Park (H). Main image: the three field sites H1, H2, H3 at Hattah. Figure derived from Fernando et al. (2018)

dunes encroaching onto the lake system along the western margins of the National Park.

Floodplain eucalypt woodlands occur on the lacustrine sediments of Hattah Mega-lake, with River Red Gum woodlands on lower-lying areas, and Black Box woodlands on slightly higher and less frequently-flooded areas (MDBA 2012). Black Box woodlands also occur in some inter-dunal swales (Kotsonis et al. 1999). Three sites utilised in an earlier pilot study by Fernando et al. (2018) were included here (Fig. 2), with sites H1 and H2 on the riparian fringes of Lakes Konardin and Waterap, and H3 on a flood-out area ~200 m west of Lake Mourmpall. All three sites are on lacustrine sediments overlain by dune sands (Kotsonis et al. 1999). Climate for the study area is semi-arid, with low-variable rainfall and high temperatures (Fig. 3). Annual rainfall at the closest weather station, Ouyen, was 374.2 mm in 2017, compared with the long-term mean of 329.6 mm (Bureau of Meteorology, Australia (<http://www.bom.gov.au/>)).

Five trees within each site were sampled between July 4, 2017 and July 13, 2018. Eight field visits were undertaken during this time, commencing just prior to water release to the Chalka Creek in July 2017. Environmental water allocation to the Hattah Lakes system was made by pumping water from the Murray River into the Chalka Creek. Surface inundation at the study sites were variously observed between August and December 2017 (Table 1). Since assessment of below-ground movement of floodwaters was beyond the scope of this study, surface floodwater at each site was taken as an indication that water was accessible to the root systems of all five study trees per site, with maximum surface water coverage for the entire study area observed in October 2017 at all sites (Table 1; Fig. 4). These woodlands were structurally open, with sparse woody shrub understories and insubstantial ground litter ranging from light to virtually absent. Field identification Black Box trees was undertaken by DF in consultation

**Fig. 3** Climate summary for Hattah from the Ouyen Post Office weather station (076047), 40 km S of Hattah (Bureau of Meteorology, Australia (<http://www.bom.gov.au/>))



with the Chief Ranger of the Hattah Kulkyne National Park.

**Plant and Soil Sampling and Analysis**

For each tree, young and mature leaf samples were obtained as two separate bulk samples, each consisting of around 10–20 leaves. Youngest fully expanded leaves at the growing tips of branchlets were selected for the ‘young’ category, while oldest, highly sclerophyllous, largest leaves lower down along branchlets were selected for the ‘mature’ category. These two categories were visually very distinct. Youngest fully expanded leaves at the growing tips of branchlets were selected for the ‘young’ category, while oldest highly sclerophyllous largest leaves lower down branchlets were selected for the ‘mature’ category. They were oven dried (70 °C), ground, and 0.2 g microwave-digested in 10 mL of concentrated nitric acid. Digests were diluted to 35 mL with de-ionised water for elemental quantification against equivalently acidified standard solutions (Fernando et al. 2018). Inductively coupled plasma mass spectroscopy (ICP MS)

(Perkin Elmer NexION, Waltham MA) was used to analyse C, nitrogen (N), phosphorus (P), potassium (K), sulphur (S), Ca, Mg, sodium (Na), copper (Cu), zinc (Zn), Mn, iron (Fe), boron (B), silicon (Si) and Al. While Al is not a plant nutrient, a previous study at this field site showed that soil Al concentration became slightly elevated with inundation (Fernando et al. 2018). Soils were sampled and analysed as described by Fernando et al. (Fernando et al. 2018). Pooled soil samples collected from within the top 10 cm soil layer excluding surface litter (Fig. 5). Deeper soil excavation for sampling under trees was out of scope of this study because of collection permit restrictions designed to prevent damage to trees. Soil sampling was done here on the basis that eucalypts widely known for their ability to acquire water and nutrients close to the soil surface (Eamus et al. 2002; Moroni et al. 2003). The soil samples were processed, and acid digested in 10 mL of Aqua Regia (1:3 concentrated HNO<sub>3</sub>/HCl). Soil pH, electrical conductivity (EC), free-water content, DTPA-extractable Zn, Mn, Fe and Cu; and total C, N, Zn, Mn, Al and P were also measured as previously described (Fernando et al. 2018). Total soil C and N were

**Table 1** Records of observed surface water around trees

sampling number	Dates 2017–2018	Site H1					Site H2					Site H3				
		Trees (T1-T5 per site)														
		T1	T2	T3	T4	T5	T1	T2	T3	T4	T5	T1	T2	T3	T4	T5
1	Jul 4th	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
2	Aug 5th	C	Y	Y	C	N	N	N	N	N	N	N	N	N	N	N
3	Sep 9th	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
4	Oct 5th	Y*	Y*	Y*	Y*	Y*	Y*	Y*	Y*	Y*	Y*	Y*	Y*	Y*	Y*	Y*
5	Dec 9th	C	Y	Y	C	C	N	Y	Y	N	N	N	N	N	N	Y
6	Feb 24th	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
7	Apr 4th	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
8	Jul 13th	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

(N - no surface water; Y - surface water present; C - surface water close, ~ 1-2m). \*Deepest observed waters



◀ **Fig. 4** Trees T1-T5 at each of sites H1-H3 just prior to water release (July 2017), and at highest encountered floodwaters around trees during sampling (October 2017). Note, paired photos of dry and wet sites not taken from the same angle

analysed by high-temperature combustion in a LECO Trumac analyser (LECO Corporation, St Joseph MI). The reason DTPA-extractable trace metal nutrients were measured in soil was to estimate their bioavailability to trees.

### Data Analysis

Standard errors of the means ( $\pm$ SE) of the leaf and soil analytical data were calculated using Microsoft Excel

**Fig. 5** Oven-dried soil samples collected from beneath individual black box trees (1 to 5) when wet and dry, at each of Sites H1, H2, and H3



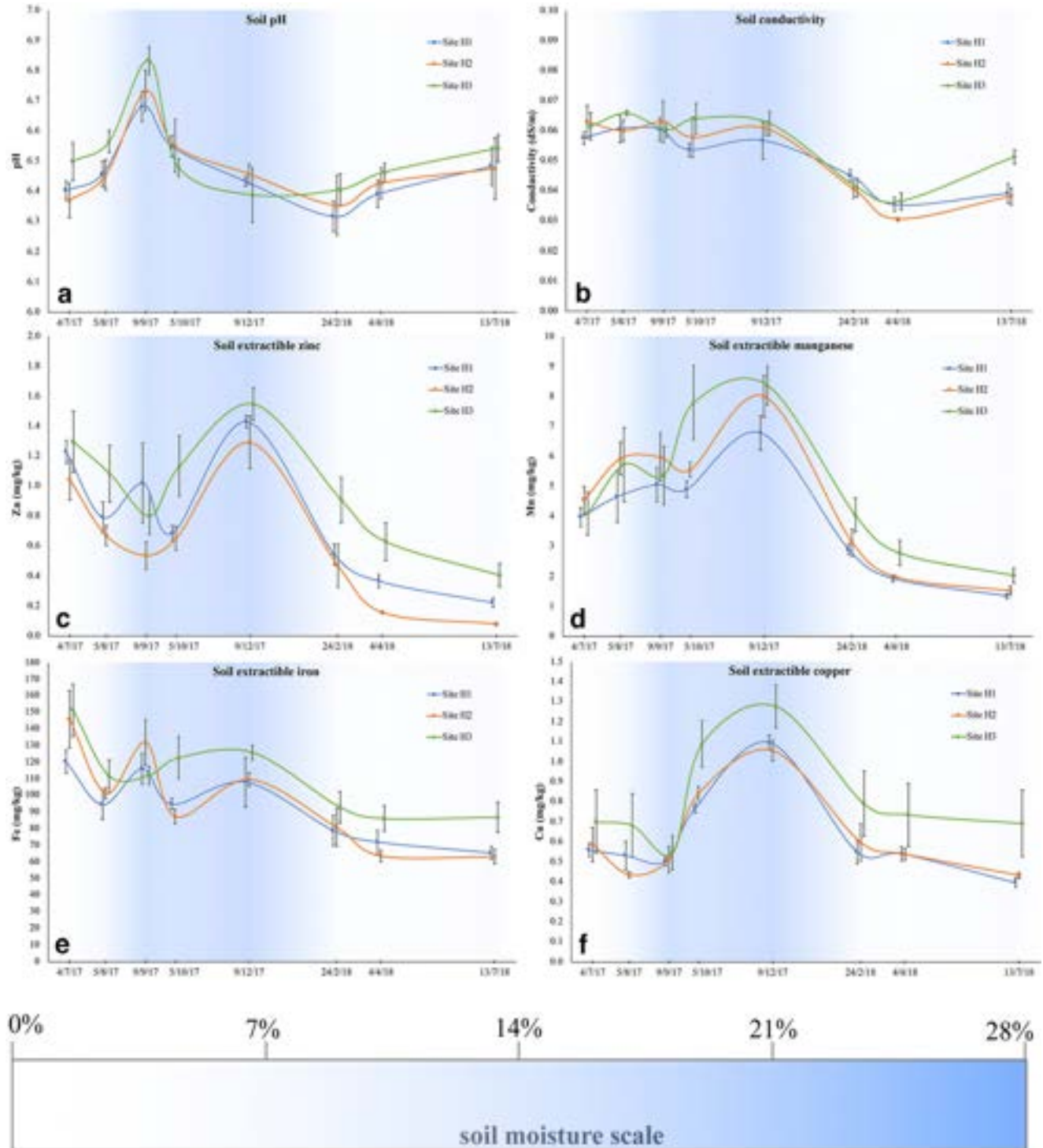
(2020) software. Statistical analysis was conducted in IBM SPSS Statistics version 26 (IBM Corp.). It was necessary to log transform the dataset to normality and homogeneity of variance. The sites H1, H2, and H3 were treated as replicates and analysed with general linear model analysis for each of the ions with sampling time (8 levels) and medium (3 levels: soil, young leaves and mature leaves) as fixed factors. Pair-wise comparisons between mediums and sampling times were made with Tukey's post hoc tests. Relationships between soil extractable DTPA Cu, Fe, Mn and Zn and young and mature leaves were explored with Pearson bivariate correlations ( $r$ ).

**Results**

Figures 6, 7, 8 and 9 display broad patterns in empirical soil (Figs. 6 and 7) and plant (Figs. 8 and 9) data relative to season and inundation, while results of statistical analyses of these data are shown in Table 2.

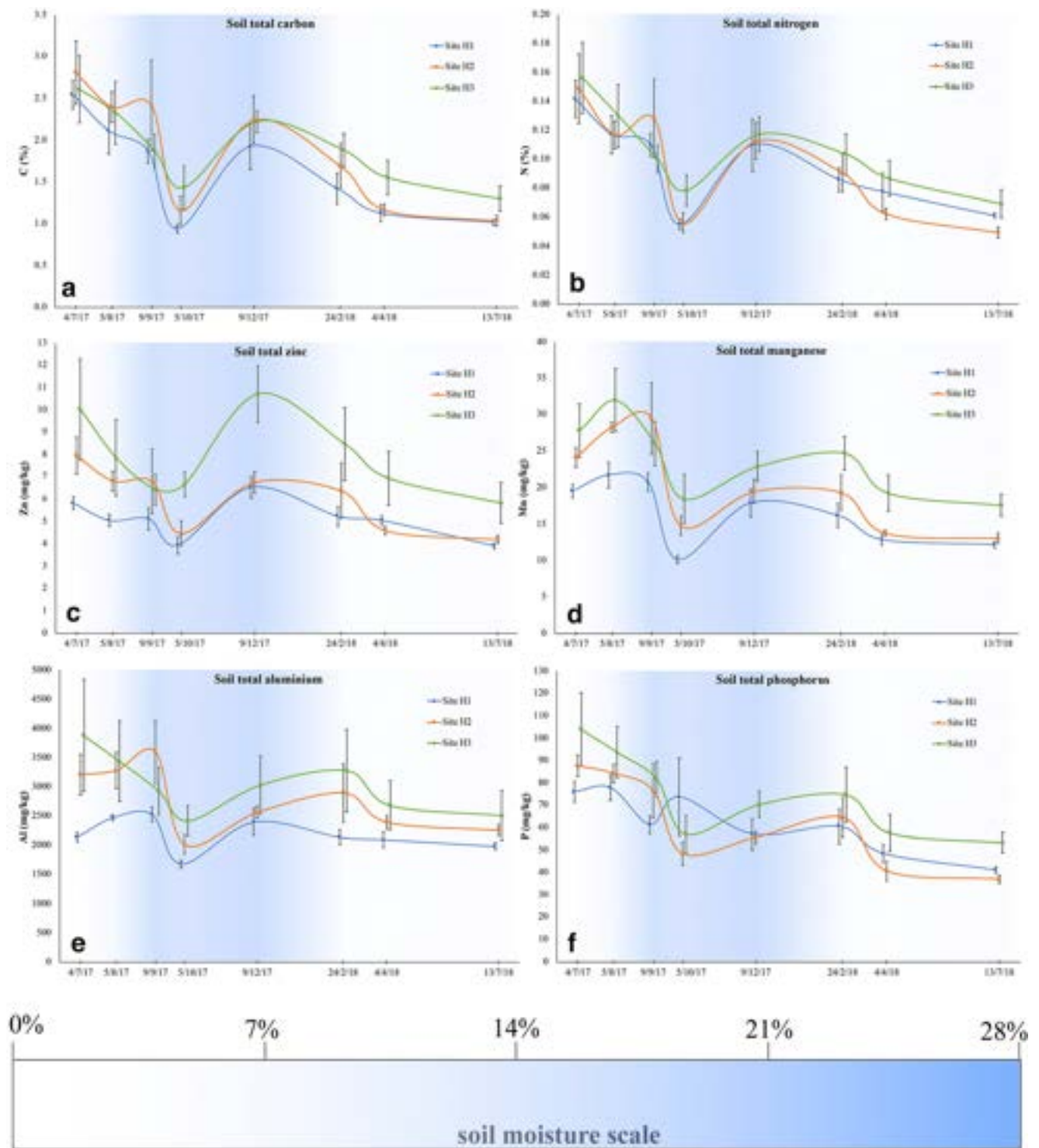
**Soil pH, Conductivity, Moisture**

Although surface water was most visibly abundant at sampling 4 (Table 1), the highest soil moisture content values as depicted in the embedded scales (Figs. 6 and 7) were recorded at samplings 3 and 5, with the second soil moisture peak in



**Fig. 6** Soil traits on sites H1, H2, H3 during the study period (July 2017–2018), with the presence of surface water shaded blue: pH (a), conductivity (deciSiemen/m) (b), DTPA-extractable Zn (c), Mn (d), Fe (e), Cu (f) concentrations ( $\pm$  SE). Soil moisture content (%) below

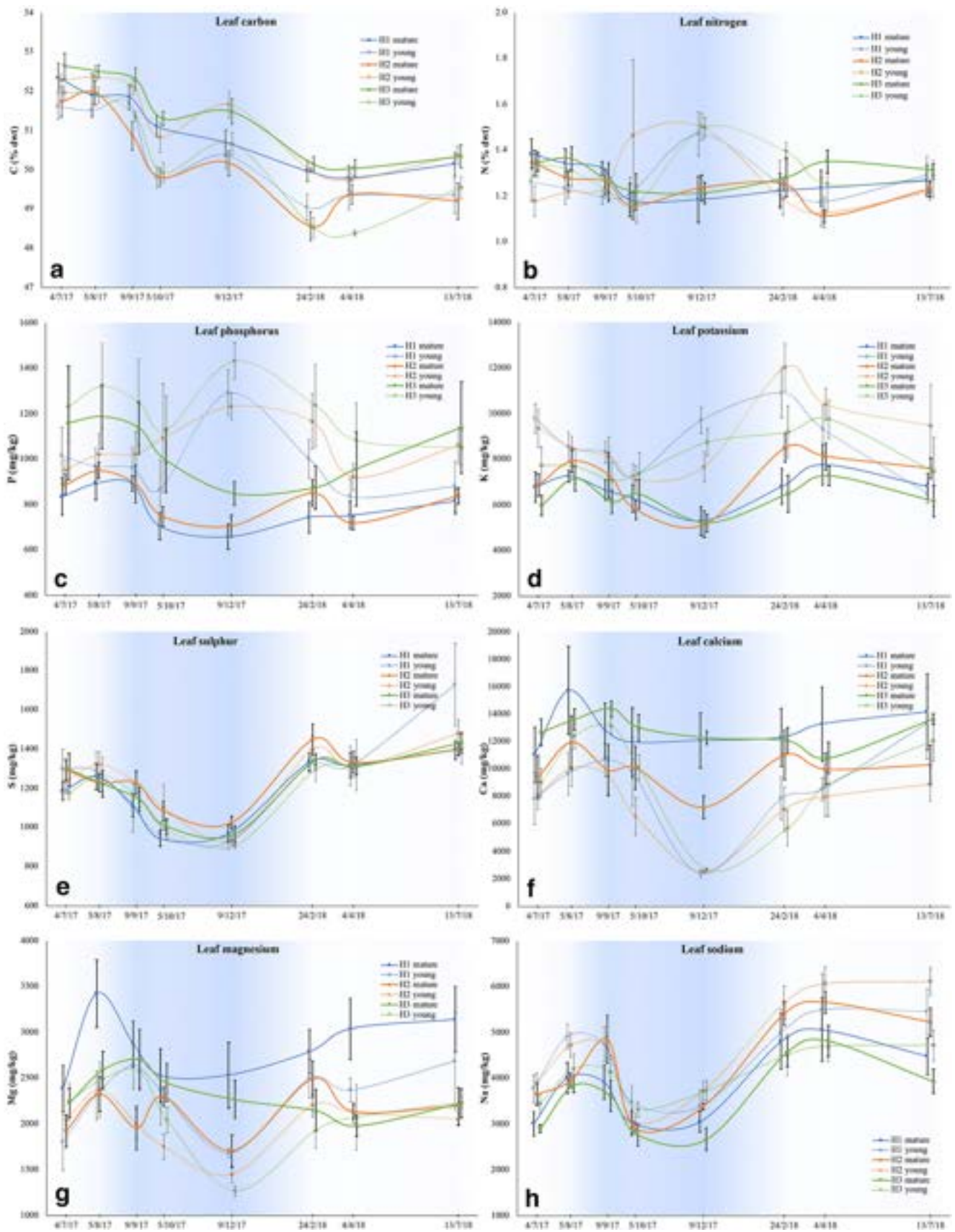




**Fig. 7** Total concentrations of elements in soils on sites H1, H2, H3 during the study period (July 2017–2018), with the presence of surface water shaded blue: C (a), N (b), Zn (c), Mn (d), Al (e), P (f) concentrations ( $\pm$  SE). Soil moisture content (%) below

December 2017 concomitant with the highest rainfall month across the entire 12-mont sampling period (Fig. 3). Maximum soil-pHs (Fig. 6a) of around 6.63 to 6.87 were observed at sampling 3, two months post water release, and coinciding

with the first moisture peak with surface water present (Table 1). A decrease in pH ( $\sim$ 6.3–6.5) occurred due to inundation and increased back toward a pH range comparable to sampling 1, just before water release.



**Fig. 8** Dry weight concentrations of C (a), N (b), P (c), K (d), S (e), Ca (f), Mg (g) and Na (h) ( $\pm$  SE) in young and mature leaves on sites H1, H2, H3 during the study period (July 2017–2018), with the presence of surface water shaded blue

## Soil Extractable Zn, Mn, Fe and Cu

Plant bioavailabilities of the trace metal plant nutrients Zn (Fig. 6c), Mn (Fig. 6d), and Cu (Fig. 6f) were highest at sampling 5, when soils were nearing their lowest pH (Fig. 6a). As a measure of free ions in the soil solution, EC (Fig. 6b) serves as a reliable proxy for salinity. Values here of around 0.03–0.07 dS/m were typically low as expected for loamy sands (Smith and Doran 1996), with an observed net lowering at the second moisture peak (sampling 5) into the drying period. The bioavailabilities of soil-Fe (Fig. 6e) at all sites decreased from sampling 1, to peak at the first soil moisture maximum (sampling 3) during surface inundation, before decreasing into the post-flood drying phase, with an overall net decrease across the entire study period.

## Total Soil C, N, Zn, Mn, Al, P

Fluctuation patterns of total soil C (Fig. 7a), N (Fig. 7b), Mn (Fig. 7d) concentrations at all 3 sites were similar. The data depict an initial lowering of these concentrations from samplings 1 to 4 during the first part of the surface inundation period, rising at sampling 5 at the second moisture peak. Net decreases in total soil C, N, Mn and P (Fig. 7f) concentrations were observed between samplings 1 and 8, however, shifts in total soil Al (Fig. 7e) concentrations were less clear. Total soil Zn concentrations (Fig. 7c) showed that site H3 had the highest values that decreased until sampling 4 before reaching a maximum at the second soil-moisture peak at sampling 5. Lower total Zn at sites H1 and H2 showed a similar trend, albeit with minor increases at sampling 3.

## Leaf C, N, P, K

A net decrease in total leaf C concentrations (Fig. 8a) was observed in both young and mature leaves at all study sites across the entire study period. Site H2 was the exception where total C in young leaves exceeded that of mature leaves. Leaf N (Fig. 8b) for mature leaves at all sites did not differ significantly across the study period, while there was a prominent increase in young leaf N concentrations over the latter part of the inundation period, reaching a maximum at around the second moisture peak at sampling 5. Similarly, P concentrations (Fig. 8c) in young leaves were at a maximum in sampling 5. Young leaf P concentrations generally exceeded those of mature leaves, as did K (Fig. 8d), which peaked after surface water subsided (Table 1).

## Leaf S, Ca, Mg, Na

Leaf S concentrations (Fig. 8e) across all sites and both leaf ages decreased to lowest recorded values during inundation, then increased into the soil drying phase. Leaf Ca

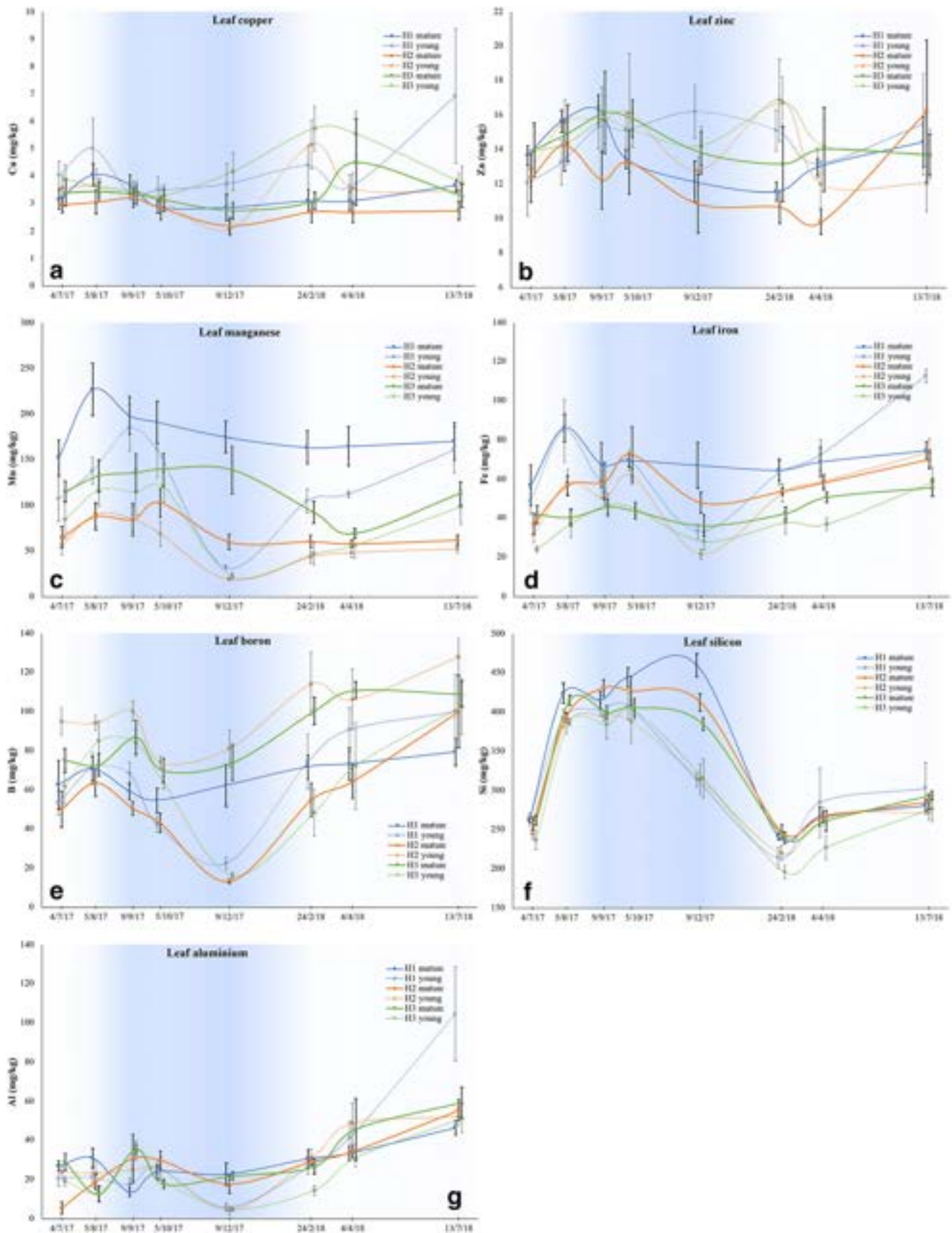
concentrations (Fig. 8f) were lower in young leaves compared to mature leaves at all sites, reaching lowest values at the second soil moisture peak at sampling 5. The data suggest an initial increase in mature leaf Ca at sites H1 and H2 at sampling 2, when surface water was first observed (Table 1). At site H2, leaf-Ca concentrations in mature leaves decreased similar to young leaves from all sites. This was not noted for mature leaves at H1 and H3. Trends in total leaf Mg concentrations (Fig. 8g) across the study period were similar to that of Ca, with an initial increase in mature leaf Mg at sites H1 and H2, a decrease in mature leaf Ca at H2, and decreases in young leaf Ca to minima at all three sites at the second soil moisture peak (sampling 5). Like leaf Ca, leaf Mg concentrations were lower in young leaves compared to mature leaves. Trends in leaf Na concentrations (Fig. 8h) during the study period were comparable across leaf age and study site, decreasing down to minimum recorded values when surface water was present.

## Leaf Cu, Zn, Mn

Leaf Cu concentrations (Fig. 9a) were generally higher in young leaves compared to mature leaves. Young leaf Cu concentrations peaked on either side of the inundation period at site H1, while for sites H2 and H3 they only peaked post surface inundation, at sampling 6, in February 2018 (Table 1). Leaf Zn concentrations (Fig. 9b) showed some increases in young foliage Zn beyond December 2017, however these trends are unclear. In general, leaf Mn concentrations (Fig. 9c) were higher in mature leaves than in young leaves, with some increases in mature and young leaf Mn concentrations before and during the early stages of surface water presence.

## Leaf Fe, B, Si, Al

Leaf Fe concentrations (Fig. 9d) increased after water release, from when surface water was first recorded in August 2017 (Table 1), with a decrease in young leaf Fe concentrations at the second soil moisture peak at sampling 5. While slight net increases in mature and young leaf B concentrations (Fig. 9e) at all three sites across the study period were observed, there was a prominent decrease in site H1 and H3 young leaves and site H2 mature leaves. Leaf Si concentrations (Fig. 9f) rose sharply after water release in July 2017 (sampling 1, Table 1), from ~250 mg/kg to ~375–475 mg/kg by sampling 2, returning to initial levels by sampling 6 into the drying period. Between the first and second soil moisture peaks at sampling 3 and 5, young leaf Si concentrations at all three sites were comparably lower than those of older leaves. Leaf Al concentrations (Fig. 9g) were similar for leaves of both ages at all sites up to the post flood drying period, with some net increase across the study period.



**Fig. 9** Dry weight concentrations of Cu (a), Zn (b), Mn (c), Fe (d), B (e), Si (f) and Al (g) ( $\pm$  SE) in young and mature leaves on sites H1, H2, H3 during the study period (July 2017–2018), with the presence of surface water shaded blue

**Table 2** Statistical analyses of soil and leaf (mature and young) elemental empirical data

Sample	<i>n</i>	Element/ trait	Means ± SD (log value ± SD; actual value ± SD) SD	<i>F</i>	Sig.
Soil	24	Al	3.41 ± 0.009; 2655.71 ± 568.98	1.95	0.13
		C	0.23 ± 0.15; 1.79 ± 0.57	17.58	0.000
		conductivity	-1.29 ± 0.10; 0.05 ± 0.01	23.23	0.000
		Cu	-0.19 ± 0.14; 0.69 ± 0.23	6.43	0.001
		Fe	1.99 ± 0.11; 100.78 ± 24.76	10.54	0.000
		Mn	0.60 ± 0.23; 4.50 ± 2.05	32.12	0.000
		moisture	0.80 ± 0.50; 11.33 ± 11.15	76.30	0.000
		N	-1.02 ± 0.14; 0.1 ± 0.03	21.99	0.000
		P	1.80 ± 0.12; 65.86 ± 17.45	7.83	0.000
		pH	0.81 ± 0.01; 6.49 ± 0.12	16.05	0.000
Young leaves	24	Zn	-0.19 ± 0.32; 0.79 ± 0.41	6.56	0.001
		Al	1.35 ± 0.33; 28.97 ± 12.76	19.25	0.000
		B	1.75 ± 0.23; 84.33 ± 19.69	22.84	0.000
		C	1.70 ± 0.11; 51.13 ± 1.03	30.90	0.000
		Ca	3.89 ± 0.21; 11892.0 ± 1876.38	31.09	0.000
		Cu	0.59 ± 0.10; 3.16 ± 0.49	1.53	0.228
		Fe	1.69 ± 0.18; 56.96 ± 13.37	3.81	0.013
		K	3.93 ± 0.07; 6734.71 ± 900.44	4.10	0.009
		Mg	3.32 ± 0.08; 2428.96 ± 410.60	5.15	0.003
		Mn	1.88 ± 0.26; 123.21 ± 49.92	5.07	0.003
		N	0.11 ± 0.04; 1.27 ± 0.08	3.41	0.020
		Na	3.64 ± 0.09; 3979.16 ± 912.96	12.71	0.000
		P	3.03 ± 0.06; 882.5 ± 151.29	1.84	0.148
		S	3.08 ± 0.07; 1219.58 ± 155.59	25.18	0.000
Mature leaves	24	Si	2.48 ± 0.10; 340.79 ± 80.96	56.15	0.000
		Zn	1.16 ± 0.05; 13.54 ± 1.74	3.20	0.026
		Al	1.41 ± 0.22; 28.97 ± 12.76	2.25	0.085
		B	1.91 ± 0.10; 84.33 ± 19.69	1.60	0.207
		C	1.71 ± 0.01; 51.13 ± 1.03	26.89	0.000
		Ca	4.07 ± 0.07; 11892 ± 1876.38	0.79	0.607
		Cu	0.50 ± 0.07; 3.16 ± 0.49	1.54	0.223
		Fe	1.74 ± 0.10; 56.96 ± 13.37	0.77	0.621
		K	3.82 ± 0.06; 6734.71 ± 900.44	6.70	0.001
		Mg	3.38 ± 0.07; 2428.96 ± 410.60	0.61	0.739
		Mn	2.05 ± 0.19; 123.21 ± 49.92	0.39	0.892
		N	0.10 ± 0.03; 1.27 ± 0.08	1.90	0.137
		Na	3.59 ± 0.10; 3979.17 ± 912.96	13.57	0.000
		P	2.94 ± 0.07; 882.50 ± 151.29	1.58	0.211
S	3.08 ± 0.06; 1219.58 ± 155.59	31.70	0.000		
Si	2.52 ± 0.11; 340.79 ± 80.96	110.04	0.000		
Zn	1.13 ± 0.06; 13.54 ± 1.74	1.74	0.171		

Values presented as mean ± standard deviation (SD) of the 8 samplings; where *n* = 24 (3 mediums, 8 samplings). Both log and actual values shown. DTPA-extractable soil concentrations used for Cu, Fe, Mn and Zn; total soil concentrations used for Al. *F* and significance (Sig.) are from general linear model analysis. C, N, moisture as %; and conductivity in dS/m

Soil moisture was similar in September, October and December 2017 (22–28 %), significantly different to July and August 2017 (3–5 %) and February to July 2018 (2–3 %) (*P* values were all 0.000). There was a drop in

conductivity for February to July 2018 ( $0.034 \pm 0.001$  to  $0.43 \pm 0.004$  dS/m) significantly different to the previous months ( $0.058 \pm 0.003$ – $0.062 \pm 0.002$  dS/m) with  $P$  values ranging from 0.000 to 0.005. Young leaf concentrations of N, P and K spiked in December 2017 (N = 1.5 %, P =  $1319.0 \pm 59.57$  mg/kg, K =  $8697.67 \pm 585.22$  mg/kg), significantly different from mature leaf concentrations of these elements (N = 1.2 %, P =  $737.67 \pm 57.37$  mg/kg, K =  $5213.33 \pm 39.77$  mg/kg;  $P = 0.000$ , 0.005 and 0.002 respectively). Foliar Al, B, Ca, Mg and Mn concentrations dropped significantly in young leaves (Al =  $4.90 \pm 0.42$  mg/g, B =  $17.0 \pm 2.65$  mg/kg, Ca =  $2496.33 \pm 81.42$  mg/kg, Mg =  $1463.67 \pm 119.88$  mg/kg, and Mn =  $24.67 \pm 3.84$  mg/kg compared to mature leaves (Al =  $21.0 \pm 1.53$  mg/g, B =  $72.33 \pm 5.55$  mg/kg, Ca =  $10504.33 \pm 1653.50$  mg/kg, Mg =  $2166.33 \pm 244.45$  mg/kg, and Mn =  $124.67 \pm 33.96$  mg/kg respectively;  $P = 0.000$ , 0.003, 0.001, 0.05, 0.013 respectively). Foliar elemental concentrations in August and September 2017 compared to July 2018 were significantly different in young leaves, Al =  $19.0 \pm 3.06$  and  $25.67 \pm 5.21$  mg/g compared with  $69.0 \pm 17.50$  mg/g respectively,  $P = 0.002$  and 0.016), Cu ( $4.07 \pm 0.47$  and  $3.39 \pm 0.01$  compared with  $4.7 \pm 1.10$  mg/kg, both  $P = 0.000$ ), and S ( $1292.67 \pm 14.62$  and  $1156.67 \pm 36.92$  compared with  $1535.33 \pm 9$  mg/kg,  $P = 0.032$  and 0.000). This is such that the ion concentrations remain similar in mature leaves for August, September 2017 and July 2018, whereas they were higher in concentration in younger leaves in July 2018 indicating they had responded to inundation. Young leaves in February 2018 had increased concentrations in Cu ( $5.10 \pm 0.38$ ,  $2.97 \pm 0.13$  mg/kg  $P = 0.004$ ) and Zn ( $16.33 \pm 0.67$ ,  $12.0 \pm 0.58$  mg/kg  $P = 0.008$ ) compared to mature leaves. The increase in copper in young leaves corresponded to significantly higher soil concentrations in the preceding October and December ( $0.89 \pm 0.11$  and  $1.13 \pm 0.07$  mg/kg respectively), significantly higher than the other 6 months sampled, ranging from 0.51 to 0.65 mg/kg with  $P$  values ranging from 0.002 to 0.038. A Pearson correlation confirmed that soil copper was significantly correlated with copper in mature leaves ( $0.69 \pm 0.05$  and  $3.16$  mg/kg  $\pm 0.1$ ;  $r = 1.00$   $P = 0.000$ ). Soil Fe was significantly correlated with concentrations in young and mature leaves ( $100.78 \pm 5.05$  with  $52.67 \pm 4.30$ , mg/kg  $r = -0.681$   $P = 0.000$  and  $56.96 \pm 2.73$ ,  $r = -0.588$   $P = 0.002$  respectively). The increase in Zn in young leaves corresponded to significantly depleted soil zinc in July 2018 ( $0.24 \pm 0.1$  mg/kg) compared to the previous 5 sampling times from July 2017 to December 2017 ( $1.17 \pm 0.08$ ,  $0.84 \pm 0.12$ ,  $0.79 \pm 0.13$ ,  $0.83 \pm 0.16$ ,  $1.42 \pm 0.07$  mg/kg) with  $P$  values for July 2018 ranging from 0.001 to 0.026.

## Discussion

Black Box foliage and soil data over the 12-month study period compared to the presence of surface water inundation, indicated inundation-associated fluxes in soil mineral and metal ions, shifts in other soil traits, and changes in leaf nutrient concentrations. It is well established that climatic and/or other environmental factors, primarily temperature, rainfall, pollution and flooding can effect soil chemical changes (CSIRO 1983; White 1997); the latter of which was evaluated here. Most evidently, changes to soil free-water content and pH, elevated trace element bioavailabilities in these nutrient poor loamy sands. Surface inundation through environmental watering and/or rainfall paralleled changes in foliage concentrations of macronutrients such as N, P, K and S. With a few exceptions, nutrient concentrations across the study period fluctuated relative to flooding without net change. In focusing on plant-soil interactions relative to the presence of surface water, we seek to explain observed trends with reference to the literature, and highlight consistencies in field-data patterns despite considerable variation therein.

## Soil Traits

While soil characteristics were largely similar for all three sites, elevated metal concentrations at H3 may have been due to disturbance from an adjacent unsealed vehicular track. The well drained loamy sand layers on these sites were found to be typically nutrient poor and lacking substantial surface leaf litter. At all sites, a rise in soil pH (Fig. 6a) during the two months post water release (July 2017) through to a maximum observed pH of around 6.9 at the first soil-moisture peak (sampling 3) align with findings of submergence studies that report a gradual pH rise to around 7.0 for loamy sands (Ponnamperuma 1972; Narteh and Sahrawat 1999). These studies report initial pH lowering preceding a rise to a plateau at around pH 7 during flooding. That plateauing effect was not observed here. Furthermore, the initial pH depression at the onset of flooding observed in those studies would not have been detected here because of the 4–6-week sampling regime of the present study. However, this very early pH-depression effect was noted in a pilot study of these identical Hattah Lakes sites, when soils were sampled just days after surface water had reached the study sites (Fernando et al. 2018). Based on the findings of Ponnamperuma (1972) it is plausible that pH elevation observed in this study was due to soil reduction, followed by soil oxidation that gradually lowered pH. These studies suggest that generalisations about soil-pH changes caused by submergence should be interpreted with some caution given the inherent variability of soil. The small peak in soil extractable Fe concentrations (Fig. 6e) at four weeks of inundation, concurrent with soil pH maxima for all sites (Fig. 6a) aligns with other studies (Ponnamperuma 1972;

Satawathananont et al. 1991; Narteh and Sahrawat 1999) that describe a state of equilibrium at around the fourth week of submergence, where pH is initially elevated by the loss of H<sup>+</sup> ions when soil Fe (III) is reduced to Fe (II).

Depression of soil EC by flooding (Fig. 6b) values already typically low for loamy sands (Smith and Doran 1996) suggest that low free ion concentrations in soil solutions at sites H1-H3 were further diluted by floodwaters, with movement deeper down the soil profile with water recession. Increases in bioavailability of the plant trace-metal nutrients Zn, Mn and Cu in soils at all sites during surface inundation (Fig. 6c, d, f) was caused in part at least by pH lowering during that period (5a), and possibly to increased redox potential not measured here. Fluctuations in characteristically low total soil C (TC) and N (TN) concentrations across the study period, with net decreases of ~ 50 % (Fig. 7a, b) at the final sampling were similar to reports from a study in which total organic carbon (TOC) and TN were found to be much lower in flooding soils compared to non-flooding soils (Paradis and Saint-Laurent 1993). Soils are generally more C- and N-rich at their surface horizons compared to further down the profile, largely because of surface litter (Paradis and Saint-Laurent 1993), which was not abundant on the present study sites. While the direct comparability of TOC and TC for Hattah soils is unknown, sandy soils are generally regarded as lacking in inorganic C (Carlyle 1993). Similarly, the net depletion of total soil Mn and P concentrations (Fig. 7d, f) indicated loss of these plant nutrients from the surface soil horizon because of flooding. Increases in total soil Zn concentrations (Fig. 7c) at all sites during surface inundation may have been due to transportation via floodwaters, or, Zn from deeper down the soil profile entering the surface horizon. As mentioned earlier, site H3 had more elevated levels of soil-Zn, possibly due to adjacent land disturbance (Fernando et al. 2018). While total soil Al concentrations (Fig. 7e) did not change overall, it is possible as previously observed in a pilot study at this site (Fernando et al. 2018) that slow-moving floodwaters transported aluminosilicate clay particles, leading to an initial elevation in total soil Al concentrations. It is plausible that these aluminosilicate particles were moved deeper down the sandy soil profile by subsiding floodwaters, past the soil-sampling zone of this study.

### Leaf Elemental Changes

Vegetation growth-flushes ensuing flooding can rapidly increase foliage biomass, with a temporary ‘diluting’ of foliar trace mineral nutrients when biomass production rates exceed those of nutrient assimilation, even though concentrations of macro-nutrients such as N, P and K, integral to new growth, can spike under conditions suited to growth; an effect generally seen in young leaves (Tinker 1981; Marschner 2002; Maathuis 2009). Consistent with those studies are

observations here of N, P (Fig. 8b, c) and K (Fig. 8d) concentration maxima, and Mn (Fig. 9c), Fe (Fig. 9d) and B (Fig. 8e) concentration minima in young leaves. Based on crop studies, Mn, Fe and B are generally known to be less concentrated in young leaves than in older leaves due to Mn and Fe phloem immobility, and possibly the role of B in structural linkages such cell walls, lignin etc. (Marschner 2002). Trends in leaf Cu (Fig. 9a) and Zn (Fig. 9b) suggest some elevation in young leaves by the end of surface inundation, which may be a lag-phase uptake response to elevated soil bioavailabilities of these metals midway through surface flooding (Fig. 6c, f). Net decrease in mature and young leaf C (Fig. 8a) during the study period cannot be explained from the literature. Whether pre-flooded trees at their driest were drought-stressed, and/or had greatest overall leaf thickening and leaf oil concentrations that possibly contributed to highest leaf-C at the start of the study; and/or, whether flooding-induced sugar (carbohydrate) production in leaves led to O-dilution of C cannot be determined here. However, these are avenues for future investigations.

Simultaneous depression of S concentrations in all leaves (Fig. 8e) during inundation is not easily explained with reference to the literature; and may have arisen from soil-S dilution at the root-soil interface, or, a vegetation biomass ‘diluting’ effect. Mature leaves generally have higher Ca concentrations than in young leaves (Marschner 2002), its role in structural linkages such as in cell walls among its many essential roles. Leaf Ca concentrations here (Fig. 8f) clearly reflected this, with some inundation-induced depression effects likely due to rapid biomass production. Trends in leaf-Mg concentrations with respect to inundation (Fig. 8g), which were very similar to that of Ca (Fig. 8f) and Mn (Fig. 9c) despite their different plant nutritional roles might reflect similarity of these divalent cation macronutrients in terms of their ionic radii and plant uptake pathways, although direct competition can occur when one or more are in oversupply (Marschner 2002). Notably, both Mg and Mn are integral to photosynthesis, among their numerous respective macro- and micro- nutritional roles. Leaf Na (Fig. 8h) concentrations around 3000–6000 mg/kg while seemingly high according crop salinity stress indices (Marschner 2002), were within normal range for other eucalypts (Judd et al. 1996), and agreed with a previous study of these trees (Fernando et al. 2018).

Despite no significant collective change in leaf Cu and Zn concentrations (Fig. 9a, b) over the duration of this study, coincidence at sampling 6 of maxima in young leaf concentrations with lowest soil pH (Fig. 6a) suggests that soil inundation facilitated plant uptake, in addition to soil Cu and Zn bioavailabilities reaching maxima at sampling 5. Given that both Cu and Zn are phloem mobile (Marschner 2002), these soil conditions likely favoured their accumulation in actively growing young leaves. There is no clear explanation for the distinct leaf Si concentration fluxes (Fig. 9f), common

to all leaf samples, where they reached levels almost 1.5 their original values, only during surface inundation. A net increase in leaf Al concentrations (Fig. 9g) to at least double the original values is not easily explained, even though it was possible that aluminosilicate clay particles in floodwaters may have contributed to this effect (Fernando et al. 2018). However, according to crop science data at least, a high degree of soil acidification, e.g. around  $\text{pH} < 5$  is required to render soil-Al available to plants (Graham et al. 1988); whereas here the lowest soil pH recorded was around 6.3 (Fig. 6a). According to Marschner (2002), low levels of Al may be beneficial to plant growth even though its toxic effects are well known.

The results of this study suggest that inundation is nutritionally beneficial to Black Box trees, although not all responses to flooding were understood with reference to the literature. There was evidence that inundation enhanced the soil bioavailabilities of Zn, Mn, Fe and Cu; trace mineral nutrients essential for plant function. There were consistencies with other sandy floodplain studies, where an increase in soil-extractable Fe was observed in the fourth week of submergence. There was also agreement with previous findings that soil TOC and TN in sandy floodplain soils devoid of much surface litter are generally low. While interpretation of plant nutritional data largely hinges on basic knowledge drawn from early crop experiments, patterns here in changes to foliar concentrations of plant macronutrients such as N, P, K to flooding were consistent with new growth. Interestingly, foliage Na concentrations recorded here were relatively high with reference to crop salinity stress limits, yet normal for eucalypts. Current knowledge about the plant toxicity of Al is solely based on crops, with results of this study warranting further investigation into increased Al accumulation in Black Box foliage as a result of flooding. The duration of inundation examined here was appropriate for generating a range of Black Box tree-soil interactions that were clearly beneficial, with some yet to be better understood. These findings offer preliminary evidence of the nutritional advantage to Black Box trees from small to intermediate sized floods most impacted by water resource development.

Except for rainfall, the separation of other individual weather effects such as air temperature on nutrient assimilation by Black Box trees was not possible and/or unlikely to confound the effects of a major managed water release because: (a) there is no evidence that eucalypt nutrition is affected by climate, however it is well known generally that plant nutrition can be affected by soil wetting/waterlogging (CSIRO 1983; Leeper and Uren 1997; White 1997; Marschner 2002), (b) the most likely climatic factor to affect nutrient assimilation here would be substantial rainfall that would indirectly affect nutrient availability in a similar manner to managed

water release, (c) the sampling regime of this study was insufficient to capture natural seasonal variations, (d) the climate in the arid to semi-arid region of Australia examined here is generally consistently hot and dry without any major fluctuation other than water availability, and (e) flooding is intrinsic to trees in floodplain habitats.

## Conclusions

By shedding light on the dynamics of certain interrelated variables within Black Box woodland systems of the Hattah Lakes floodplain, this study informs on nutrient acquisition aided by the flood-induced chemical diversification of these nutrient poor soils. Foliar elemental concentrations in mature and young leaves, traits of the loamy sand layer on which these trees grow, and the presence of surface water before during and after flooding were examined alongside each other, demonstrating that an integrated approach is essential to gaining even a basic overview of tree-soil interactions within such complex multidimensional frameworks. There is a need for assimilating similar data from a variety of soil types and phenotypes at least, so that Black Box response to flooding can be interpreted in the context of inundation-driven changes to the variety of soils on which it occurs. Further studies at Hattah, and new studies at other sites should incorporate deeper subsurface soil sampling in order to examine the spatial heterogeneity of soils associated with the root systems of Black Box trees, as well as floodwater retention. This would also enable investigation into floodwater subsidence and its possible role in shifting plant nutrients along the soil profile, where they may be variously accessible to tree roots. Soils not only underpin the availability of essential nutrients to trees but can drive toxic stress depending on pedology and other external factors. Whether phenotypic variability in Black Box affects its mineral nutrition is yet unknown, and also warrants investigation.

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**Data Availability** Data will be made publicly available or by contacting authors directly.

**Code Availability** Yet to be determined, authors can be contacted directly.



## Declarations

**Conflict of Interest** The authors declare no conflict of interest.

**Consent to Participate** All authors consent to participate.

**Consent for Publication** All authors give their consent for publication.

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