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Manganese Accumulation and Tissue-level Distribution in the Australian Hyperaccumulator *Gossia Bidwillii* (Myrtaceae)

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Abstract

The manganese (Mn) hyperaccumulator *Gossia bidwillii* is a tree species native to subtropical eastern Australia where it occurs on Mn-rich soils. Here, we conducted the first Mn accumulation and tissue-level distribution study on wild and experimentally grown *G. bidwillii*. *Gossia bidwillii* plants were subjected to different levels of Mn (250 µg g^{-1}, 500 µg g^{-1}, 1000 µg g^{-1}) soil dosing treatments, whereas the wild *G. bidwillii* was sampled from growing on highly Mn-enriched natural soils. We used laboratory-based micro-X-ray Fluorescence (µXRF) elemental mapping to elucidate in situ distribution patterns of Mn and other elements in hydrated wild and Mn-dosed *G. bidwillii* leaves. The data from wild *G. bidwillii* revealed that it can be strongly Mn-hyperaccumulating with foliar Mn concentrations of 39 000 µg g^{-1} and 24 000 µg g^{-1} in old and young leaves, respectively. In the Mn dosing trial, *G. bidwillii* accumulated 24 400 µg g^{-1} in old leaves and 17 100 µg g^{-1} in young leaves in the highest treatment level. The laboratory based µXRF data revealed that Mn is uniformly enriched throughout the laminae and petioles of both young and old leaves in wild *G. bidwillii*; while in Mn-dosed *G. bidwillii*, the foliar Mn distribution was primarily concentrated at the leaf-tip and lamina. The approach employed by combining data from the field and controlled experiments was especially meaningful for investigating Mn accumulation in this species and gaining added insight into the phenomenon of Mn hyperaccumulation.

Keywords Elemental distribution · Hyperaccumulator · Manganese · *Gossia bidwillii*

Introduction

Hyperaccumulators are highly unusual plants that are able to concentrate extremely high concentrations of specific trace elements in their foliage and other aerial parts (Baker and Brooks 1989; Reeves 2003; van der Ent et al. 2013). They are able to attain such high levels of accumulation due to their enhanced uptake and translocation mechanisms that are yet to be fully understood (Baker 1981, 1987). Manganese (Mn) hyperaccumulation is recognised at the notional threshold concentration of 10 000 µg g^{-1} Mn in dry weight shoot tissue (van der Ent et al. 2013). The hyperaccumulation of Mn is a rare trait documented primarily within the genera *Alyxia* (Apocynaceae), *Denhamia* (*Maytenus*) (Celastraceae), *Gossia* (Myrtaceae), *Grevillea, Macadamia* and *Virotia* (Proteaceae) distributed over eastern Australia and New Caledonia (Losfeld et al. 2015; Fernando et al. 2008, 2009b; Jaffré 1977, 1980), Malaysia (Nkrumah et al. 2018) and recently from Papua New Guinea (Do et al. 2019).

There are 20 Australian *Gossia* species with a wide latitudinal distribution, ranging from northern New South Wales (32^0S) to the northern Cape York Peninsula (13^0S) in Queensland (Snow et al. 2003). *Gossia bidwillii* (Myrtaceae) has a smooth bark which is irregularly covered with relatively large, coloured patches. This species, as well as *G. acmenoides*, *G. lucida* and *G. grayi,* are called "python bark *Gossias*" due to the resemblance of their bark to the skin colouring of the python snake (Snow et al. 2003). *Gossia bidwillii* is the only Australian *Gossia* to thrive

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on ultramafic soils (McLay et al. 2019), and also the first Mn hyperaccumulator described in Australia (Bidwell et al. 2002). That discovery instigated subsequent research on Mn hyperaccumulation in several other Australian *Gossia* species (Fernando et al. 2007, 2008a, 2009b, 2013; McLay et al. 2019). All these published studies have been based on freshly collected field samples or preserved material obtained from herbaria. There have been consistent observations in the Mn hyperaccumulative trait in *Gossia bidwillii*, as captured in a recent phylogenetic study of *Gossia* (McLay et al. 2019), and in fresh field material where foliar Mn concentrations reached up to 19 200 μg g^{-1} in this species (Bidwell et al. 2002).

Recent growth experiments on *G. fragrantissima* have shown that it can take up to 545 μg g^{-1} Co, 17 400 μg g^{-1} Mn and 13 000 μg g^{-1} Zn (Abubakari et al. 2021a), whereas freshly collected field samples of *G. grayi* and *G. shepherdii* were observed to contain up to 13 700 μg g^{-1} and 11 000 μg g^{-1} foliar Mn, respectively (Fernando et al. 2018). In vivo cryo-scanning electron microscopy (SEM)/energy dispersive X-ray analysis (EDS) showed Mn localization in *G. bidwillii* to be different from other hyperaccumulating species. Foliage hyperaccumulated metals are usually known, with rare exceptions, to accumulate in non-photosynthetic tissues, in most species in epidermal cells and in some species in trichomes and trichome bases (Vázquez et al. 1992; Küpper et al. 2000, 2001; Krämer et al. 1997; Mesjasz-Przybylowicz et al. 2001; Bhatia et al. 2003; Bidwell et al. 2004; Broadhurst et al. 2004), whereas in *G. bidwillii* Mn was found to be primarily localised in photosynthetic cells (Fernando et al. 2006a, b, 2007). Laboratory and synchrotron micro-X-ray Fluorescence (μXRF) analysis have revealed marginal accumulation of Co, Mn, and Zn in leaves, with localization of Co, Mn, and Zn in epidermal cells of *G. fragrantissima* (Abubakari et al. 2021a).

To date, no attempt has been made to examine the effects of Mn dosing treatments on *G. bidwillii* under controlled experimental conditions, mainly due to the relatively slow growth rate of this species. Furthermore, there has not been any study to examine tissue-level Mn distribution in *G. bidwillii* originating from growing on extremely high Mn-enriched soils. This study aims to: (i) measure the response of propagated *G. bidwillii* plants to Mn treatment under controlled conditions, and (ii) assess Mn (and other elements) uptake and accumulation in *G. bidwillii* plants from naturally occuring highly Mn-enriched soils. By employing laboratory μXRF analysis to determine in situ distributions of Mn and other elements in wild *G. bidwillii* and Mn-dosed *G. bidwillii,* this study also investigates leaf tissue Mn distribution patterns, as well as within-species age-related distributional differences, *i.e.*, between their young and old leaves.

Results

Elemental Concentrations in Wild and Mn-dosed *G. bidwillii* All plant tissue elemental concentrations presented in this study are based on dry weight (Fig. 1). The bulk elemental concentrations in young leaves, old leaves, and twigs of the wild *G. bidwillii* and the dosed *G. bidwillii* are shown in Tables 1 and 2, respectively, and that of the concentrations of Mn in young leaves, old leaves, and twigs of *G. bidwillii* in Fig. 2. The concentrations of Mn in the wild *G. bidwillii* were remarkably high, with a mean value of 39 000 μg g^{-1} (SE ± 3540) in old leaves, and 24 000 μg g^{-1} (SE ± 410) in young leaves and 5840 μg g^{-1} (SE ± 2820) in the twigs ($p < 0.05$) (Table 1). The concentrations of Ca and Na in wild *G. bidwillii* were higher in old leaves (9250 ± 785 μg g^{-1} Ca, 710 ± 70.0 μg g^{-1} Na) than in young leaves (5590 ± 145 μg g^{-1} Ca, 660 ± 190 μg g^{-1} Na) and twigs (4700 ± 400 μg g^{-1} Ca, 170 ± 35.0 μg g^{-1} Na) (Table 1). The concentrations of Fe and Mg in old leaves of wild *G. bidwillii* were 75.0 μg g^{-1} Fe (SE ± 17.0) and 1290 μg g^{-1} Mg (SE ± 108), respectively compared to 45 μg g^{-1} Fe (SE ± 0.70) and 840 μg g^{-1} Mg (SE ± 38.0) in its young leaves. However, no significant difference was observed between Mg in old leaves and twigs of wild *G. bidwillii* (Table 1). Concentrations of Al were higher in old leaves (200 ± 40.0 μg g^{-1} Al) than in young leaves (55.0 ± 0.85 μg g^{-1} Al) and in twigs (6.0 ± 10.5 μg g^{-1} Al). The concentrations of K were higher in young leaves (9380 ± 1040 μg g^{-1} K) than old leaves (3660 ± 160 μg g^{-1} K) and twigs (2710 ± 570 μg g^{-1} K). No significant differences ($p > 0.05$) were observed for Ni, P and Zn in young leaves, old leaves, and twigs of wild *G. bidwillii* (Table 1).

In the old leaves of the Mn-dosed *G. bidwillii*, the mean Mn concentrations in the **T4**, **T3** and **T2** treatment levels were 24 400 μg g^{-1} (SE ± 10 700), 21 800 μg g^{-1} (SE ± 8850) and 14 100 μg g^{-1} (SE ± 12 600), respectively. These concentrations are more than two-fold the Mn hyperaccumulation threshold in the **T4** and **T3** treatment levels (Fig. 2 and Table 2). The old leaves of *G. bidwillii* in the control (**T1**) had up to 10 900 μg g^{-1} Mn (mean 5490 μg g^{-1}). In the young leaves, the Mn concentrations in the **T4** and **T3** treatment levels were 17 100 μg g^{-1} (SE ± 8440) and 12 600 μg g^{-1} (SE ± 11 000), respectively (Fig. 2 and Table 2). The Mn concentrations of young leaves in the **T2** treatment level (8300 μg g^{-1}) were two-fold higher than that in the control **T1** (Fig. 2 and Table 2). In the twigs, the highest treatment level (**T4**) contained 17 400 μg g^{-1} (SE ± 6690) Mn, which is more than threefold higher than that in the **T3** (5000 ± 1150 μg g^{-1}), **T2** (4300 ± 1220 μg g^{-1}) and the control **T1** (2900 ± 1300 μg g^{-1}) treatment levels (Fig. 2 and Table 2).

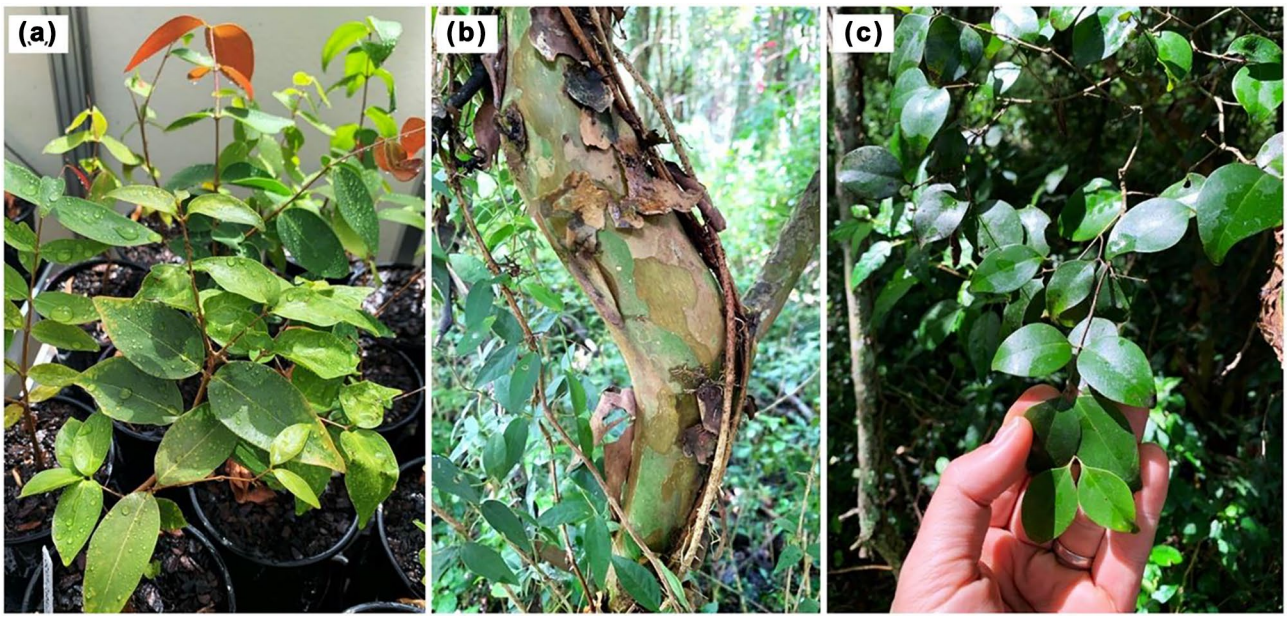

Fig. 1 Visual appearances of experimental and wild plants: (**a**) Experimental *Gossia bidwillii*, (**b**) field *G. bidwillii* tree trunk, and (**c**) field *G. bidwillii* foliage

The concentrations of Ca in the dosed *G. bidwillii* were higher in the young leaves compared to the old leaves ($p < 0.05$) (Table 2). The twigs had higher concentrations of Ca in the control **T1**, **T2** and **T4** treatment levels than in the young and old leaves, with mean concentrations of 17 100 µg g^{-1} (SE ± 1580), 11 100 µg g^{-1} (SE ± 2970) and 11 700 µg g^{-1} (SE ± 3780), respectively (Table 2). The concentrations of Al in old leaves of *G. bidwillii* were higher in the **T3** and **T4** treatment levels, with concentrations of 9000 µg g^{-1} (SE ± 5400) and 10 700 µg g^{-1} (SE ± 5370), respectively, compared to that in the young leaves and twigs (Table 2). The concentrations of K in the control **T1** (22,100 ± 2960 µg g^{-1} K) treatment level in young leaves was significantly higher than those observed in the other treatment levels (**T2**, **T3**, **T4**) in old leaves and twigs ($p < 0.05$). The concentrations of Mg were higher in old leaves in the control **T1** treatment level (4790 ± 1900 µg g^{-1} Mg) compared to that in the other treatment levels (**T2**, **T3**, **T4**) (Table 2). The concentrations of Na in twigs in the **T4** treatment level (1100 ± 840 µg g^{-1} Na) were significantly higher than that in young leaves (440 ± 260 µg g^{-1} Na) and old leaves (630 ± 140 µg g^{-1} Na) ($p < 0.05$) (Table 2). The concentrations of Fe and Zn were low in all treatment levels compared to other elements in young leaves, old leaves, and twigs (Table 2). The concentrations of P were below the limit of detection (< 6.00 µg g^{-1}) in young leaves, old leaves, and twigs in all treatment levels (Table 2).

Soil Chemical Properties The soil pH of the wild *G. bidwillii* was lower (pH = 4.20) compared to that of the Mn-dosed *G. bidwillii* pot soils (pH 5.27–5.95) (Table 3). Extractable Mn concentrations for DTPA and $Sr(NO_3)_2$ increased with the dose levels, as expected. Variability in Mn values for DTPA and $Sr(NO_3)_2$ can be explained by the strong binding of Mn^{2+} to the organic matter of the potting mix, thereby making it less available.

µXRF Elemental Mapping of Wild and Mn-dosed *G. bidwillii* Manganese in wild *G. bidwillii* from Mn-enriched soils in Amamoor is strongly enriched throughout the leaf blade and petiole of young and old leaves, while the veins and midrib have relatively lower concentrations (Figs. 3 and 4). Calcium and K are strongly enriched in the veins and midrib, with high concentrations of Ca in the stem and petiole and low K in the leaf margins of young and old leaves (Fig. 3). In the Mn-dosed *G. bidwillii*, the distribution of Mn is high at the apex and lamina, but lower in the midrib, margin, and veins (Fig. 4). However, K tends to be concentrated at the tip/margin, midrib, and petiole and veins whereas Ca is high in the midrib, margin, and veins, but lower in the leaf lamina (Fig. 4).

Discussion

Foliar Mn concentrations observed in the wild and experimental *G. bidwillii* were at least three-fold higher than the Mn hyperaccumulation threshold of 10 000 µg g^{-1} (van der Ent et al. 2013), which aligns with published data for of this species (Bidwell et al. 2002; Fernando et al. 2006b; Abubakari et al. 2021a). A weak relationship between

Table 1 Elemental concentrations in wild *G. bidwillii* leaves and twigs. Values are average of three replicates ± standard error (except for young leaves where n = 2). Values with different small letters in the same column are significantly different ($p < 0.05$)

Species Mn levels (μg g^{-1})	Elemental concentrations (μg g^{-1})									
	Al	Ca	Fe	K	Mg	Mn	Na	Ni	P	Zn
Gossia bidwillii	**Young leaves**									
In situ collection	53 ± 0.85^{b}	5590 ± 145^{b}	45.0 ± 0.70^{a}	9380 ± 1040^{a}	840 ± 38.0^{b}	24 000 ± 410^{b}	660 ± 190^{b}	10 ± 1.74^{a}	445 ± 83.0^{a}	8 ± 1.40^{a}
	Old leaves									
In situ collection	200 ± 38.0^{a}	9250 ± 785^{a}	75.0 ± 17.0^{a}	3660 ± 160^{b}	1290 ± 108^{a}	39 000 ± 3540^{a}	710 ± 70.0^{a}	25 ± 3.10^{a}	410 ± 63.0^{a}	15 ± 7.07^{a}
	Twigs									
In situ collection	6.0 ± 10.5^{b}	4700 ± 400^{b}	17.0 ± 6.00^{b}	2710 ± 570^{b}	1130 ± 725^{a}	5840 ± 2820^{c}	170 ± 35.0^{c}	90 ± 85.5^{a}	440 ± 60.0^{a}	20 ± 9.10^{a}

substrate Mn supply and foliar Mn concentrations, is also consistent with past field observations (Bidwell et al. 2002; Fernando et al. 2006b, 2007). The ability of *G. bidwillii* to over-accumulate Mn, even in very low soil-supply, is a common characteristic feature for hyperaccumulators, *i.e.,* their ability to accumulate metals in shoot tissues is consistent across a wide range of host substrate concentrations (Baker 1981; Fernando et al. 2007). The acidity of the wild *G. bidwillii* host soil (pH 4.20, Table 3) sampled for this study warrants consideration in the context of Mn availability at the root-soil interface. However, it is unclear why such high accumulation occurs and further investigation of specific Mn transporters associated with uptake at the root-soil interface and translocation from roots to shoots is required. It is also plausible that the apparently high Mn accumulation by wild *G. bidwillii* reflects specific rhizosphere effects such as acidification and/or microbial associations unique to the location that renders soil-Mn highly bioavailable.

Heterogeneity of Mn accumulation has been described in several species of the Mn hyperaccumulators; *G. grayi* and *G. shepherdii* (Fernando et al. 2013, 2018), *G. hillii* (Abubakari et al. 2021a, b; McLay et al. 2019), and *Denhamia founieri* (Fernando et al. 2008; Jaffré 1977) through field and herbarium analysis of their samples (Pollard et al. 2002; Baker et al. 1994; Macnair 2002). In contrast, the wild and Mn-dosed *G. bidwillii* in the present study, and previous observations through field sampling across the natural distribution and analysis of herbarium material, has found that the Mn-hyperaccumulation trait is consistent across its extensive natural range (Bidwell et al. 2002; Fernando et al. 2007).

In the Mn-dosed *G. bidwillii*, the behaviour of Mn in the oldest leaves resembled that of Ca, Mg and Na, which remained high at all treatment levels but contrasted with that of K and P which decreased after maturity. Similarly, in wild *G. bidwillii*, K was higher in young leaves than in old leaves, whereas Ca, Mg, Na and Mn were higher in old leaves. The high concentrations of Mn, Ca, Mg and Na in old leaves of the wild and Mn-dosed *G. bidwillii* could be attributed to phloem immobility of the aforementioned elements (Marschner 2002; Graham et al. 1988) and *vice versa* for K. Moreover, this behaviour could be due to the similarities in divalent cations of Mn, Ca, and Mg. Similar observations of high Mn in old leaves has been reported in *G. bidwillii* (Bidwell et al. 2002) and in other Mn hyperaccumulators including *Phytolacca americana* (Xu et al. 2006), *Macadamia integrifolia* (Fernando et al. 2009a), *G. fragrantissima* (Abubakari et al. 2021a, b) and in crop plants (Millikan 1951). In contrast, *G. grayi* and *G. shepherdii* were reported to accumulate higher Mn concentrations in young leaves than in older leaves (Fernando et al. 2018). A previous report by Bidwell et al. (2002) of decreased Ca and Mg with an increase in Mn concentration in old leaves of *G. bidwillii* contradicts the findings of this study. The nutritional

Table 2 Elemental concentrations in young leaves, old leaves, and twigs of experimental *G. bidwillii* after exposure to different levels of Mn treatments. Values are average of three replicates ± standard error. Values with different small letters in the same column are significantly different ($p < 0.05$)

Species Mn levels (μg g^{-1})	Elemental concentrations (μg g^{-1})									
	Al	Ca	Fe	K	Mg	Mn	Na	Ni	P	Zn
Gossia bidwillii	**Young leaves**									
Control	20 ± 12.0^{h}	9400 ± 2280^{e}	40 ± 18.0^{a}	22 100 ± 2960^{a}	3800 ± 540^{b}	4640 ± 1725^{h}	1400 ± 500^{a}	< LOD	< LOD	225 ± 140^{b}
200	14 200 ± 7640^{a}	5700 ± 1820^{f}	30 ± 5.8^{a}	12 800 ± 2830^{c}	3400 ± 400^{b}	8300 ± 4270^{f}	1050 ± 520^{a}	320 ± 95.0	< LOD	65 ± 13^{g}
500	2800 ± 1600^{f}	12 400 ± 2790^{b}	60 ± 7.0^{a}	5000 ± 2640^{g}	2500 ± 260^{d}	12 600 ± 11 000^{e}	835 ± 480^{b}	25 ± 7.0	< LOD	95 ± 20^{d}
1000	7400 ± 3710^{e}	11 200 ± 4000^{c}	40 ± 10^{a}	13 200 ± 2180^{c}	2300 ± 1130^{d}	17 100 ± 8440^{c}	440 ± 260^{d}	< LOD	< LOD	90 ± 40^{d}
	Old leaves									
Control	40 ± 20.0^{h}	10 100 ± 4200^{d}	50 ± 20^{a}	10 500 ± 2770^{d}	4790 ± 1900^{a}	5490 ± 2740^{g}	1640 ± 1270^{a}	< LOD	< LOD	15 ± 3.3^{h}
200	1700 ± 1060^{g}	8680 ± 1730^{e}	34 ± 5.5^{a}	15 600 ± 12 200^{b}	3200 ± 2210^{b}	14 100 ± 12 600^{d}	1040 ± 740^{a}	< LOD	< LOD	55 ± 20^{g}
500	9000 ± 5400^{d}	10 500 ± 2540^{d}	40 ± 8.0^{a}	12 200 ± 4720^{c}	2400 ± 620^{d}	21 800 ± 8850^{b}	430 ± 110^{d}	< LOD	< LOD	70 ± 27^{e}
1000	10 700 ± 5370^{c}	8170 ± 1330^{e}	45 ± 6.0^{a}	12 500 ± 3600^{c}	3090 ± 540^{c}	24 400 ± 10 700^{a}	630 ± 140^{c}	< LOD	< LOD	100 ± 60^{c}
	Twigs									
Control	8000 ± 220^{e}	17 100 ± 1580^{a}	25 ± 3.60^{a}	8000 ± 220^{f}	1460 ± 270^{c}	2900 ± 1300^{i}	240 ± 49.0^{e}	< LOD	< LOD	445 ± 200^{a}
200	12 700 ± 4150^{b}	11 100 ± 2970^{c}	35 ± 5.50^{a}	7150 ± 3600^{f}	2600 ± 1280^{d}	4300 ± 1220^{h}	840 ± 603^{b}	100 ± 34.0	< LOD	130 ± 65.0^{c}
500	8000 ± 520^{e}	10 800 ± 3700^{d}	60 ± 8.02^{a}	7500 ± 4100^{f}	2270 ± 430^{d}	5000 ± 1150^{h}	650 ± 235^{c}	< LOD	< LOD	60 ± 35^{g}
1000	3100 ± 1700^{f}	11 700 ± 3780^{c}	50 ± 10.4^{a}	8600 ± 3900^{e}	2040 ± 790^{e}	17 400 ± 6690^{c}	1100 ± 840^{a}	< LOD	< LOD	80 ± 33.0^{e}

< LOD = below Limit of Detection; LOD for Ni = 0.03 μg g^{-1}, LOD for P = 6.00 μg g^{-1}

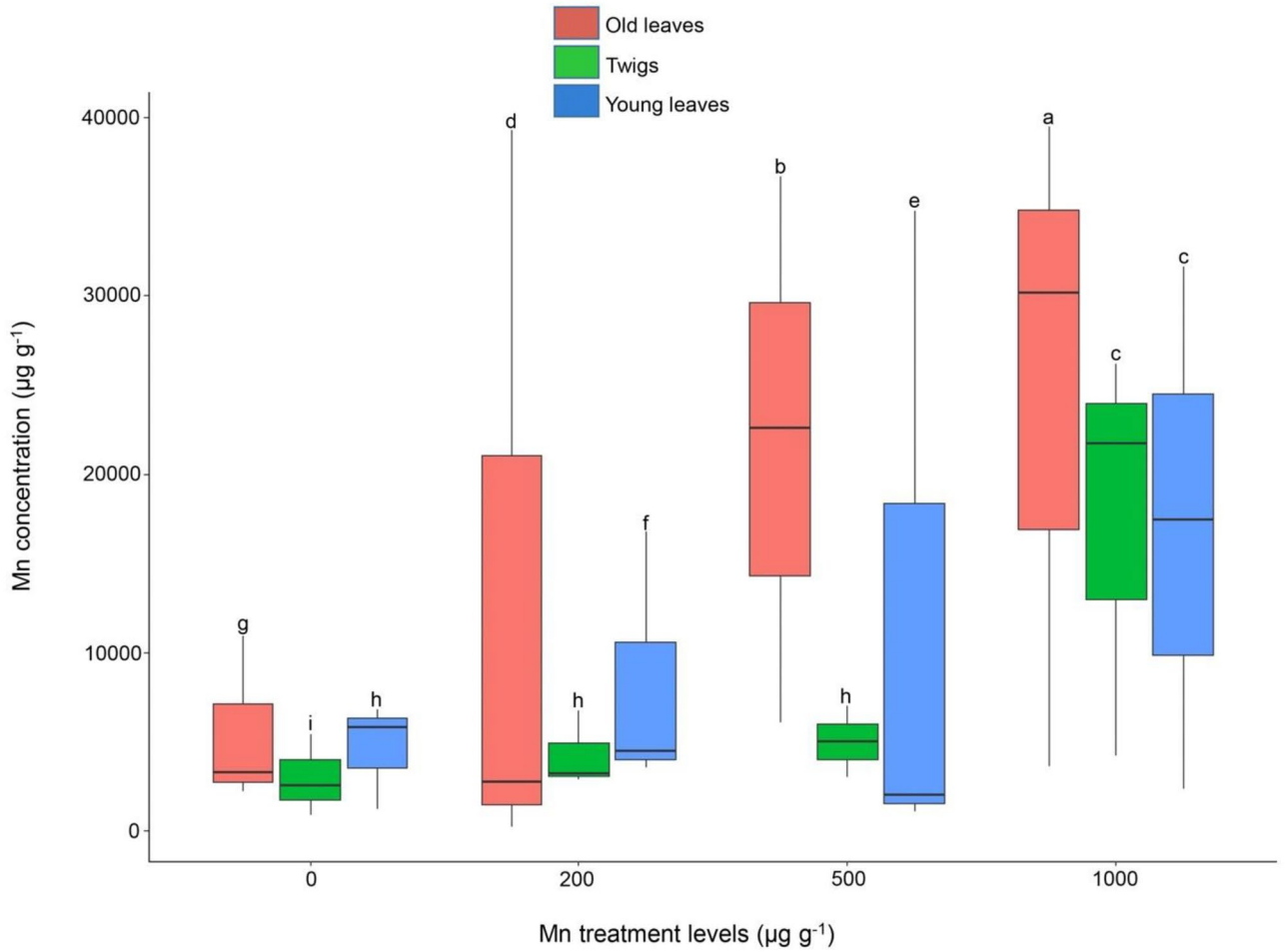

Fig. 2 Manganese concentrations in young leaves, old leaves, and twigs of experimental *Gossia bidwillii* plants. Keys to symbol of boxplots: open squares are the 25% to 75% quartiles, lines within the boxes indicate the median, whereas the whiskers mark the maximum and minimum values. Values with different small letters are significantly different ($p < 0.05$)

dynamics of plants as unusual as metal hyperaccumulators are yet to be fully understood, and clearly cannot be assumed to align with broader understanding of plant nutrition largely drawn from crop models (Marschner 2002). Another possible factor warranting consideration is possible seasonal shifts in foliar nutrient and metal concentrations that may contribute to observed variations across different studies.

Table 3 pH, diethylenetriaminepentaacetic acid (DTPA) and strontium nitrate ($Sr(NO_3)_2$) extractable concentrations in soils of different treatment levels of Mn of experimental *G. bidwillii* at harvest and soil of wild *G. bidwillii*. Values with different small letters in the same column are significantly different ($p < 0.05$)

Mn-dosed *Gossia bidwillii*	**pH**	**DTPA-extractable concentrations (µg g^{-1})**			**$Sr(NO_3)_2$-extractable concentrations (µg g^{-1})**		
Treatment levels (µg g^{-1})		**Co**	**Mn**	**Zn**	**Co**	**Mn**	**Zn**
Control	5.95	1.60 ± 0.07^{c}	< LOD	32.5 ± 4.24^{c}	1.00 ± 0.01^{c}	< LOD	7.30 ± 1.24^{d}
Mn 200	5.80	5.80 ± 0.30^{b}	< LOD	44.5 ± 10.0^{c}	1.20 ± 0.04^{c}	6.52 ± 3.40	12.6 ± 6.70^{b}
Mn 500	5.61	4.90 ± 0.75^{b}	27.2 ± 6.03	60.0 ± 13.4^{b}	3.45 ± 0.22^{b}	10.1 ± 5.60	18.9 ± 3.40^{b}
Mn 1000	5.27	12.7 ± 2.04^{a}	37.30 ± 9.01	126 ± 56.07^{a}	6.70 ± 1.90^{a}	17.8 ± 8.01	30.3 ± 7.55^{a}
Filed collected Gossia bidwillii	**pH**	**DTPA-extractable concentrations (µg g^{-1})**			**$Sr(NO_3)_2$-extractable concentrations (µg g^{-1})**		
		Co	**Mn**	**Zn**	**Co**	**Mn**	**Zn**
	4.20	0.98	1580	12.2	0.19	1650	3.00

< LOD = below Limit of Detection; LOD for Mn = 0.07 µg g^{-1}

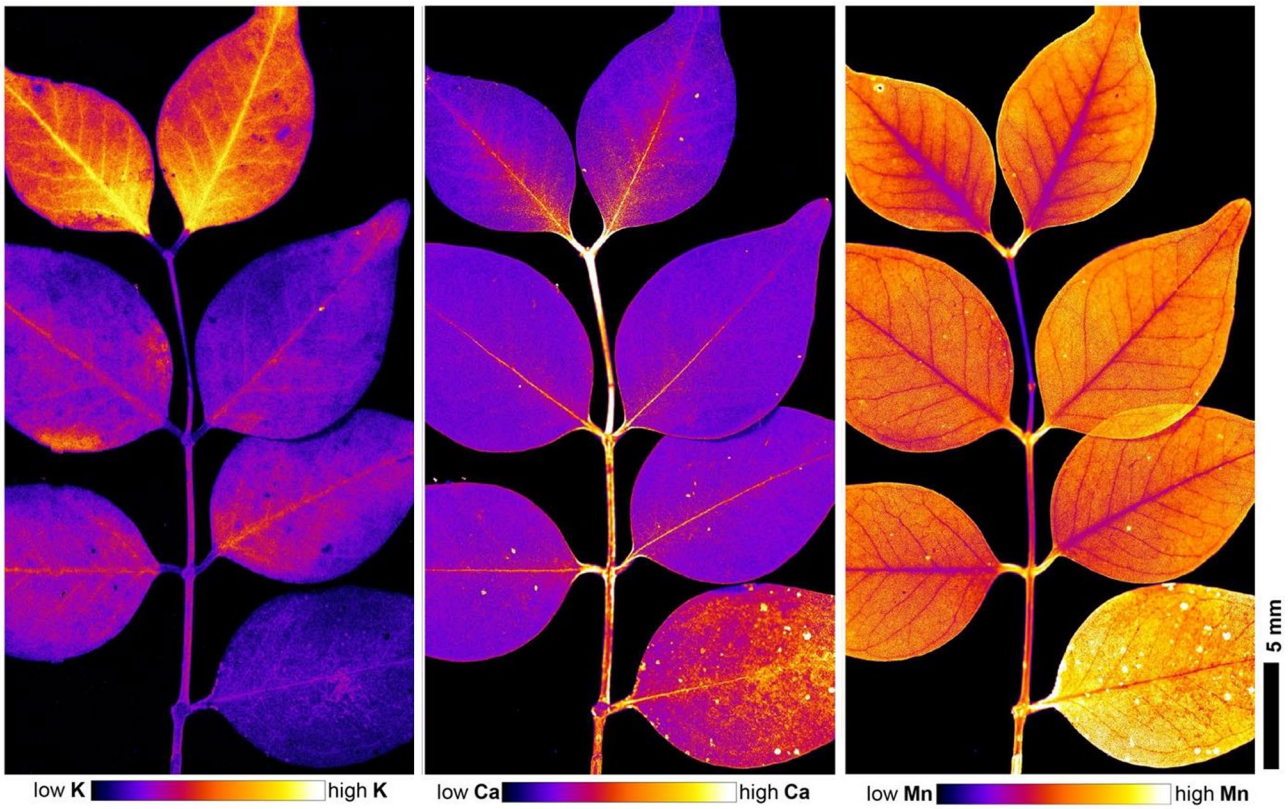

Fig. 3 Laboratory µXRF maps of K, Ca, and Mn of a whole fresh/hydrated wild *G. bidwillii* branchlets

The phytoavailability of Al and Mn are known to occur in soils of low pH (< 5). However, the pH of soils on which the Mn-dosed *G. bidwillii* was cultivated in this study shows that the solubility of Al was low, yet *G. bidwillii* was able to take up high concentrations of Al in old leaves which qualifies it as a potential Al hyperaccumulator, with concentrations six-fold higher than the Al hyperaccumulation threshold set at 3000 µg g^{-1} (Jansen et al. 2003, 2001). Exceptionally high Al concentrations in old *G. bidwillii* leaves, even under Mn treatment, could be facilitated by organic anions involved in Mn transport (Bidwell et al. 2002). This suggests that *G. bidwillii* may be able to take up Al via an anion channel, a mechanism that appears to be a peculiar trait among Al tolerant species (Zhang et al. 2001; Ryan et al. 1997; Piñeros and Kochian 2001; Kollmeier et al. 2001). It should be cautioned here that these observations of Al over accumulation by *G. bidwillii* have been made under experimental conditions, and have not been observed in the field, even on lateritic soils rich in Al (Fernando, Bidwell etc.). The notable limit of Al uptake in wild *G. bidwillii,* even though the soil pH was suitable for Al mobilisation, was most likely due to the very low soil Al concentrations. Furthermore, it has also been suggested that Al inhibits uptake of Ca and Mg in non-Al accumulators (Kochian et al. 2005; Ryan and Kochian 1993; Rengel and Zhang 2003). This was an unexpected observation in old leaves of the wild *G. bidwillii* in this present study. This present observation of Al accumulation in Mn-dosed *G. bidwillii* and its absence in wild *G. bidwillii* warrants further investigation. Species within the Myrtaceae have been listed to contain Al hyperaccumulators (Jansen et al. 2003, 2001) and previous studies by Fernando et al. (2009b) have shown that other *Gossia* spp., including *G. hillii*, *G. inophloia*, *G. lewensis* and *G. macilwraithensis,* can be Al hyperaccumulators under experimental conditions.

The distribution patterns of Mn in leaves of wild *G. bidwillii* was strongly enriched throughout the young and old leaves (Fig. 3). In the Mn-dosed *G. bidwillii*, Mn was concentrated in the apical point of the leaf as well as across the broader laminal area (Fig. 4). Similar observations have been reported for the Mn hyperaccumulators *Acanthopanax sciadophylloides* (Memon et al. 1980), *G. fragrantissima* (Abubakari et al. 2021a), and *D. silvestris* and *D. cunninghamii* (Abubakari et al. 2021b). This study used a dosing trial for the first time to demonstrate the Mn-hyperaccumulation trait in *G. bidwillii* under controlled experimental conditions. The Mn concentrations in young leaves, old leaves, and twigs of the Mn-dosed *G. bidwillii* increase with increasing soil Mn concentrations. Laboratory µXRF analysis revealed different Mn distribution patterns in the leaves of wild and Mn-dosed *G. bidwillii*. Further work should be

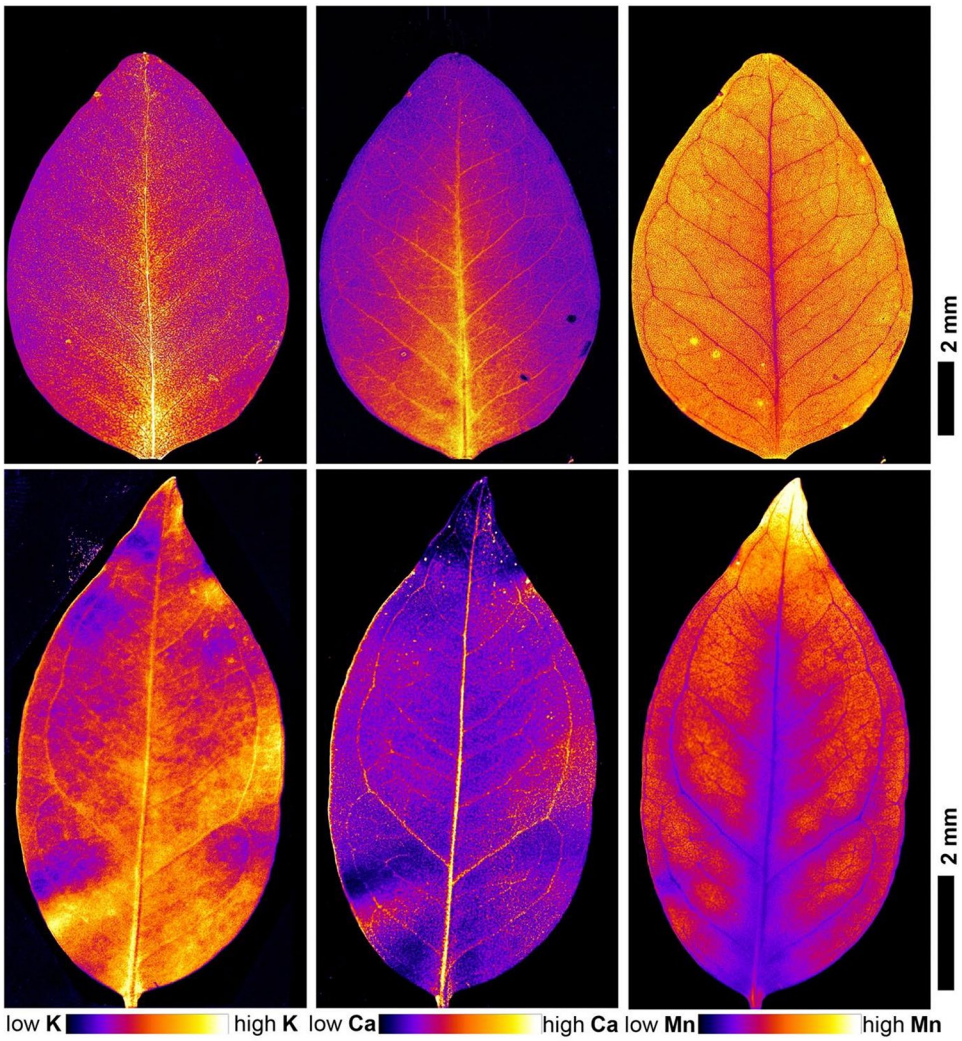

Fig. 4 Laboratory µXRF maps of K, Ca, and Mn of fresh/hydrated leaves of wild *G. bidwillii* (top) and Mn-dosed *G. bidwillii* (below)

undertaken using synchrotron X-ray Fluorescence Microscopy with higher resolution to investigate Mn distribution at the cellular and subcellular levels in order to elaborate hypotheses for its metabolic pathways to be coupled to molecular biological studies (*e.g.,* transcriptomics) to identify putative Mn-transporters in *G. bidwillii*.

Methods

Habitat and Ecology of the Studied Species *Gossia bidwillii*, grows up to 15 m high with a smooth trunk with "python"-coloured patches. Its leaves are oval to elliptical, apex acuminate, lower surface glabrous, and oil glands densely crowded (Snow et al. 2003; PlantNET). The leaves tend to be sticky when crushed (Fig. 1). *Gossia bidwillii* is the most widespread Australian species in the genus and occurs from the Hunter River (32° S) in New South Wales to near Coen (13° S) in far northern Queensland. It mostly occurs east of the Great Dividing Range with an altitudinal range from 300–800 m (Snow et al. 2003). It grows in subtropical drier rainforests and can be very common on ridgetops and slopes in Araucarian microphyll vineforests, complex notophyll forests, semi-evergreen vineforest thickets, dry scrub, and gallery rainforests on a range of soils (Snow et al. 2003).

Plant Dosing Trial *Gossia bidwillii* plants, approximately 20 cm in height, were obtained from Coastal Dry Tropics Landcare (Pallarenda Road, Townsville, Queensland) and cultivated in a temperature and humidity-controlled glasshouse at the University of Queensland, Brisbane, Australia, Central Glasshouse Service. The plants were kept at 24ºC and 80% Relative Humidity (RH), with a photosynthetic photon flux density at 13:00 h of 1600 µmol m^2 s^{-1} (measured with an Apogee MQ-500 instrument). After three weeks the plants were transferred to 15 cm pots containing a mixture of 9 parts composted pine bark (5–10 mm) and one part coco peat (Bassett Barks Pty Ltd, Queensland, Australia). Fertilisers and other additives were mixed with the potting compost at the following amounts per m^3, including

1.2 kg Yates Flowtrace, 1 kg iron sulphate heptahydrate ($FeO_4SO_4.7H_2O$), 0.1 kg superphosphate ($Ca(H_2PO_4)_2$), 1.5 kg gypsum ($CaSO_4$), and 1.5 kg dolomite ($CaMg(CO_3)_2$) (per m^3). The Flowtrace contained 24 weight percent iron (Fe) as $FeSO_4$, 14 weight percent sulphur (S) as SO_4, 0.75 weight percent copper (Cu) as $CuSO_4$, 0.5 weight percent manganese (Mn) as $MnSO_4$, 0.2 weight percent zinc (Zn) as $ZnSO_4$, 0.04 weight percent molybdenum (Mo) as Na_2MoO_4, 0.033 weight percent boron (B) as $Na_2B_4O_7$. The soils with final dosed Mn^{2+} concentrations of 200 g g^{-1} (**T2**), 500 g g^{-1} (**T3**), and 1000 g g^{-1} (**T4**) in addition to the control (**T1**) were duplicated three times, resulting in a total of 12 experimental groups. Over a period of 12 months, each treatment was given as aqueous $MnSO_4.H_2O$ solutions, with a similar volume of water added each time to the control. To avoid losses of the treatments, the individual pots were placed on saucers, and hand watered daily to field capacity.

Field Sampling *Gossia bidwillii* was sampled within the Amamoor State Forest in Queensland, Australia (26°20′41.0"S 152°37′7.0"E). The geology of this area is predominantly volcanic rock (andesite) overlaying variably silicified shale or tuffs containing Mn-rich (~ 30–50 wt%) minerals such as bixbyite and pyrolusite. Krasnozem soils derived from this parent rock contain Mn to levels as high as 40 wt% (Isbell 1994). Old and young *G. bidwillii* leaves (10–20 each) were collected for total elemental analysis, while small branchlets with old and young leaves were detached and stored fresh for the laboratory µXRF analyses. Soil samples were collected from beneath the trees (< 10 cm depth) at three different points (free of surface litter).

Chemical Analysis of Soil and Plant Samples After harvesting experimental *G. bidwillii*, soils were extracted in each pot and emptied into plastic bags. Soils on which wild *G. bidwillii* was growing were also collected as described above. All soils were air dried and later passed through a 2 mm sieve. After 2 h of shaking, the pH of a 1 to 2.5 soil to water mixture was determined. The extraction of immediately available trace elements uses a 0.01 M $Sr(NO_3)_2$ solution at a soil:solution ratio of 1:4 (10 g soil with 40 mL solution) and a 2 h shaking period were adapted from Kukier and Chaney (2001). Diethylenetriaminepentaacetic acid (DTPA) solution was used to extract "phytoavailable" trace elements, based on the original method by Lindsay and Norvell (1978), but modified as follows: no triethanolamine (TEA), pH set to 5.3, and 5 g soil with 25 mL extractant, with an extraction time of 1 h.

Plant material samples were oven dried at 60 °C for three days and weighed, powdered and 300 mg per sample digested with 4 mL HNO_3 (70%) in a microwave oven (Milestone Start D) for a 45-min programme. Digests were then brought to volume (45 mL) with ultrapure water (Millipore 18.2 MΩ·cm at 25 °C) for analysis with Inductively coupled plasma atomic emission spectroscopy (ICP-AES) using a Thermo Scientific iCAP 7400 instrument for macro-elements (Al, Na, Mg, K, P, Ca) and trace-elements (Fe, Ni, Mn, Co, Zn). Certified reference material (Sigma-Aldrich Periodic Table mix 1 for ICP TraceCERT®) and Standard Reference Material (NIST Apple 1515) were used as quality controls.

Laboratory µXRF Elemental Mapping Freshly excised branchlets from 1000 µg Mn g^{-1} treated experimental *G. bidwillii* plants and from wild *G. bidwillii* trees from the Amamoor State Forest were used for the µXRF analysis. The University of Queensland µXRF facility contains a modified IXRF ATLAS X system with a 50 W X-ray source (Mo-tube producing 17.4 keV X-rays) focussing to 25 µm and two silicon drift detectors of 150 mm^2. Measurements were conducted at atmospheric temperature (~ 20 °C) and to limit dehydration, the hydrated foliar samples were tightly mounted between two sheets of 4 m Ultralene thin film.

Data processing and Statistical Analysis The data on the µXRF instrument were acquired in mapping mode using the instrument control package Iridium (IXRF systems) from the sum of counts at the position of the principal fluorescence peak for each element. These were each exported into ImageJ as greyscale 8-bit TIFF files, internally normalised so that each image covered the entire dynamic range and displayed using ImageJ's "Fire" lookup table. The concentrations of Mn presented as boxplots were generated using R version 3.6.1 (2019–07-05). Tests presented in the Tables were conducted using One-Way ANOVA and means compared with Tukey's honestly significant difference (HSD) Post Hoc Test in the IBM SPSS Statistics 27 software package (IBM, New York, USA). Values with different small letters are significantly different ($p < 0.05$).

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Author Contributions FA, PNN and AVDE designed and conducted the experiment. FA collected the samples and undertook the chemical analysis of the samples. FA, PNN and AVDE performed data processing and analysis. All authors contributed to writing of the manuscript.

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Declarations

Conflicts of Interest The authors have no conflicts of interest to declare that are relevant to the content of this article.

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