Manganese accumulation and foliar distribution in the Australian hyperaccumulators Gossia bidwillii and Gossia acmenoides

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Abstract

The known manganese (Mn) hyperaccumulator Gossia bidwillii, and G. acmenoides unknown to hyperaccumulate Mn are tree species native to subtropical eastern Australia, where they co-occur on Mn-rich soils. Here, we investigate Mn accumulation and distribution in G. acmenoides collected from its natural habitat, and propagated G. bidwillii plants in a Mn dosing trial. Gossia bidwillii were subjected to different levels of Mn (250 µg g-1, 500 µg g-1, 1000 µg g-1) treatments whereas G. acmenoides were sampled from Mn rich soil. We used laboratory-based X-ray Florescence Microscopy (XFM) to elucidate in situ distribution patterns of Mn and other elements in hydrated G. acmenoides and G. bidwillii tissues. Data from G. acmenoides revealed that contrary to existing knowledge, 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 at the highest treatment level. The XFM data revealed clear interspecies differences in foliar Mn distribution patterns, with wild G. acmenoides leaves uniformly enriched throughout the laminae and petioles of both young and old leaves; while in G. bidwillii, the foliar Mn distribution was primarily concentrated at the apex and lamina. The approach employed of combining data from the field and controlled experiments was especially effective for comparing Mn accumulation in these two species and gaining added insight into the phenomenon of Mn hyperaccumulation.

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Abstract

The known manganese (Mn) hyperaccumulator *Gossia bidwillii* , and*G. acmenoides* unknown to hyperaccumulate Mn are tree species native to subtropical eastern Australia, where they co-occur on Mn-rich soils. Here, we investigate Mn accumulation and distribution in *G. acmenoides* collected from its natural habitat, and propagated *G. bidwillii* plants in a Mn dosing trial. *Gossia bidwillii* were subjected to different levels of Mn (250 μg g^{-1}, 500 μg g^{-1}, 1000 μg g^{-1}) treatments whereas *G. acmenoides* were sampled from Mn rich soil. We used laboratory-based X-ray Florescence Microscopy (XFM) to elucidate*in situ* distribution patterns of Mn and other elements in hydrated*G. acmenoides* and *G. bidwillii* tissues. Data from *G. acmenoides* revealed that contrary to existing knowledge, 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 at the highest treatment level. The XFM data revealed clear interspecies differences in foliar Mn distribution patterns, with wild *G . acmenoides* leaves uniformly enriched throughout the laminae and petioles of both young and old leaves; while in *G. bidwillii* , the foliar Mn distribution was primarily concentrated at the apex and lamina. The approach employed of combining data from the field and controlled experiments was especially effective for comparing Mn accumulation in these two species and gaining added insight into the phenomenon of Mn hyperaccumulation.

KEYWORDS

elemental distribution, hyperaccumulator, manganese, *Gossia bidwillii, Gossia acmenoides*

INTRODUCTION

Hyperaccumulators are unique plants able to concentrate extraordinarily high concentrations of specific trace elements in their foliage and other aerial parts (Baker & Brooks, 1989; Reeves, 2003; van der Ent*et al.* , 2013). They achieve such extreme levels of accumulation*via* enhanced uptake and translocation mechanisms that are yet to be fully understood (Baker, 1981; Baker, 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* , *Denhamia (Maytenus)* , *Gossia, Grevillea, Macadamia* and*Virotia* distributed over eastern Australia and New Caledonia (Losfeld et al., 2015, Fernando et al., 2008, Jaffré, 1977, Jaffré, 1980, Fernando et al., 2009b), 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 tip of the Cape York Peninsula (10^0S) in Queensland (Snow et al., 2003). *Gossia bidwillii* and *G. acmenoides* (Myrtaceae) have a very smooth bark which is irregularly covered with relatively large, coloured patches. These species, as well as *G. lucida* and *G. grayi,* are called "python bark" *Gossia's* 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 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; Fernando *et al.* , 2008a; Fernando *et al.* , 2009b; Fernando *et al.* , 2013, McLay 2018). All these published studies have been based on freshly collected field samples or preserved material obtained from herbaria. There have been certain consistent observations throughout, for example, the Mn hyperaccumulative trait in *Gossia bidwillii* , and the Mn non-hyperaccumulation by *G. acmenoides,* as captured in a recent phylogenetic study of *Gossia* (McLay et al 2018). While these two species are known to be sympatric on Mn-rich soils, they appear taxonomically partitioned into separate clades (McClay et al (2018).

Studies on fresh field material have reported foliar Mn concentrations of 19 200 μg g^{-1} in *G. bidwillii* (Bidwell et al., 2002). Recent growth experiment on *G. fragrantissima* has shown it can take up to 545 μg g^{-1} Co, 17 400 μg g^{-1} Mn and up to 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 to accumulate in non-photosynthetic tissues such as the epidermal cells and associated dermal structures including trichomes and leaf hairs (Vázquez et al., 1992, Küpper et al., 2000, Küpper et al., 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., 2006b, Fernando et al., 2006a, Fernando et al., 2007). Laboratory and synchrotron X-ray Florescence Microscopy (XFM) 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 woody species such as this. Neither have there been any studies to examine Mn accumulation of *G. bidwillii* to Mn accumulation by a closely related species. *Gossia acmenoides* was selected here for a field investigation into Mn accumulation in its natural habitat because it is commonly sympatric to *G. bidwillii* . This study aims to: i) measure the response of propagated *G. bidwillii* plants to Mn treatment under controlled conditions, and ii) assess Mn uptake and accumulation in the aforementioned *G. bidwillii* plants with that of *Gossia acmenoides* on naturally Mn enriched soils. By employing laboratory XFM to determine *in situ* distributions of Mn and other elements in *G. bidwillii* and *G.acmenoides,* this study also investigates Mn distribution patterns in the leaf tissues of these two species, as well as test within-species age-related distributional differences, *i.e* ., between their young and old leaves.

MATERIALS AND METHODS

Habitat and ecology of the studied species – *Gossia acmenoides* and *G. bidwillii* are small to medium trees up to ˜18 m tall, which occur in drier scrub patches and rainforests. *Gossia acmenoides* has a brown/green bark, shedding in patches; terminal buds silky. Leaves are oval to elliptical quickly tapering to a pointed or rounded tip, simple, opposite, blade moderately glossy above, translucent, venation distinct, old dots visible with the naked eye (Snow et al., 2003). Unlike *G. bidwillii* , the leaves of*G. acmenoides* are not sticky when crushed. It is a hardy plant with dense and glossy foliage. On the other hand, *G .bidwillii* have almost round, very smooth, shiny and a cinnamon smell when crushed (Fig. 1).

Plant dosing trial of *G. bidwillii* – *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. Plants were kept at 20 ºC and 80% Relative Humidity (RH), with and 13:00 hours of PAR light (1600 µM photons s^{-1}) at the Central Glasshouse Services at The University of Queensland, Brisbane, Australia. After three weeks, the plants were transferred into 15 cm pots containing a ratio of 9:1 mixture of Composted Pine Bark 5–10 mm and Coco Peat (Bassett Barks Pty Ltd, Queensland, Australia). The media was mixed with low-level fertilizers and other augments consisting (per m^3) of 1.2 kg Yates Flowtrace, 1 kg iron sulphate heptahydrate ($FeO_4SO_4{\cdot}7H_2O$), 0.1 kg superphosphate ($Ca(H_2PO_4)_2$), 1.5 kg gypsum ($CaSO_4$) and 1.5 kg dolomite ($CaMg(CO_3)_2$). The composition of the Flowtrace was 24 wt% iron (Fe) as $FeSO_4$, 14 wt% sulfur (S) as SO_4, 0.75 wt% copper (Cu) as $CuSO_4$, 0.5 wt% manganese (Mn) as $MnSO_4$, 0.2 wt% zinc (Zn) as $ZnSO_4$, 0.04 wt% molybdenum (Mo) as Na2MoO4, 0.033 wt% boron (B) as $Na_2B_4O_7$ and also contains zeolite, to ensure flowability (Yates Australia, Padstow, NSW, Australia). Soluble Mn was applied to the plants in a randomised block design. The applied treatments were the control (**T1**), and soils with final dosed Mn^{2+} concentrations of 200 µg g^{-1} (**T2**), 500 µg g^{-1}(**T3**) and 1000 µg g^{-1} (**T4**) replicated three times yielding a total of 12 experimental groups. Each treatment was administered monthly as aqueous $MnSO_4.H_2O$ solutions for a period of 12 months; a similar volume of water was added to the control each time. The individual pots were placed on saucers and hand watered daily to field capacity to prevent loss of treatment solutions.

Field sampling of *G. acmenoides* – *Gossia acmenoides* was sampled at a field site within the Amamoor State Forest in Queensland, Australia (26°20'41.0"S 152°37'7.0"E). The geology of this subtropical 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. acmenoides*leaves (10–20 each) were harvested for total mineral nutrient analysis, while small branchlets with old and young leaves were detached and stored fresh for XRF 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*G. bidwillii* , soils were extracted in each pot and emptied into respective plastic bags. Soils on which *G. acmenoides* was growing were also collected as described above. All soils were oven dried at 60º C and later sieved using the 2mm sieve. Soil pH was obtained in a 1 to 2.5 soil to water mixture after 2 hr shaking. Exchangeable trace elements were extracted in 0.1 M $Sr(NO_3)_2$ at a soil:solution ratio of 1:4 (10 gram soil with 40 mL solution) and 2 hr shaking time was adapted from Kukier and Chaney (2001). As a means of estimating potentially phytoavailable trace elements, the DTPA-extractant was used according to Dai *et al.* (2004) which was adapted from the original method by Lindsay and Norvell (1978), with the following modifications: excluding TEA, adjusted at pH 5.3, 5 g soil with 25 mL extractant, and extraction time of one hr.

Plant material samples were oven dried at 60°C for three days and then weighed, ground to fine powder and (300 mg) digested using 4 mL HNO_3 (70%) in a microwave oven (Milestone Start D) for a 45-minute programme. Digests were then diluted to 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) in radial and axial modes, depending on the element and expected analyte concentration. In-line internal standardization using yttrium was used to compensate for matrix-based interferences. Quality controls included matrix blanks, certified reference material (Sigma-Aldrich Periodic Table mix 1 for ICP TraceCERT®, 33 elements, 10 mg L^{-1} in HNO_3) and Standard Reference Material (NIST Apple 1515 digested with HNO_3).

Λαβορατορψ μΞΡΦελεμενταλ μαππινγ – Live samples (a whole branch) from *Gossia bidwillii*originating from the Mn1000 treatment and *G. acmenoides* collected from Amamoor, Queensland were used for the microXRF scanning. The UQ microXRF facility contains a modified IXRF ATLAS X system, mounting two 50W X-ray sources fitted with polycapillary focussing optics: XOS microfocus Mo-target tube producing 17.4 keV X-rays (flux of 2.2×10^8 ph s^{-1}) focussing to 25 μm and a Rh-target tube producing 20.2 keV (flux of 1.0×10^7ph s^{-1}) focussing to 5 μm. The system is fitted with two silicon drift detectors of 150 mm^2. Typical energy resolution is <145 eV with a maximum input count rates of 2 M counts per second. The motion stage can address areas up to 300 × 300 mm. Measurements were conducted at atmospheric temperature (˜20°C), using the Mo 25 μm X-ray source at a 40 kV, 1000 uA, with a rise time of 0.25 μs and a per-pixel dwell of 100 ms. The hydrated foliar samples were mounted between two sheets of 4 μm Ultralene thin film in a tight sandwich to limit evaporation and analysed within 10 minutes after excision. The mounted samples between Ultralene thin film were stretched over a Perspex frame magnetically attached to the x-y motion stage at atmospheric temperature (˜20°C). The possibility of radiation-induced damage in μ-XRF analysis (especially in fresh hydrated samples) is an important consideration, but such damage was not observed because the source produced a flux of 2.2×10^8photons s^{-1} in a 25 μm beam spot, at a maximum dwell of 100 ms this results in a deposited radiation dose of just 6.6 Gy.

Data processing and statistical analysis – The XRF spectra on the UQ microXRF facility were acquired in mapping mode using the instrument control package, Iridium (IXRF systems) from the sum of counts at the position of the principal peak for each element. These were each exported into ImageJ as greyscale 8-bit TIFF files, internally normalised such that each image covered the full dynamic range and displayed using ImageJ's "Fire" lookup table.

The concentrations of Mn presented as boxplots were performed using R version 3.6.1 (2019-07-05). Concentrations of elements presented in Tables as mean ± standard error 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).

RESULTS

Elemental concentrations in *G. acmenoides* and *G. bidwillii* – All plant tissue elemental concentrations presented in this study are based on dry weight. The bulk elemental concentrations in young leaves, old leaves and twigs of the wild *G.acmenoides* and the dosed *G. bidwillii* are shown in Table 1 and 2 respectively, and that of the concentration of Mn in young, old leaves and twigs of *G. bidwillii* in Figure 2. The concentrations of Mn in the wild *G. acmenoides* were remarkable, with significantly (p <0.05) high mean value of 39 000 μg g^{-1} (SE ± 3540) in old leaves, compared to 24 000 μg g^{-1} (SE ± 410) in young leaves and 5840 μg g^{-1} (SE ± 2820) in twigs (Table 1). Similarly, the concentrations of Ca and Na in *G. acmenoides* tend to be high 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 *G. acmenoides* were also high with concentrations of 75.0 μg g^{-1} Fe (SE ± 17.0) and 1290 μg g^{-1} Mg (SE ± 108) respectively compared to 840 μg g^{-1} Mg (SE ± 38.0) and 45 μg g^{-1} Fe (SE ± 0.70) in its young leaves. However, no significant difference was observed between Mg in old leaves and twigs of *G.acmenoides* (Table 1). Values of Al tend to be also high in old leaves (200 ± 38.0 μg g^{-1} Al) than young leaves (53.0 ± 0.85 μg g^{-1} Al) and twigs (6.0 ± 10.5 μg g^{-1} Al). Nonetheless, the concentration of K was high 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 difference (p>0.05) was observed for Ni, P and Zn in young, old leaves and twigs of *G. acmenoides* (Table1).

In the old leaves of *G. bidwillii* , the mean Mn concentrations at 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 are more than 2-fold the Mn hyperaccumulation threshold at the **T4** and **T3** treatment levels and greater than 1-fold the Mn hyperaccumulation threshold at the**T2** treatment level (Fig. 2 and Table 2). The old leaves of*G. bidwillii* at the control (**T1**) also contained up to 10 900 μg g^{-1} Mn (mean 5490 μg g^{-1}). In the young leaves, however, Mn concentration at the **T4** and**T3** treatment levels were 17 100 μg g^{-1} (SE ± 8440) and 12 600 μg g^{-1} (SE ± 11 000) respectively which are more than 1-fold the Mn hyperaccumulation threshold (Fig. 2 and Table 2). On the other hand, the **T2** treatment level contained 8300 μg g^{-1} (SE ± 4270) which is almost around double the **T1** value (4600 μg g^{-1}) in young leaves (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 1.5-fold the Mn hyperaccumulation threshold than that observed at the **T3** (5000 ± 1150 μg g^{-1}), **T2** (4300 ± 1220 μg g^{-1}) and the **T1** (2900 ± 1300 μg g^{-1}) treatment levels (Fig. 2 and Table 2).

Unlike *G. acmenoides* , the concentration of Ca in the dosed*G. bidwillii* was high in the young leaves with concentrations of 9400 μg g^{-1} (SE ± 2280) at **T1** , 5700 μg g^{-1} (SE ± 1820) at **T2** , 12 400 μg g^{-1} (SE ± 2790) at **T3** and 11 200 μg g^{-1} (SE ± 4000) at **T4** treatment level compared to that in the old leaves (Table 2). The twigs on the other hand, contained high amounts of Ca at the **T1** , **T2** and**T4** treatment levels than in young and old leaves with mean values 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) except at the **T3** treatment level where the concentration of Ca was high in young leaves than in old leaves and twigs. The concentration of Al in old leaves of *G. bidwillii* tend to be high at 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 concentration of K at the **T1** (22100 ± 2960 μg g^{-1} K) treatment level in young leaves was significantly (p <0.05) higher than those observed at the other treatment levels (**T2** ,**T3** , **T4**) in old leaves and twigs. However, Mg tend to be high at the **T1** (4790 ± 1900 μg g^{-1} Mg) treatment level in old leaves compared to those observed at the other treatment levels (**T2** , **T3** , **T4**) in young leaves and twigs (Table 2). Whereas no significant difference was observed between the concentration of Na at the **T1** and **T2**treatment levels of young and old leaves, the concentration of Na at the**T4** treatment level in twigs (1100 ± 840 μg g^{-1} Na) was significantly (p <0.05) higher compared to that in young leaves (440 ± 260 μg g^{-1} Na) and old leaves (630 ± 140 μg g^{-1} Na) (Table 2). The values for Fe and Zn were low at all treatment levels compared to other

elements in young, old leaves and twigs (Table 2). Nonetheless, the concentration of P was below the limit of detection (<6.00 μg g^{-1}) in young, old leaves and twigs at all treatment levels, and this could probably be due to the low P demand in the experimental *G. bidwillii* (Table 2).

Soil chemical properties – The soil pH of the wild *G. acmenoides* was low (pH = 4.20) compared to that of *G. bidwillii*pot soils (pH 5.27–5.95) (Table 3). Extractable Mn values 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.

μΞΡΦ ελεμενταλ μαππινγ of wild *G.acmenoides* and *G. bidwillii* – Manganese in the wild*G* . *acmenoides* collected from the Mn-enriched substrate in Amamoor has strong enrichment throughout the leaf blade and petiole of young and old leaves, while veins and midrib have relatively lower concentrations (Figs. 3 and 4). Calcium and K are strongly enriched in the veins and midrib, and 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 *G. bidwillii* , the distribution of Mn is high at the apex and lamina but low 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 low in the leaf lamina (Fig. 4).

DISCUSSION

This study details the first-ever growth experiment on the Mn hyperaccumulating tree, *G. bidwillii* , exposed here to a range of Mn treatments and reports for new discovery of the Mn hyperaccumulative trait in wild *G. acmenoides* , on the highly acidic Mn-rich soils is intriguing given past analyses of samples sourced from a range of other eastern Australian locations had not detected Mn hyperaccumulation in this species (Fernando et al. 2009b). Foliar Mn concentrations in experimental *G. bidwillii* to as high as ˜3-fold Mn hyperaccumulation threshold of 10 000 μg g^{-1} (van der Ent et al. 2013) aligns with field data from earlier studies of this species (Bidwell et al. 2002; Fernando et al. 2006b). A weak relationship between substrate Mn supply and foliar Mn concentrations, most notably, high Mn uptake by control plants is also consistent with past field observations (Bidwell et al., 2002, Fernando et al., 2006b, Fernando et al., 2007). The ability of *Gossia bidwillii* as shown experimentally here and as previously noted in the field to vastly over-accumulate Mn even in very low soil-supply is a common characteristic hyperaccumulation, *i.e.,* the ability to scavenge from host substrates (Baker 1981; (Fernando et al., 2007).

Contrasting findings of previous field studies that have not found*G. acmenoides* to hyperaccumulate Mn, and this present field study describing strong Mn hyperaccumulation by this species may be attributable to genetic differences, as also observed in *Denhamia founieri* from New Caledonia by Fernando et al. (2008). The present findings on *G. acmenoides* suggests heterogeneity across the species, warranting further investigation of the genetic basis of Mn hyperaccumulation in *Gossia,* for example, as interrogated by Pollard et al (2002) for ubiquitous metal hyperaccumulating herbs of the Northern Hemisphere. Variability of Mn hyperaccumulation trait in*G. bidwillii* has previously been reported, however there is greater consistency across its broad natural range in comparison to the emerging picture of *G. acmenoides* (Fernando et al., 2007). Heterogeneity of metal accumulation has also been described in several species of hyperaccumulators of metals other than Mn (Pollard et al., 2002, Baker et al., 1994, Macnair, 2002). The extreme acidity of the host soil (pH 4.20, Table 3) from which *G. acmenoides* was sampled for this study warrants consideration in the context of Mn availability at the root-soil interface. It is plausible that the apparently high variation in Mn accumulation by *G. acmenoides* reflects specific rhizosphere effects such as acidification and/or microbial associations unique to a particular site location that renders soil-Mn highly bioavailable. These findings yet again highlight the gaps in knowledge around metal hyperaccumulation, with woody species such as *Gossia*spp. poorly understood. At locations where *G. acmenoides*has previously been found to accumulate\souts low levels of Mn, there may also be ion competition among similar divalent cations such as Ca^{2+}, Mg^{2+} and Mn^{2+} in addition to less acidic soil conditions (Fernando et al., 2013, McLay et al., 2019, Bidwell et al., 2002). It is notable that high concentrations of Ca and Mg relative to Mn were reported in leaves of *G. acmenoides* (Bidwell et al. (2002).

In *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. However, in *G. acmenoides* , K was high 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 *G. bidwillii* and *G. acmenoides* could be attributed to phloem immobility of the aforementioned elements (Marschner, 2002, Graham et al. 1988) and vice versa for K in *G . acmenoides* and*G. bidwillii* . Moreover, this behaviour could be due to the similarities in divalent cations of Mn, Ca and Mg. Similar observation 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. 2021) 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 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).

The phytoavailability of Al and Mn are known to occur in soils of low pH (<5), however the pH of soils on which *G. bidwillii* was cultivated in this present study shows that the solubility of Al was low (>5), yet *G. bidwillii* was able to take up high amounts of Al in old leaves which qualifies it as an Al hyperaccumulator with concentration 6-fold higher than the Al hyperaccumulation threshold set at 3000 μg g^{-1} (Jansen et al., 2003, Jansen et al., 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 while these observations of Al over accumulation by *G. bidwillii*have been made under experimental conditions, it has not been observed in the field, even on lateritic soils rich in Al (Fernando, Bidwell etc). The notable limit in uptake of Al in *G. acmenoides* even though its soil pH was suitably low for Al mobilisation for plant uptake, 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), and this was found in old leaves of*G. acmenoides* in this present study. Species within the Myrtaceae family have been listed to contain Al hyperaccumulators (Jansen et al., 2003, Jansen et al., 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 after they were exposed to Al treatments.

The distribution patterns of Mn in leaves in wild *G. acmenoides*and Mn-dosed *G. bidwillii* have been shown by XFM to be distinctly different (Figs. 3 and 4). Strong Mn distribution throughout young and old leaves in wild *G. acmenoides* , while highly concentrated at the apex and lamina of treated *G. bidwillii* suggest that Mn movement with the transpiration stream rather than against it. This indicates that, increasing transpiration rate throughout the leaves of*G. acmenoides* , and towards the apex and lamina of *G. bidwillii* led to higher Mn accumulation in those parts of the studied species. Similar observations to that of *G. bidwillii* have been reported for the Mn hyperaccumulators *Acanthopanax sciadophylloides* (Memon et al., 1980) and *G. fragrantissima*(Abubakari et al., 2021a).

This study newly revealed the strong and facultative Mn hyperaccumulative trait in *G. acmenoides* and confirmed it in*G. bidwillii* , demonstrating both species to be strong Mn hyperaccumulators with concentrations in both young and old leaves well exceeding the Mn hyperaccumulation threshold. Laboratory based-XFM revealed distinct Mn distribution patterns in the leaves of *G. acmenoides* and *G. bidwillii* . Further work should be undertaken using synchrotron X-ray Florescence Microscopy with more precision and higher resolution to investigate Mn distribution at the cellular and subcellular levels in order to elaborate hypotheses for its metabolic pathways to be elucidated with genetic studies.

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AUTHOR CONTRIBUTIONS

FA, PNN and AVDE designed and conducted the experiment. FA and AVDE 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.

CONFLICT OF INTEREST

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

DATA AVAILABILITY STATEMENT

Data can be accessed from the Dryad Digital Repository.

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Figure 1. Visual appearance\souts of experimental and field plants: **A** Experimental *Gossia bidwillii* , **B** ; field *G. acmenoides* tree trunk, and **C** field *G. acmenoides* foliage.

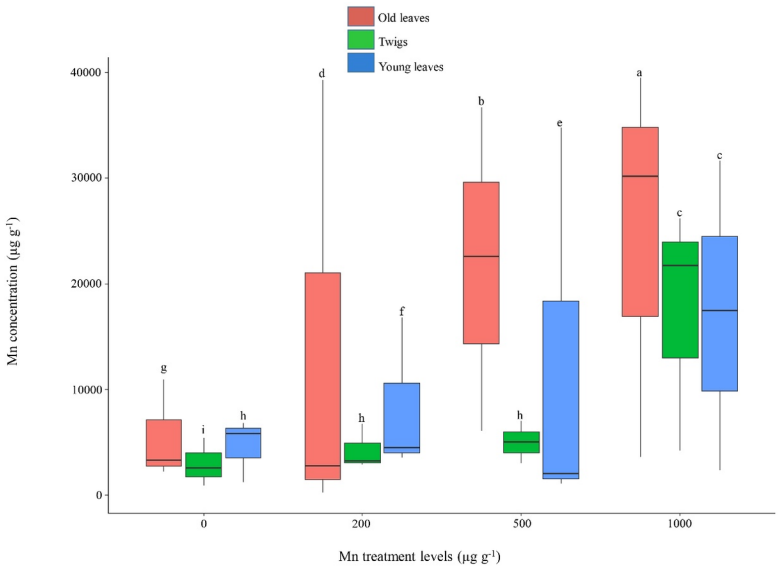

Figure 2. Manganese concentrations in young leaves, old leaves, and twigs of experimental *Gossia bidwillii* plants. Values are in ranges and means of three replicates. Keys to symbol of boxplots: open squares are the 25% to 75% quartiles; whiskers are the mean ± standard error and circles are outliers. Values with different small letters are significantly different (p <0.05).

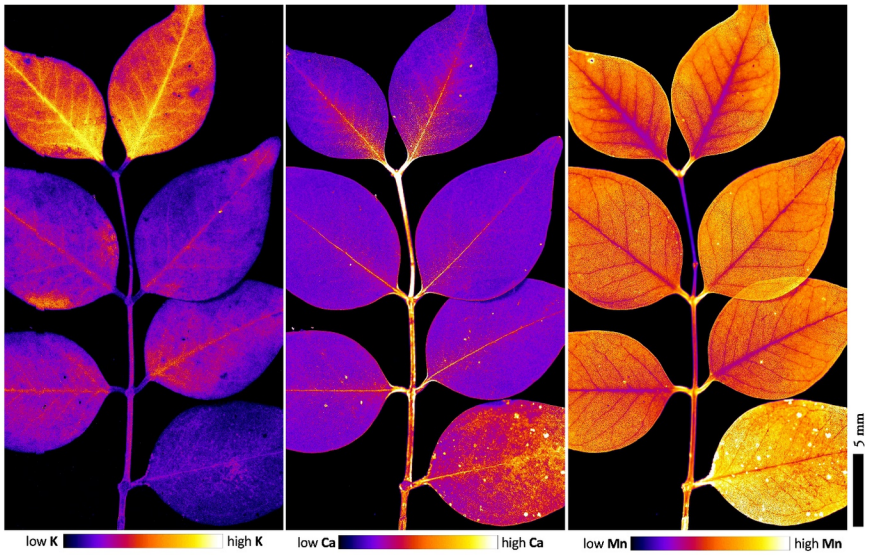

Figure 3 . Laboratory μXRF maps of K, Ca and Mn of a whole fresh/hydrated *G. acmenoides*branchlets.

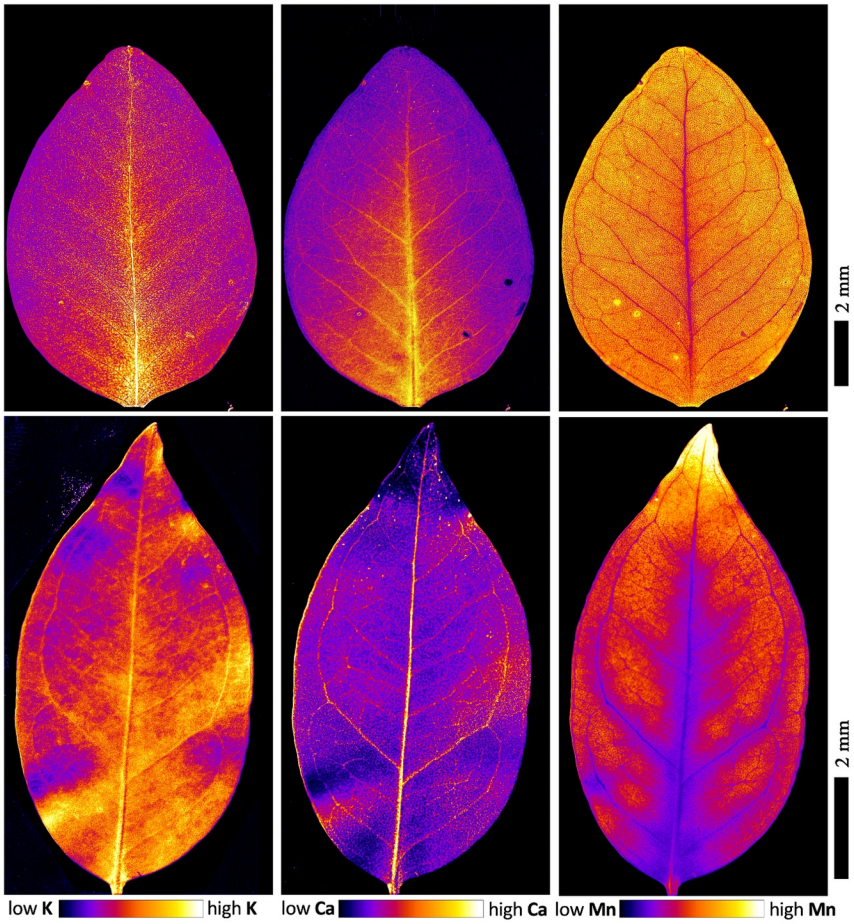

Figure 4. Laboratory μXRF maps of K, Ca and Mn of fresh/hydrated leaves of *G. acmenoides* (top) and *G. bidwillii* (below).

Table 1. Elemental concentrations in field samples of *G. acmenoides* young leaves, old 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	Element concentrations (μg g^{-1})								
	Al	**Ca**	**Fe**	**K**	**Mg**	**Mn**	**Na**	**Ni**	**P**
Gossia acmenoides	**Young leaves**	**Young leaves**	**Young leaves**	**Young leaves**	**Young leaves**	**Young leaves**	**Young leaves**	**Young leaves**	**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}
	Old leaves	**Old leaves**	**Old leaves**	**Old leaves**	**Old leaves**	**Old leaves**	**Old leaves**	**Old leaves**	**Old leaves**
In situ collection	200±38.0^{a}	9250±785^{a}	75.0±17.0^{a}	3660±160^{b}	1290±108^{a}	39000±3540^{a}	710±70.0^{a}	25±3.10^{a}	410±63.0^{a}
	Twigs	**Twigs**	**Twigs**	**Twigs**	**Twigs**	**Twigs**	**Twigs**	**Twigs**	**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}

Table 2. Elemental concentrations in young leaves, old leaves and twigs of *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	Element concentrations (μg g^{-1})								
	Al	**Ca**	**Fe**	**K**	**Mg**	**Mn**	**Na**	**Ni**	**P**
Gossia bidwillii	**Young leaves**	**Young leaves**	**Young leaves**	**Young leaves**	**Young leaves**	**Young leaves**	**Young leaves**	**Young leaves**	**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
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
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
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
	Old leaves	**Old leaves**	**Old leaves**	**Old leaves**	**Old leaves**	**Old leaves**	**Old leaves**	**Old leaves**	**Old leaves**

Species Μν λεελς (μγ γ^{-1})	**Ελεμενταλ ςονςε-ντρα-τιονς (μγ γ^{-1})**	**Ελεμενταλ ςονςε-ντρα-τιονς (μγ γ^{-1})**	**Ελεμενταλ ςονςε-ντρα-τιονς (μγ γ^{-1})**	**Ελεμενταλ ςονςε-ντρα-τιονς (μγ γ^{-1})**	**Ελεμενταλ ςονςε-ντρα-τιονς (μγ γ^{-1})**	**Ελεμενταλ ςονςε-ντρα-τιονς (μγ γ^{-1})**	**Ελεμενταλ ςονςε-ντρα-τιονς (μγ γ^{-1})**	**Ελεμενταλ ςονςε-ντρα-τιονς (μγ γ^{-1})**	**Ελεμενταλ ςονςε-ντρα-τιονς (μγ γ^{-1})**
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
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
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
1000	10 700±5370^{c}	8170±1330e	45±6.0^{a}	12 500±3600^{c}	3090±540^{c}	24 400±10 700^{a}	630±140^{c}	<LOD	<LOD
	Twigs	**Twigs**	**Twigs**	**Twigs**	**Twigs**	**Twigs**	**Twigs**	**Twigs**	**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
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
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
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

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

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

Gossia bidwillii	**pH**	**ΔΤΠΑ-εξτρασταβλε ςονςεντρατιονς (μγ γ^{-1})**	**ΔΤΠΑ-εξτρασταβλ**
Τρεατμεντ λεελς (μγ γ^{-1})		**Co**	**Mn**
Control	5.95	1.60±0.07^{c}	<LOD
Mn 200	5.80	5.80±0.30^{b}	<LOD
Mn 500	5.61	4.90±0.75^{b}	27.2±6.03
Mn 1000	5.27	12.7±2.04^{a}	37.30±9.01
Gossia acmenoides	**pH**	**ΔΤΠΑ-εξτρασταβλε ςονςεντρατιονς (μγ γ^{-1}) Co**	**ΔΤΠΑ-εξτρασταβλ Mn**
	4.20	0.98	1580

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