CSIRO PUBLISHING

RESEARCH PAPER
https://doi.org/10.1071/BT22033

Australian Journal of Botany

Root foraging in the leguminous zinc hyperaccumulator *Crotalaria novae-hollandiae* from Queensland, Australia

Fuyao Chen[A], Philip Nti Nkrumah[B], Roger H. Tang[B] and Antony van der Ent[B,C,*]

For full list of author affiliations and declarations see end of paper

***Correspondence to:**
Antony van der Ent
Centre for Mined Land Rehabilitation, Sustainable Minerals Institute, The University of Queensland, St Lucia, Qld, Australia
Email: a.vanderent@uq.edu.au

Handling Editor:
Dick Williams

ABSTRACT

Context. Root foraging by hyperaccumulator plants in response to patchily distributed metals has been observed in several obligate hyperaccumulators, but it is not known whether facultative hyperaccumulators respond similarly. **Aims.** This study investigated the root-growth behaviour in the leguminous zinc (Zn) hyperaccumulator *Crotalaria novae-hollandiae* compared with the non-accumulating *Crotalaria cunninghamii* in response to localised soil Zn enrichment in the soil to observe foraging versus avoidance responses. **Methods.** We conducted rhizotron experiments in which we exposed the *Crotalaria* species pair to juxtaposed treatments, which were either homogenous (each half of the treatments containing same Zn concentrations) or heterogenous (different Zn concentrations in each half of the treatments). The Zn concentrations were 0 μg Zn g^{-1} (control), 2000 μg Zn g^{-1} and 5000 μg Zn g^{-1} in the form of zinc carbonate). **Key results.** We found that none of the treatments had significantly different rooting density and root biomass, regardless of the *Crotalaria* species. This finding contrasts with increased root proliferation in Zn-rich patches found for other obligate hyperaccumulator species. **Conclusions and implications.** The no-preference root response towards Zn in *Crotalaria* may partly explain the facultative hyperaccumulation mechanism displayed by these species. This root response towards Zn may ultimately affect Zn phytoextraction efficacy when utilising *Crotalaria* species in a heterogenous Zn soil substrate. These findings highlight the need for rhizosphere investigations prior to field phytoextraction applications.

Keywords: *Crotalaria*, hyperaccumulation, metal tolerance, microXRF, phytoextraction, root avoidance, root foraging, zinc.

Introduction

Plants colonising metalliferous soils, so called metallophytes, have evolved physiological mechanisms to tolerate potentially toxic metals. These plants adopt three main tolerance strategies and are as therefore classified as (i) excluders, (ii) indicators and (iii) (hyper) accumulators (*sensu* Baker 1981). In excluder plants, shoot metal concentrations are maintained low over a wide range of soil metal gradients, whereas those of indicator plant species reflect soil metal concentrations, and, finally, in accumulators shoot concentrations are (much) higher than are soil metal concentrations (Baker 1981). Hyperaccumulator plants attain extremely high shoot metal(loid) concentrations through highly enhanced metal uptake and translocation mechanisms (Reeves and Baker 2000; Reeves 2006). Notional threshold values for delimiting hyperaccumulators have been set at 100 μg g^{-1} for cadmium (Cd), selenium (Se) and thallium (Tl), at 300 μg g^{-1} for cobalt (Co) and copper (Cu), at 1000 μg g^{-1} for arsenic (As) and nickel (Ni), at 3000 μg g^{-1} for zinc (Zn), and at 10 000 μg g^{-1} for manganese (Mn; van der Ent *et al.* 2013). There are currently >700 hyperaccumulator species known globally, the majority of which are Ni hyperaccumulators (~70%), with only about 20 Zn hyperaccumulators known (Reeves *et al.* 2018). Hyperaccumulators can be 'obligate' (the species in question consistently hyperaccumulates and is often restricted to metalliferous soils) or 'facultative' hyperaccumulator (it occurs on

Received: 24 March 2022
Accepted: 25 February 2023
Published: 30 March 2023

Cite this:
Chen F *et al.* (2023)
Australian Journal of Botany, **71**(4), 167–174.
doi:10.1071/BT22033

both metalliferous and non-metalliferous soils, but is hyperaccumulating only on the latter (Pollard *et al.* 2014).

The rhizosphere processes underlying hyperaccumulation are still poorly understood. The capacity of root differential growth between microenvironments has been demonstrated in a range of plant species (Robinson 1994, Haines 2002). Plant roots tend to proliferate towards regions of the soil where resources (such as nutrients and water) are abundant and increase lateral root growth in nutrient-rich zones to optimise nutrient uptake in soils with diverse microenvironments (Fitter 1994; Hutchings and John 2003; Guan *et al.* 2014). Interestingly, some accessions of the intensively studied *Noccaea caerulescens* (J. Presl & C. Presl) F.K. Mey. (Brassicaceae) positively forage for Zn, Cd and Ni (Schwartz *et al.* 1999; Whiting *et al.* 2000; Haines 2002; Tognacchini *et al.* 2020), suggesting that these hyperaccumulator plants may have a higher requirement for these metals. Notably, localised root growth ('root foraging') is one of the adaptations responsible for highly efficient metal uptake in the obligate Ni/Zn hyperaccumulator *N. caerulescens* (Prayon accession; Schwartz *et al.* 1999; Haines 2002; Whiting *et al.* 2000). A recent study showed that different accessions of *N. caerulescens* respond differently to soil Ni enrichment, with the Ni accession (Bergenbach) actively foraging for Ni, whereas the non-Ni accession (Plombieres) avoided the Ni-enriched soil (Tognacchini *et al.* 2020). However, the roots of the obligate Ni hyperaccumulator plant *Berkheya coddii* Roessler (Asteraceae) showed no preference for localised Ni-enriched zones (Moradi *et al.* 2009). Similarly, the facultative As hyperaccumulator *Pityrogramma calomelanos* (L.) Link does not specifically forage for As-contaminated soil (Corzo Remigio *et al.* 2021), but the obligate Se hyperaccumulator *Neptunia amplexicaulis* Domin. (Fabaceae) does preferentially forage in soluble Se-enriched soil, which leads to an increase in growth (Pinto Irish *et al.* 2021).

There are only two Zn hyperaccumulator plant species known from Australia, namely, *Gomphrena canescens* R.Br. (Amaranthaceae) from the Bulman Prospect in the Northern Territory (Farago *et al.* 1977) and *Crotalaria novae-hollandiae* DC. (Fabaceae) from the Dugald River outcrop in Queensland (Cole *et al.* 1968; Tang *et al.* 2022). These species are both widespread on non-metalliferous soils and, hence, facultative hyperaccumulators. *C. novae-hollandiae* is an annual shrub with bright yellow flowers in spiked inflorescences, and brown club-shaped pods that rattle when mature and contain toxic alkaloids (Holland 2002). It is widespread in the semi-arid central and northern part of Australia in the states of Queensland and the Northern Territory (Atlas of Living Australia (ALA) 2022). *C. novae-hollandiae* is a facultative hyperaccumulator that hyperaccumulates Zn only when growing on highly Zn-enriched soils, such as the Dugald River Zn–Pb gossan near Cloncurry and can accumulate up to 16 200 μg Zn g^{-1} in its leaves (Tang *et al.* 2022). *C. novae-hollandiae* has desirable traits for Zn phytoextraction on the basis of its high biomass production, nitrogen-fixing ability and high shoot Zn concentrations when occurring on Zn-enriched soils. It is presently unknown whether roots of *C. novae-hollandiae* actively forage for Zn, like some accessions of *N. caerulescens*. Because there are about 30 *Crotalaria* species native to Australia, it is germane to also assess how other species within this genus respond to Zn-enriched soils. *Crotalaria cunninghamii* R.Br., which is a moderately common species widespread in inland areas of Australia and not known to be a Zn hyperaccumulator, is thus a potentially suitable candidate. Investigating root responses of *Crotalaria* species towards Zn-enriched regions in the growing media have major implications for understanding Zn-uptake mechanisms in these species and their potential use in phytoextraction of Zn-rich substrates such as base metal-mine tailings.

In this study, we investigated whether *C. novae-hollandiae* displays active root foraging or, in contrast, shows avoidance towards localised soil Zn enrichment, in comparison to the non-metallophyte *C. cunninghamii*. To that end, rhizotron experiments were conducted where *C. novae-hollandiae* was exposed to homogeneously and heterogeneously spiked soils in two different concentrations, in comparison with a non-metallophyte *C. cunninghamii*. The aim of this study was to address the following questions: (i) does the Zn hyperaccumulator *C. novae-hollandiae* (metalliferous accession) preferentially forage towards Zn-enriched zones; (ii) does a positive root-foraging response to Zn enhance Zn accumulation in *C. novae-hollandiae*; (iii) how do the root responses of *C. novae-hollandiae* towards Zn compare with that of the non-metallophyte *C. cunninghamii*?

Materials and methods

Experimental plant species

Two species were used in the experiments, namely, *C. novae-hollandiae* (metalliferous accession) and *C. cunninghamii* (non-metalliferous accession). Seeds of *C. novae-hollandiae* were collected from the Dugald River Lode Zn outcrop in Queensland, Australia (Tang *et al.* 2022), whereas seeds of *C. cunninghamii* were obtained from the Nindethana Seed Company (King River, WA, Australia). Seeds were germinated in perlite–vermiculite mix (ratio 1:1). Once the cotyledons formed, the seedlings were transplanted into the rhizotrons, with the root pointing directly downward.

Soil preparation and analyses

Local soil (from the University of Queensland St Lucia Campus) characterised by low total and plant-available Zn was selected for the experiment (Tognacchini *et al.* 2020). The soil was oven dried at 40°C for 24 h, sieved at 1 mm then mixed with sand and peat moss (70% soil, 25% sand, 5% peat moss), divided into equal parts of 11.84 kg.

One aliquot of soil was amended with Zn and the remaining soil was kept as a control. We chose insoluble, but easily weatherable Zn carbonate ($ZnCO_3$, 99.9%, Sigma-Aldrich) for the soil Zn enrichment to avoid substantial Zn diffusion in the control soil. Moist autoclaved soil was mixed with the $ZnCO_3$ and manually homogenised, then re-dried and re-moistened for 7 days to allow for equilibration. Half of the untreated soil to be used as a control was limed with $CaCO_3$ to obtain the same pH as in the soil spiked with Zn carbonate, so as to avoid pH changes owing to the spiking. Soil pH was measured in ultra-pure water (w:v ratio 1:2.5) with a pH-metre in two replicates. The $Sr(NO_3)_2$ extraction (0.01 M) was performed using a method adapted from Kukier and Chaney (2001) to determine cation exchange capacity (CEC)-exchangeable trace element concentrations in the soil solution. Extraction *via* diethylenetriamine pentaacetate (DTPA) excluding triethanolamine (TEA) was performed using a solid:liquid ratio (*m/v*) of 1:5 at pH 5.3, so as to determine available pool of trace elements with an equilibrium time of 2 h (adapted from Lindsay and Norvell 1978). To determine pseudo-total concentrations, 300 mg of the soil samples were digested for 16 min at 50% power by using a ColdBlock system (CB15S 15 channel system, ColdBlock Technologies Inc.) with high-intensity infrared irradiation (Wang *et al.* 2014). The digests were brought to volume of 50 mL and filtered (Whatman® Grade 1 filter paper) before analysis with inductively coupled plasma–atomic emission spectroscopy (ICP–AES).

Rhizotron experiment

Rhizotrons consisted of Petri dishes (12 cm × 12 cm) with juxtaposed treatments, which were either homogenous (each half of the treatments containing the same Zn concentrations) or heterogenous (different Zn concentrations in each half of the treatments). The Zn concentrations were 0 μg Zn g^{-1} (control), 2000 μg Zn g^{-1} and 5000 μg Zn g^{-1} in the form of zinc carbonate. The limed soil was used as a control for the Zn carbonate treatments. The soil surface of the rhizotrons was carefully compacted to ensure homogeneity. Seedlings were germinated in a vermiculite mix and transplanted into the rhizotrons as soon as cotyledons appeared. The rhizotrons were then closed and wrapped with aluminium foil to protect the roots from light and inclined at a 45° angle, with the rooted surface facing down. Each treatment had three replicates, making a total of 48 rhizotrons. The experiment was conducted in a growth cabinet with a 12-h day of light, a temperature of 20–25°C (day–night), 75% humidity and with a photosynthetic photon flux density of 350 μmol m^{-2} s^{-1}. *C. novae-hollandiae* (metalliferous) was grown for 26 days, and *C. cunninghamii* (non-metalliferous) was grown for 21 days to account for differences in growth rates between the respective species. The physico-chemical properties of the rhizotron soils at the end of the experiment are given in Table 1.

Measurement of root biomass, shoot biomass and root density

At the end of the experimental period and before the shoots were harvested, roots were harvested from each half of the rhizotrons, thoroughly rinsed to remove soil particles and oven dried at 40°C for 3 days. Dry weight was recorded and the root density in each side was measured as a percentage of the total root density for each rhizotron. The harvested shoots were also dried in an oven at 40°C for 3 days, and the dry weights were recorded.

Bulk chemical analysis of plant-tissue samples

Plant-tissue samples were weighed in 6 mL polypropylene tubes. These samples were pre-digested using 2 mL HNO_3 (70%) for 24 h before being digested in a block heater (Thermo Scientific™ digital dry bath) for a 2-h program (1 h at 70°C, followed by 1 h at 125°C) and brought to 10 mL with ultrapure water (Millipore) before analysis with ICP–AES with a Thermo Scientific iCAP 7400 instrument, as described erlier (Tognacchini *et al.* 2020).

Table 1. Soil pH, and total DTPA- and $Sr(NO_3)_2$-extractable zinc and manganese concentrations (μg g^{-1}) in the juxtaposed control and Zn-enriched soil treatment.

Element	Extraction	2000 (mg kg^{-1})		5000 (mg kg^{-1})	
		Zn side	Control side	Zn side	Control side
Soil pH		7.14	7.47	6.70	6.32
Zn	Total	1990	60	3450	120
	DTPA	340	15	380	40
	$Sr(NO_3)_2$	15	<LOD	80	<LOD
Mn	Total	2670	2750	4120	4340
	DTPA	215	285	110	635
	$Sr(NO_3)_2$	45	15	70	55

<LOD, below limit of detection.

Instrumental X-ray fluorescence elemental mapping

The University of Queensland (UQ) microXRF facility has a 50 W X-ray focussing to 25 μm and is fitted with two silicon drift detectors (SDD) of 150 mm^2, as described earlier (Corzo Remigio *et al.* 2021). Measurements were conducted at atmospheric temperature (~20°C) with a per-pixel dwell of 100 ms. The XRF spectra on the UQ microXRF facility were acquired in mapping mode, by using the instrument-control package, Iridium (IXRF Systems) and the XRF data were exported into ImageJ as TIFFs and visualised with the built-in 'Fire' LUT (Schneider *et al.* 2012).

Statistical analyses

Statistical analyses were performed using OriginPro 2021 (https://www.originlab.com/). Significant differences were determined by two-way ANOVA (Zn concentration, and species (*C. novae-hollandiae* and *C. cunninghamii*), separated by Tukey's honestly significant difference (HSD) test ($P < 0.05$) and indicated by different letters.

Results

The rooting density, root biomass and root Zn concentrations for all the treatments are shown in Figs 1, 2 and 3. None of the treatments had significantly ($P > 0.05$) different rooting density or root biomass (Figs 1–3). Moreover, the roots of both *C. novae-hollandiae* and *C. cunninghamii* did not proliferate towards Zn-rich parts of the various treatments (Figs 4, 5). The Zn concentrations in the roots of both *C. novae-hollandiae* and *C. cunninghamii* were significantly ($P < 0.05$) higher in the homogenous Zn treatments (both high-Zn and low-Zn treatments) than in their respective homogenous control treatments (Fig. 3). For the low Zn treatment, the root Zn concentrations of *C. cunninghamii* in the Zn-treated portion were significantly ($P < 0.05$) higher than those in the control portion (Fig. 3). For the low homogenous Zn treatment, there was no significant ($P > 0.05$) difference in the root Zn concentrations between *C. novae-hollandiae* and *C. cunninghamii* (Fig. 3*a*). However, in the high-Zn treatment, the root Zn concentrations of *C. cunninghamii* were significantly ($P < 0.05$) higher than those of *C. novae-hollandiae* (Fig. 3*b*). For *C. novae-hollandiae* under the high-Zn treatment, there was a significant ($P < 0.05$) difference in the root Zn concentrations between homogenous Zn treatment and heterogenous Zn treatment (Fig. 3*b*).

The shoot biomass and shoot Zn concentrations are shown in Figs 6, 7. The shoot biomass did not vary significantly ($P > 0.05$) between *C. novae-hollandiae* and *C. cunninghamii* in the respective treatments (Fig. 6). There was a significant ($P < 0.05$) difference in the shoot biomass of *C. cunninghamii* between the high- and low-Zn treatments (Fig. 6). However, in the low-Zn treatment, there was no significant ($P > 0.05$) difference in the shoot biomass of *C. cunninghamii* in all the subtreatments (Fig. 6). The shoot Zn concentrations of *C. cunninghamii* in the low homogenous

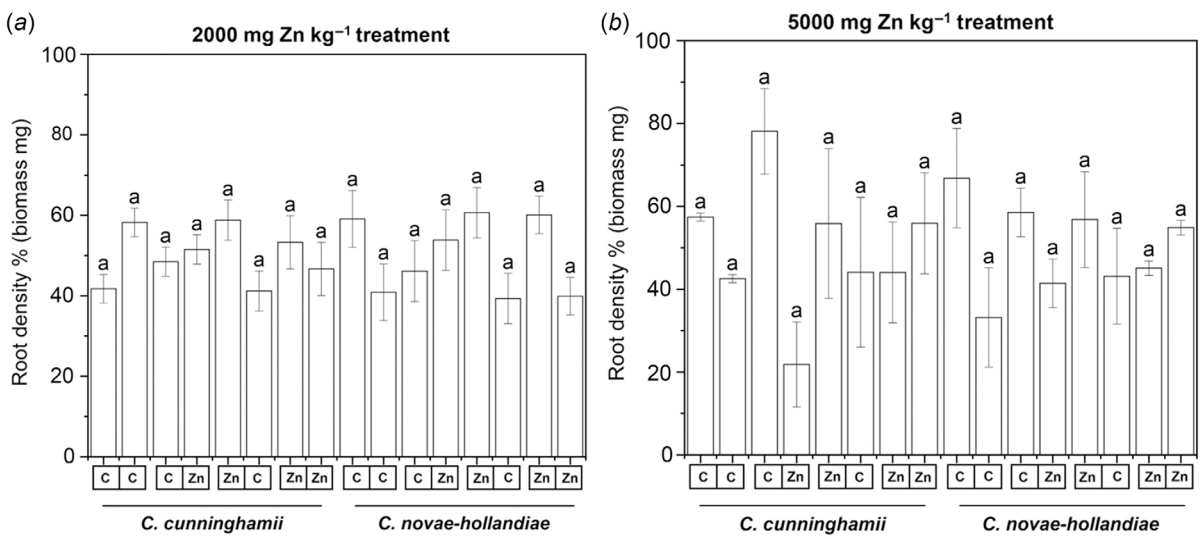

Fig. 1. Root density % in the two areas of the rhizotron (control (C) and zinc-enriched (Zn)) calculated from root biomass (mg). (*a*) 2000 mg Zn kg^{-1} treatment and (*b*) 5000 mg Zn kg^{-1} treatment. Each of the bars compares the rooting density in the two halves of the rhizotron side by side. These are either control or Zn-enriched, with treatment (Zn enrichment) applied to either the left- or right-hand side of the rhizotron to correct for potential preferential rooting directionality. Key to symbols of bar plots: columns are the mean and whiskers are ±s.e. Mean ± s.e. followed by the same letter are not significantly different (at $P = 0.05$) according to the Tukey test.

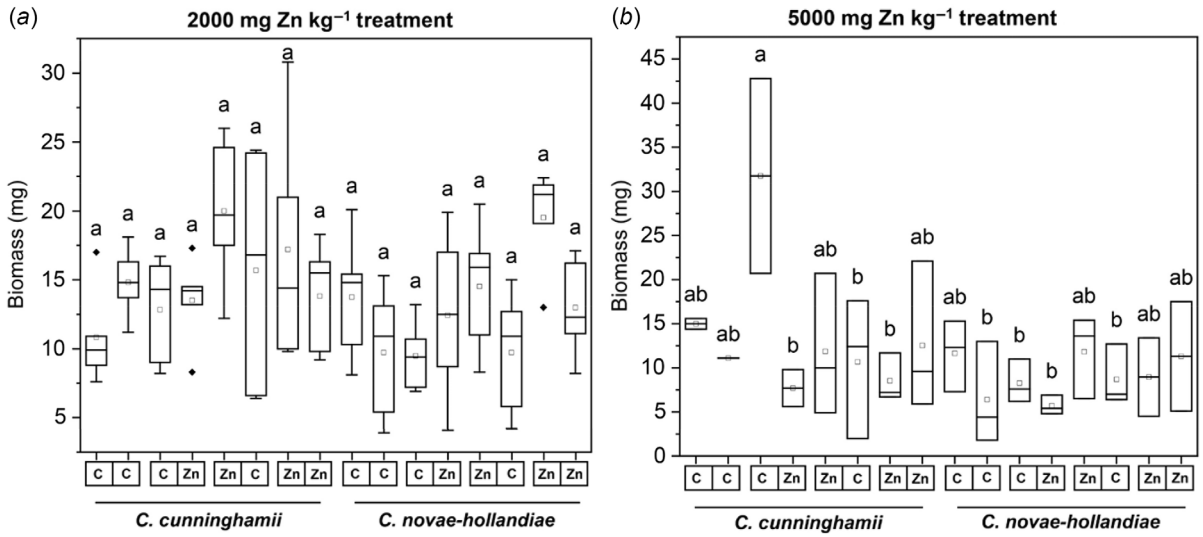

Fig. 2. Root biomass (mg) in the two areas of the rhizotron (control (C) and zinc enriched (Zn)) under low- and high-Zn treatments. (*a*) 2000 mg Zn kg^{-1} treatment and (*b*) 5000 mg Zn kg^{-1} treatment. Each of the bars compares the root biomass in the two halves of the rhizotron side by side. These are either control or Zn-enriched, with Zn enrichment applied to either the left- or right-hand side of the rhizotron to correct for potential preferential rooting directionality. Key to symbols of boxplots: open squares indicate the ±mean, whiskers are ±s.e. and diamonds are outliers. Mean ± s.e. followed by the same letter are not significantly different (at $P = 0.05$) according to the Tukey test.

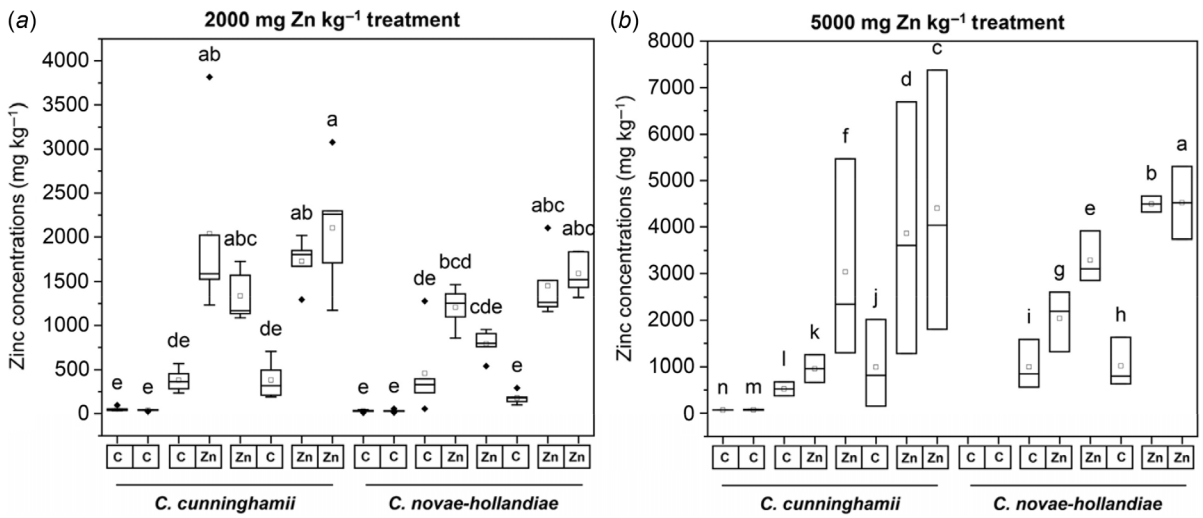

Fig. 3. Root zinc concentrations (mg kg^{-1}) in the two areas of the rhizotron (control (C) and zinc enriched (Zn)) under low and high Zn treatments (panel *a*: 2000 mg Zn kg^{-1} treatment and panel *b*: 5000 mg Zn kg^{-1} treatment). Each of the bars compares the concentrations in the two halves of the rhizotron side by side. These are either control or Zn-enriched, with Zn enrichment applied to either the left or right hand side of the rhizotron to correct for potential preferential rooting directionality. Key to symbols of boxplots: open squares indicate the ±mean, whiskers are ±s.e. and diamonds are outliers. Mean ± standard error followed by the same letter are not significantly different ($P > 0.05$) according to the Tukey test.

Zn treatment were significantly higher than in the respective homogenous control treatment ($P < 0.05$) (Fig. 7). However, the shoot Zn concentrations of *C. novae-hollandiae* in the low homogenous Zn treatment were not significantly ($P > 0.05$) higher than those in the respective homogenous control treatment (Fig. 7). In the high-Zn treatment, the shoot Zn concentrations in both *C. novae-hollandiae* and *C. cunninghamii* were significantly ($P < 0.05$) higher than those in their respective control treatments (Fig. 7). However, there was no significant difference in the shoot Zn concentrations in both *C. novae-hollandiae* and *C. cunninghamii* in the high-Zn treatments (Fig. 7).

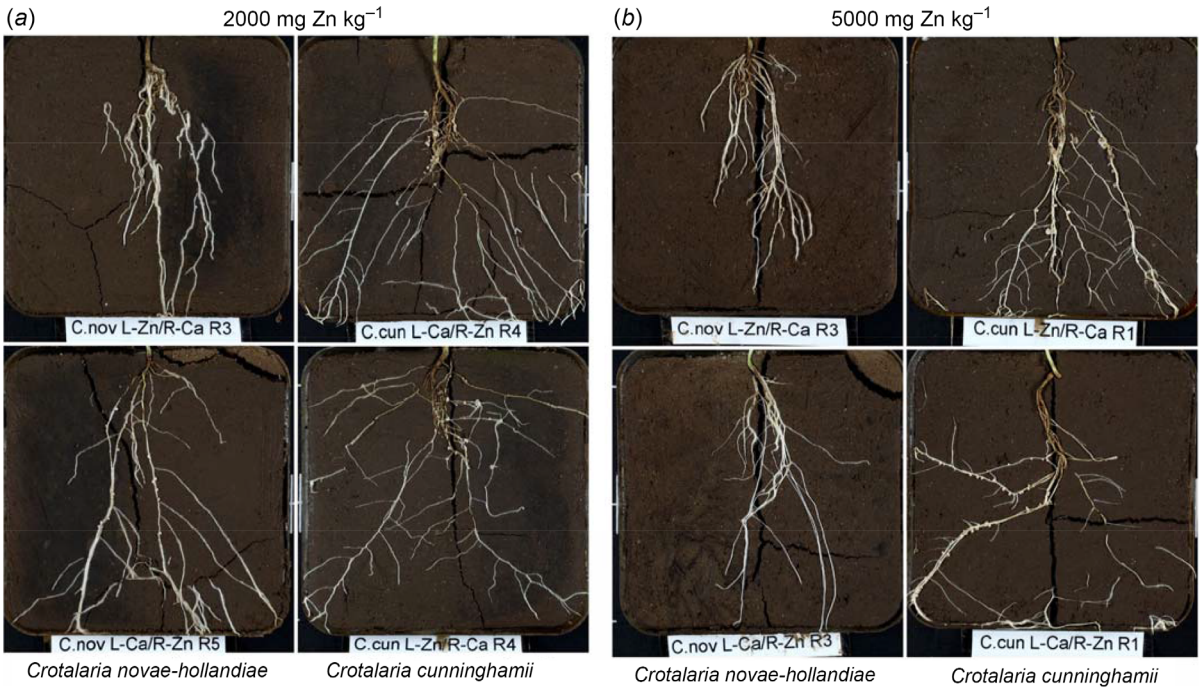

Fig. 4. Rhizotron rooted surfaces at the end of the experiment with *C. novae-hollandiae* and *C. cunninghamii* under juxtaposed control and Zn-enriched soil treatments. (*a*) 2000 mg Zn kg^{-1} treatment and (*b*) 5000 mg Zn kg^{-1} treatment. Treatments: L-Zn/R-Ca denotes left portion enriched with Zn, whereas right portion is control, and *vice versa* for L-Ca/R-Zn. R1 and R3 are replicates.

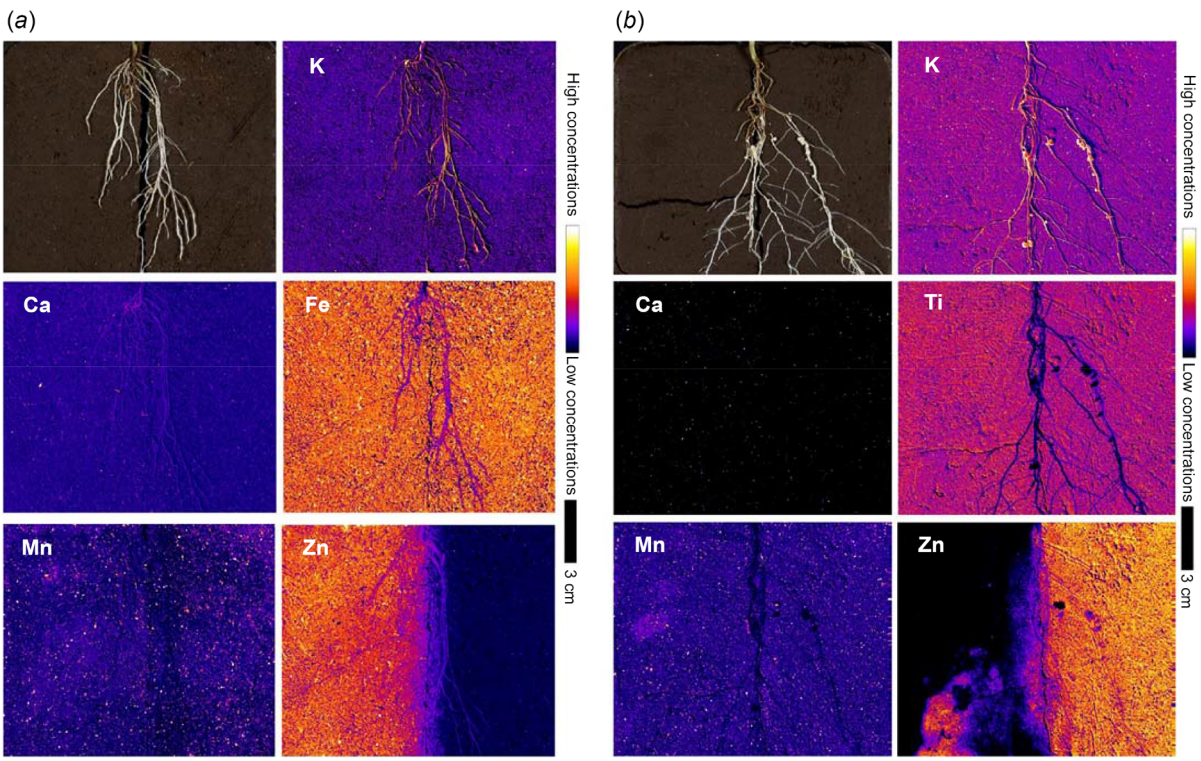

Fig. 5. Laboratory micro-X-ray fluorescence maps of the rhizotrons with *C. novae-hollandiae* exposed to juxtaposed (*a*) control (right portion) and zinc (left portion) treatment, and (*b*) control (left portion) and zinc (right portion) treatment. The Zn concentration was 5000 mg Zn kg^{-1}.

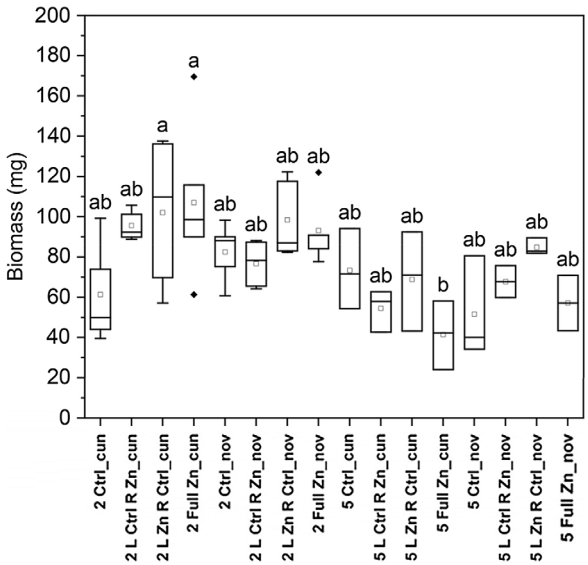

Fig. 6. Shoot biomass (mg) of *C. novae-hollandiae* and *C. cunninghamii* under homogeneous and heterogenous treatments. cun and nov represent *C. cunninghamii* and *C. novae-hollandiae* respectively, whereas Ctrl, 2 and 5 represent 0, 2000 and 5000 mg Zn kg^{-1} treatment. Ctrl, control; L,left portion of rhizotron; R, right portion of rhizotron; Full, homogeneous treatment. Key to symbols of boxplots: open squares indicate the ±mean, whiskers are ±s.e. and diamonds are outliers. Mean ± standard error followed by the same letter are not significantly different ($P > 0.05$) according to the Tukey test.

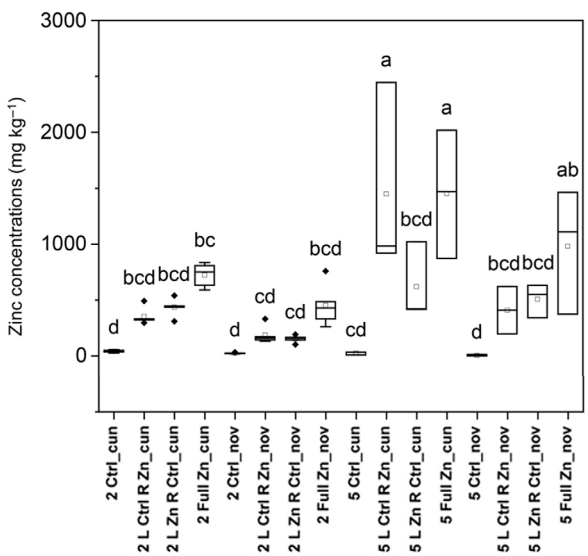

Fig. 7. Shoot zinc concentrations (mg kg^{-1}) of *C. novae-hollandiae* and *C. cunninghamii* under homogeneous and heterogenous control and Zn treatments. cun and nov represent *C. cunninghamii* and *C. novae-hollandiae* respectively, whereas Ctrl, 2 and 5 represent 0, 2000 and 5000 mg Zn kg^{-1} treatment. Ctrl, control; L, left portion of rhizotron; R, right portion of rhizotron; Full, homogeneous treatment. Key to symbols of boxplots: open squares indicate the ±mean, whiskers are ±s.e. and diamonds are outliers. Mean ± standard error followed by the same letter are not significantly different ($P > 0.05$) according to the Tukey test.

Discussion

This study showed that *C. novae-hollandiae* and *C. cunninghamii* do not prefer Zn-enriched soils for rooting. This finding is consistent with the non-metallicolous accession of *N. caerulescens* (Bradford Dale) that also did not exhibit zincophilic root foraging (Haines 2002). In contrast, the metallicolous accession of *N. caerulescens* (Prayon) showed increased root proliferation in Zn-rich patches, whereas the non-accumulator *Thlaspi arvense* L. avoids Zn-rich patches (Haines 2002; Schwartz *et al.* 1999; Whiting *et al.* 2000). Contrasting root responses (no-preference vs proliferation vs avoidance) towards Zn suggest that different physiological requirements for Zn influence their adaptations to different Zn environments. The no-preference response towards Zn by *C. novae-hollandiae* and *C. cunninghamii* suggests that *Crotalaria* species are well adapted to both normal and Zn-rich substrates. However, these species accumulate Zn only when exposed to substrates with elevated Zn concentrations (Tang *et al.* 2022), classifying them as facultative hyperaccumulators (Pollard *et al.* 2014). A recent field survey of the metallophyte *C. novae-hollandiae* occurring on the Zn–Pb–Cu Dugald River gossan in Queensland (Australia) found a strong correlation between soil total and soil-available Zn and foliar Zn (Tang *et al.* 2022), which is consistent with the shoot Zn concentrations in the low- and high-Zn treatments (Fig. 6). However, in the field-collected material, the foliar Zn concentrations reached 16 200 μg Zn g^{-1} (Tang *et al.* 2022), whereas those in this study were <3000 μg Zn g^{-1}. Notably, the growth period of *C. novae-hollandiae* in this study was only 21 days, which accounts for the difference in the foliar Zn concentrations.

The root responses in this study showed that Zn may not be a strong requirement for physiological functioning in *Crotalaria* species. The no-preference response towards Zn by the *Crotalaria* species also suggests that in a heterogenous Zn environment, their shoot Zn concentrations may not be enhanced (Fig. 6), unlike the metallophyte ecotype of *N. caerulescens* (Prayon) that actively forages towards Zn-rich patches, leading to increased shoot Zn concentrations (Haines 2002). In a heterogenous Zn-rich substrate phytoextraction approach, *N. caerulescens* (Prayon) may exhibit a more efficient removal of Zn than does *Crotalaria* species. However, considering the high biomass production of *Crotalaria* species, its overall Zn yield may be significantly higher than that of *N. caerulescens* (Prayon). These findings indicate that different Zn hyperaccumulator plants and ecotypes may behave differently in a heterogenous Zn environment, necessitating rhizosphere investigations prior to field phytoextraction application. Combining planar optodes, diffusive gradients in thin films and microXRF imaging could provide insights on the rhizosphere processes affecting Zn uptake in *C. novae-hollandiae*. Future research is also required to investigate

whether Zn tolerance and accumulation are species-wide traits in the Australian endemic *Crotalaria* species.

References

Atlas of Living Australia (ALA) (2022) *Crotalaria novae-hollandiae* DC. Available at https://bie.ala.org.au/species/https://id.biodiversity.org.au/node/apni/2888407 [Accessed 21 March 2022]

Baker AJM (1981) Accumulators and excluders: strategies in the response of plants to heavy metals. *Journal of Plant Nutrition* **3**, 643–654. doi:10.1080/01904168109362867

Cole MM, Provan DMJ, Tooms JS (1968) Geobotany, biogeochemistry and geochemistry in the Bulman-Springs area, Northern Territory, Australia. *Transactions of the Institution of Mining and Metallurgy* **77**, 81–104.

Corzo Remigio A, Edraki M, Baker AJM, van der Ent A (2021) Root responses to arsenic enrichment in the fern *Pityrogramma calomelanos* var. *austroamericana* grown in rhizotrons. *Plant Physiology and Biochemistry* **164**, 147–159. doi:10.1016/j.plaphy.2021.04.025

Farago ME, Clark AJ, Pitt MJ (1977) Plants which accumulate metals. Part I. The metal content of three Australian plants growing over mineralised sites. *Inorganica Chimica Acta* **24**, 53–56. doi:10.1016/S0020-1693(00)93850-7

Fitter AH (1994) Architecture and biomass allocation as components of the plastic response of root systems to soil heterogeneity. In 'Exploitation of environmental heterogeneity by plants: ecophysiological processes above- and belowground'. (Eds MM Caldwell, RW Pearcy) pp. 305–323. (Academic Press: San Diego, CA, USA)

Guan P, Wang R, Nacry P, Breton G, Kay SA, Pruneda-Paz JL, Davani A, Crawford NM (2014) Nitrate foraging by *Arabidopsis* roots is mediated by the transcription factor TCP20 through the systemic signaling pathway. *Proceedings of the National Academy of Sciences* **111**, 15267–15272. doi:10.1073/pnas.1411375111

Haines BJ (2002) Zincophilic root foraging in *Thlaspi caerulescens*. *New Phytologist* **155**, 363–372. doi:10.1046/j.1469-8137.2002.00484.x

Holland AE (2002) A review of *Crotalaria* L. (Fabaceae: Crotalarieae) in Australia. *Austrobaileya* **6**, 311–312.

Hutchings MJ, John EA (2003) Distribution of roots in soil, and root foraging activity. In 'Root ecology'. Ecological studies (analysis and synthesis). Vol. 168. (Eds H de Kroon, EJW Visser) pp. 33–60. (Springer: Berlin, Heidelberg, Germany)

Kukier U, Chaney RL (2001) Amelioration of nickel phytotoxicity in muck and mineral soils. *Journal of Environmental Quality* **30**, 1949–1960. doi:10.2134/jeq2001.1949

Lindsay WL, Norvell WA (1978) Development of a DTPA soil test for zinc, iron, manganese, and copper. *Soil Science Society of America Journal* **42**, 421–428. doi:10.2136/sssaj1978.03615995004200030009x

Moradi AB, Conesa HM, Robinson BH, Lehmann E, Kaestner A, Schulin R (2009) Root responses to soil Ni heterogeneity in a hyperaccumulator and a non-accumulator species. *Environmental Pollution* **157**, 2189–2196. doi:10.1016/j.envpol.2009.04.015

Pinto Irish K, Harvey M-A, Erskine PD, van der Ent A (2021) Root foraging and selenium uptake in the Australian hyperaccumulator *Neptunia amplexicaulis* and non-accumulator *Neptunia gracilis*. *Plant and Soil* **462**, 219–233. doi:10.1007/s11104-021-04843-x

Pollard AJ, Reeves RD, Baker AJ (2014) Facultative hyperaccumulation of heavy metals and metalloids. *Plant Science* **217–218**, 8–17. doi:10.1016/j.plantsci.2013.11.011

Reeves RD (2006) Hyperaccumulation of trace elements by plants. In 'Phytoremediation of metal-contaminated soils'. (Eds J-L Morel, G Echevarria, N Goncharova). pp 25–52. (Springer: Dordrecht, The Netherlands)

Reeves RD, Baker AJM (2000) Metal accumulating plants. In 'Phytoremediation of toxic metals: using plants to clean up the environment'. (Eds I Raskin, BD Ensley) pp. 193–229. (Wiley: New York, NY, USA)

Reeves RD, Baker AJM, Jaffré T, Erskine PD, Echevarria G, van der Ent A (2018) A global database for plants that hyperaccumulate metal and metalloid trace elements. *New Phytologist* **218**, 407–411. doi:10.1111/nph.14907

Robinson D (1994) The responses of plants to non-uniform supplies of nutrients. *New Phytologist* **127**, 635–674. doi:10.1111/j.1469-8137.1994.tb02969.x

Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* **9**, 671–675. doi:10.1038/nmeth.2089

Schwartz C, Morel JL, Saumier S, Whiting SN, Baker AJM (1999) Root development of the zinc-hyperaccumulator plant *Thlaspi caerulescens* as affected by metal origin, content and localization in soil. *Plant and Soil* **208**, 103–115. doi:10.1023/A:1004519611152

Tang RH, Erskine PD, Nkrumah PN, Echevarria G, van der Ent A (2022) Soil–plant relationships of metallophytes of the zinc-lead-copper Dugald River gossan, Queensland, Australia. *Plant and Soil* **471**, 227–245. doi:10.1007/s11104-021-05209-z

Tognacchini A, Salinitro M, Puschenreiter M, van der Ent A (2020) Root foraging and avoidance in hyperaccumulator and excluder plants: a rhizotron experiment. *Plant and Soil* **450**, 287–302. doi:10.1007/s11104-020-04488-2

Wang Y, Kanipayor R, Brindle ID (2014) Rapid high-performance sample digestion for ICP determination by ColdBlock™ digestion: part 1 environmental samples. *Journal of Analytical Atomic Spectrometry* **29**, 162–168. doi:10.1039/C3JA50333J

Whiting SN, Leake JR, McGrath SP, Baker AJM (2000) Positive responses to Zn and Cd by roots of the Zn and Cd hyperaccumulator *Thlaspi caerulescens*. *New Phytologist* **145**, 199–210. doi:10.1046/j.1469-8137.2000.00570.x

van der Ent A, Baker AJM, Reeves RD, Pollard AJ, Schat H (2013) Hyperaccumulators of metal and metalloid trace elements: facts and fiction. *Plant and Soil* **362**, 319–334. doi:10.1007/s11104-012-1287-3

Data availability. The data that support this study will be shared upon reasonable request to the corresponding author.

Conflicts of interest. The authors declare no conflicts of interest relevant to the content of this paper.

Declaration of funding. This research was funded by the Sustainable Minerals Institute (UQ) Complex Orebodies strategic initiative program. Roger Tang was the recipient of a UQ Graduate School Scholarship (UQGSS) from The University of Queensland.

Acknowledgements. We thank Lachlan Casey (University of Queensland) for support with the laboratory μXRF analysis. We acknowledge the support of the AMMRF at the Centre for Microscopy and Microanalysis at the University of Queensland.

Author contributions. FC and RHT conducted the rhizotron experiments and undertook the chemical analyses of the samples. PNN conducted the statistical analysis and prepared the figures. AVDE supervised the study. All authors contributed to writing the paper.

Author affiliations

[A]Environment Science and Spatial Informatics, China University of Mining and Technology, Xuzhou City, China.

[B]Centre for Mined Land Rehabilitation, Sustainable Minerals Institute, The University of Queensland, St Lucia, Qld, Australia.

[C]Université de Lorraine-INRAE, Laboratoire Sols et Environnement, 54000 Nancy, France.