

# Delimiting soil chemistry thresholds for nickel hyperaccumulator plants in Sabah (Malaysia)

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**Abstract** Nickel hyperaccumulator plants have been the focus of considerable research because of their unique ecophysiological characteristics that can be exploited in phytomining technology. Comparatively little research has focussed on the soil chemistry of tropical nickel hyperaccumulator plants to date. This study aimed to elucidate whether the soil chemistry associated with nickel hyperaccumulator plants has distinctive characteristics that could be indicative of specific edaphic requirements. The soil chemistry associated with 18 different nickel hyperaccumulator plant species occurring in Sabah (Malaysia) was compared with local ultramafic soils where nickel hyperaccumulator plants were absent. The results showed that nickel hyperaccumulators in the study area were restricted to circum-neutral soils with relatively high phytoavailable calcium, magnesium and nickel concentrations. There appeared to be a ‘threshold response’ for the presence of nickel hyperaccumulator plants at  $>20 \mu\text{g g}^{-1}$  carboxylic-extractable nickel or  $>630 \mu\text{g g}^{-1}$  total nickel, and  $>\text{pH } 6.3$  thereby delimiting their edaphic range. Two (not mutually exclusive) hypotheses were proposed to explain nickel hyperaccumulation on these soils: (1)

hyperaccumulators excrete large amounts of root exudates thereby increasing nickel phytoavailability through intense rhizosphere mineral weathering; and (2) hyperaccumulators have extremely high nickel uptake efficiency thereby severely depleting nickel and stimulating re-supply of Ni from diffusion from labile Ni pools. It was concluded that since there was an association with soils with highly labile nickel pools, the available evidence primarily supports hypothesis (2).

**Keywords** Diffusion sink · Nickel speciation · Labile pools · Root exudates

## Introduction

Hyperaccumulators are unusual plants that accumulate trace elements to extraordinarily high concentrations in their living shoots (Baker and Brooks 1989; Van der Ent et al. 2013a). Some of these hyperaccumulator species can attain up to 6 % nickel (Ni) in their leaves (Reeves et al. 1999) and up to 25 % Ni in their latex (Jaffré et al. 1976). These are amongst the highest trace element concentrations in any living plant material. Nickel hyperaccumulator plants can be utilized in phytomining technology, an approach which uses the ability of hyperaccumulators to sequester target metals in their biomass, which after incineration produces a high-grade ‘bio-ore’ that contains 10–20 % Ni (Brooks and Robinson 1998; Chaney et al. 1998; Van der Ent et al. 2015a). The high purity of such a bio-ore makes it uniquely suited for the manufacture of Ni-based catalysts for the chemical industry (Losfeld et al. 2012) or for obtaining high purity Ni chemicals for the electronics industry (Barbaroux et al. 2012; Van der Ent et al. 2015a). Critical to developing Ni phytomining, however, is a better understanding of the Ni

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pathways across the soil/plant interface. A first step in advancing this understanding is to study the ecology and habitat of Ni hyperaccumulator plants in their native ranges, particularly in relation to the characteristics of the soils in which natural populations occur (Van der Ent et al. 2013b).

Nickel phytoavailability in soils depends on the in situ Ni-bearing phases (Becquer et al. 2001; Quantin et al. 2001) with soil pH generally controlling Ni solubility (Anderson and Christensen 1988). Soil weathering of primary ultramafic minerals releases Ni, which can then be incorporated in secondary minerals (e.g. smectite clays, Fe-oxides) or adsorbed onto these phases (Massoura et al. 2006; Bani et al. 2014). In intensively weathered ultramafic soils (Ferralsols), Fe-oxyhydroxides (especially goethite), and to a lower extent Mn-oxides (for example phyllo-manganate, asbolane, lithiophorite), are the main Ni-bearing phases (Quantin et al. 2008; Siebecker and Sparks 2010; Fan and Gerson 2011; Dublet et al. 2012). Nickel adsorption is favoured on Mn-oxides because of their negative surface charge (Massoura et al. 2006; Alves et al. 2011; Bani et al. 2014). Manganese-oxides are very sensitive to redox conditions (Quantin et al. 2002). Although they are generally less abundant than Fe-oxides, the lower point of zero charge means a higher adsorption capacity at soil pH 5–7 (Cornu et al. 2005; Raous et al. 2013) and as a consequence, Ni is more easily desorbed from Mn-oxides compared to well-crystallized Fe-oxides (Quantin et al. 2001).

In less intensively weathered soils (such as hypermagnesian Cambisols) and in the saprolite layer beneath limonitic strata, Ni is mostly associated with phyllosilicates and smectites as replacement for Mg in the lattices and as outer sphere complexes (Chardot et al. 2007; Fan and Gerson 2011; Raous et al. 2013; Bani et al. 2014). Generally, Ni phytoavailability is higher in soils where phyllosilicates are the main phases for soil Ni, as opposed to soils where well-crystallized Fe-oxides/oxyhydroxides are the main Ni phase, however soils with high contents of amorphous Fe-oxides have also been shown to have high phytoavailability of Ni (Massoura et al. 2006) due to their extremely high specific area. Adsorption to mineral phases increases with increasing pH, and the adsorption edge is inversely related to the  $pK$  of the metal ion hydrolysis reaction (Basta et al. 2005). Increasing soil pH decreases Ni phytotoxicity in 'normal' plants (Siebielec et al. 2007), which can be explained by increasing Ni adsorption to soil phases, and reduced  $H^+$  competition. For example, Ni adsorption on Fe-oxyhydroxide (goethite) increases approximately 75-fold when soil acidity changes from pH 5 to pH 7 (Basta et al. 2005).

Nickel hyperaccumulators can effectively take up and translocate Ni from soils with relatively low (phytoavailable) Ni, where non-hyperaccumulator plants show no signs of Ni toxicity (Proctor 2003; Reeves 2003). Such highly efficient

uptake mechanisms suggest that the response of hyperaccumulator plants to soil Ni concentrations is vastly different from non-accumulator plants, but there is no consensus for any method to accurately predict the quantity of Ni accumulation in the living shoot of a given hyperaccumulator plant. Estimating Ni availability and uptake in plants, including hyperaccumulator plants, has been widely studied using single extraction methods [such as the  $NH_4Ac$ , DTPA,  $CaCl_2$ ,  $Sr(NO_3)_2$  extracts], Ion exchange resin methods (IER), Isotopic exchange kinetics (IEK) and Diffusive Gradients in Thin-films (DGT) (Echevarria et al. 1998; Becquer et al. 2002). The IER method was found to significantly correlate with Ni uptake in non-hyperaccumulator plants in New Caledonia and was preferred over KCl and DTPA extraction methods (Becquer et al. 2002). The IEK method showed that Ni accumulation in hyperaccumulator and non-hyperaccumulator plants was mainly related to the same Ni labile pool (Shallari et al. 2001; Massoura et al. 2004), despite the fact that hyperaccumulators can accumulate 100–1000 times more Ni in their shoots. It also confirmed the accuracy of the DTPA method to qualitatively assess labile Ni in soils (Echevarria et al. 1998, 2006).

Generally, the amount of Ni chemically extracted from a soil is inversely related to the pH of the extraction solution (Robinson et al. 1996), but experiments have shown that hyperaccumulators can extract large amounts of Ni over a wide pH range (Robinson et al. 1999). Plant-induced acidification of the rhizosphere cannot explain hyperaccumulation, as neither a reduction of pH in the rhizosphere nor the release of reductants from the roots was associated with the Ni hyperaccumulator *Alyssum* (Bernal and McGrath 1994; Bernal et al. 1994). Studies using soil amendments to evaluate the uptake of Ni in hyperaccumulators have demonstrated that the addition of Mg and Ca carbonates led to decreased Ni extractability in the soil and decreased accumulation in the plants, whereas addition of S decreased pH and increased Ni accumulation (Robinson et al. 1999). This was attributed to an increase in soil pH (for Mg addition) and to ion competition (for Ca addition). Kukier et al. (2004) showed increased Ni accumulation in a hyperaccumulator with an increased soil pH, although water-soluble soil Ni actually decreased. In contrast, in ultramafic soils from Portugal, no negative correlation of soil solution Ni with soil pH was found, which was hypothesized to be the result of specific Ni adsorption to Mn-oxides (Alves et al. 2011). This is confirmed by studies on isotopically-exchangeable Ni soil pools (' $E$  value'), which at higher pH show that Ni is more adsorbed and therefore high loadings can occur without phytotoxicity (Ma et al. 2013).

The primary question this study sought to address was whether soils on which Ni hyperaccumulator plants occur naturally have distinctive chemical characteristics and, if

so, whether Ni hyperaccumulator plants may have specific edaphic requirements compared to non-hyperaccumulator plants? To answer this question we employed a range of soil analytical techniques on samples from plots where hyperaccumulator plants were either present or absent in Kinabalu Park (Sabah, Malaysia).

## Methods

### Study area and sample collection

Between February 2010 and March 2013 the ultramafic flora of the 754 km<sup>2</sup> Kinabalu Park (Sabah, Malaysia) was intensively surveyed for the occurrence of Ni hyperaccumulator plants. A total of 12 different ultramafic outcrops in Kinabalu Park and its immediate vicinity were visited (the locations of these outcrops are provided in a map in Van der Ent et al. 2015b). Thousands of plant specimens were screened with dimethylglyoxime ('DMG') impregnated paper for significant foliar Ni concentrations. Empirical evidence shows that a colour reaction (red-magenta Ni-DMG complex) appears when foliar Ni is >500–700 µg g<sup>-1</sup>, which is well below the threshold defined for Ni hyperaccumulation at 1000 µg g<sup>-1</sup> (Van der Ent et al. 2013a). To avoid sample bias, all observed plant species in a large number ( $n = 93$ ) of plots (ranging in size from 100 × 20 m to 10 × 25 m depending on elevation, for details refer to Van der Ent et al. 2016) were tested in this manner, spanning an altitude range from 400 to 2950 m asl and thereby testing more than 10,000 plant specimens. Leaf samples were collected from all 'positive' colour reactions in the field, and the samples subsequently analysed with ICP-AES after acid digestion in the laboratory. The results of foliar analyses of the Ni hyperaccumulator species we found are described elsewhere (Van der Ent et al. 2013c, 2015c; Van der Ent and Mulligan 2015). The data in this study are from a total of 18 different Ni hyperaccumulator species, those described in Van der Ent et al. (2015c) excluding *Aporosa chalarocarpa*, *Baccaurea lanceolata*, *Cleistanthus* sp. 1, *Glochidion brunneum*, *Ptyssiglottis* cf. *fusca* and *Shorea tenuiramulosa*, which are species distributed elsewhere in Sabah. The aforesaid mentioned publication provides a detailed ecological account on the Ni hyperaccumulator species from Sabah that also form the subject of this study.

Soil samples were collected near the roots of the 18 different Ni hyperaccumulator species, and as all these species were trees or (large) woody shrubs, the soil samples were collected approximately 1 m from the stem in the mineral soil (5–20 cm depth). As such, the soil samples comprise 'rooting zone soil' and not actual 'rhizosphere soil' (the latter defined as soil directly attached and

surrounding the roots). In order to be able to subsequently compare these 'hyperaccumulator-soils', further samples were also collected from the 93 plots ( $n = 3$  per plot) in the same area and from the vicinity of these plots if no Ni hyperaccumulators were locally found. All samples were categorised as soils with no hyperaccumulators ('NON-HYP'  $n = 273$ ), or to originate from soils with hyperaccumulators present ('HYP'  $n = 63$ ). The soil samples were air-dried at room temperature to constant weight (2–3 weeks), sieved to <2 mm, shipped to Australia, and analysed at the University of Queensland after gamma irradiation for release from quarantine.

### Digestion and extraction based soil analysis

Soil sub-samples (0.3 g) were digested using freshly prepared 'reverse' Aqua Regia (9 mL 70 % nitric acid and 3 mL 37 % hydrochloric acid per sample) in a digestion microwave (Milestone Start D) for a program of 1.5 h, and diluted to 45 mL with ultra-pure water before analysis to obtain pseudo-total elemental concentrations (hereafter referred to 'total' concentrations). Additionally, soil pH and electrical conductivity (EC) was obtained in a 1:2.5 soil to water mixture following Rayment and Higginson (1992). Exchangeable cations (Al, Ca, Mg, K, Na) were extracted with silver-thiourea (Dohrmann 2006) over 16 h. Exchangeable trace elements (Ni, Co, Cr and Mn) were extracted in 0.1 M Sr(NO<sub>3</sub>)<sub>2</sub> at a soil:solution ratio of 1:4 (10 g soil with 40 mL solution) and 2 h shaking time (adapted from Kukier and Chaney 2001). As a means of estimating potentially phytoavailable trace elements, the DTPA-extractant was used according to Becquer et al. (1995), which was adapted from the original method by Lindsay and Norvell (1978), by the following modifications: excluding TEA, adjusted to pH 5.3, 5 gram soil with 20 mL extractant, and extraction time of 2 h. Another method for estimating potentially phytoavailable trace elements used a mixture of carboxylic acids (acetic, malic and citrate acid in molar ratio of 1:2:2 at 0.01 M) at a soil to solution ratio of 1:4 (10 gram soil with 40 mL solution) and 2 h shaking time (loosely based on Feng et al. 2005). Nickel partitioning over soil phases was evaluated with a five-step selective sequential extraction scheme. This scheme is based on Quantin et al. (2002), which in turn was modified mainly from Leleyter and Probst (1999). Adaptations were made here by combining step 1 and step 2, and by using HNO<sub>3</sub>/HF high-pressure microwave digests for the residual fraction (step 5) instead of an alkaline fusion as in Quantin et al. (2002). The fractions were: water soluble and exchangeable (1), bound to Mn oxides (2), bound to amorphous Fe oxides (3), bound to crystalline Fe oxides (4), and residual (5). After each extraction step, the tubes were centrifuged for 10 min at 4000 rpm and the

supernatants were then filtered through 0.45  $\mu\text{M}$  syringe-filters. The residues were washed with 20 mL of ultra-pure water, centrifuged again for 10 min at 4000 rpm, the water decanted, and the residue dried at 40  $^{\circ}\text{C}$  prior to the next extraction step.

All soil samples were analysed with ICP-AES (Varian Vista Pro II) for aluminium, calcium, chromium, cobalt, copper, iron, magnesium, manganese, nickel, potassium, phosphorus, sodium, sulfur and zinc. The quality controls included National Institute of Standards and Technology (NIST) and Australasian Soil and Plant Analysis Council (ASPAC) standards. The ICP-AES instrument was calibrated using a six-point multi-element standard prepared in each extraction solution.

### Hyperaccumulator soil transect and DGT experiment

In a second experiment, four soil samples were collected along a 1 m transect from an isolated individual of *Phyllanthus* cf. *securinegoides* at 5, 10, 50 and 100 cm from the main stem. Each sample was approximately 1 kg and collected from 10 to 20 cm depth, and kept field-moist until analysis. Sub-samples were air-dried and analysed for the same package of analytical methods as all other soil samples. In addition, DGT devices (Diffusive Gradient in Thin Films Technique) were deployed in the field-moist soils (after adding ultra-pure water to bring the samples to 100 % of water holding capacity, and equilibration for 48 h before deployment) at the same locations of the soil sample collection, as described above. The DGT devices (DGT Research Ltd, Lancaster, UK) consisted of a 0.91 mm diffusive layer with filter and a chelex-100 binding layer. The appropriate deployment time was calculated on the basis of 0.1 M  $\text{Sr}(\text{NO}_3)_2$  extractable Ni and was 4 h. After deployment, the binding gel was removed and eluted in 1 mL of 5 M  $\text{HNO}_3$ . After 48 h elution, 0.8 mL of the eluent was sampled, and brought to volume (5 mL) before analysis by ICP-MS. Pore water was also collected by centrifugation (4000 rpm at 10 min) of the wet soil matrix and filtering through 0.45  $\mu\text{M}$  syringe-filters. Finally, sub-samples were dried at 105  $^{\circ}\text{C}$  for 48 h to allow for moisture correction.

### Mineralogical analysis (XRD)

Mineral phases were identified in soil samples collected underneath four of the most common Ni hyperaccumulator plant species (*Rinorea bengalensis*, *Phyllanthus balgooyi*, *P.* cf. *securinegoides* and *Mischocarpus sundaicus*). In addition, mineral phases were also identified in bedrock samples collected under the same species (with the exception of *Mischocarpus sundaicus*). The analysis was

performed on finely ground ( $<100 \mu\text{m}$ ) soil powder on a Bruker D8 Advance X-Ray diffractometer with a copper target, diffracted-beam monochromator, and scintillation counter detector. The settings in the instrument were: 40 kv, 30 mA,  $3^{\circ}$ – $80^{\circ}$   $2\theta$ ,  $0.05^{\circ}$  step size or increment, with 10 s per step.

### Statistical analysis

The soil and rock chemistry data were analysed using the software package STATISTICA Version 9.0 (StatSoft), Excel for Mac version 2011 (Microsoft) and in Plymouth Routines In Multivariate Ecological Research (PRIMER, version 6) (Clarke and Gorley 2006). The DGT data were analysed with the 2D DIFS package (Sochaczewski et al. 2007), which is a dynamic numerical model developed for use in conjunction with DGT in soils to estimate the proportion of Ni in the solid phase. This includes the distribution ratio or partition coefficient ( $K_d$ ) with equilibration response times (TC) and ( $R_{\text{diff}}$ ).  $R_{\text{diff}}$  was then used to calculate the effective concentration (CE), which represents the Ni concentration that is available from the solution-phase and solid-phase (Harper et al. 1998). DGT-induced fluxes ( $\mu\text{g}/\text{h}/\text{m}^2$ ), effective concentrations (CE) and resupply rate (R) were measured by DGT/2D DFIS technique. The XRD data were analysed with DIFFRACplus Evaluation Search/Match Version 8.0 for mineral species identification using the International Centre for Diffraction Data's PDF-4/Minerals database.

## Results

### Soil chemistry of 'hyper-accumulator soils' versus 'non-hyperaccumulator soils'

Table 1 shows the chemistry of both hyperaccumulator and non-hyperaccumulator soils. Mean soil pH in non-hyperaccumulator soils was medium acidic at pH 5.7, whereas the mean pH of the hyperaccumulator soils was significantly ( $p < 0.001$ ) higher at pH 6.7. The electrical conductivity (EC) of the hyperaccumulator soils was also higher, indicating greater amounts of ions in the soil solution. Exchangeable Ca, Mg and K were all significantly ( $p < 0.001$ ) higher in the hyperaccumulator soils, particularly exchangeable Mg which was almost five-fold higher than in non-hyperaccumulator soils. In addition to Student's  $t$  tests for individual soil variables (Table 1) in hyperaccumulator and non-hyperaccumulator soils, the data were tested for significant differences in multivariate space (using all soil variables) between hyperaccumulator and non-hyperaccumulator soils (ANOSIM) and the sample statistic was  $R = 0.46$  at  $p < 0.001$ . This confirms that hyperaccumulator and

**Table 1** Mean (with standard error between brackets) elemental concentrations in hyperaccumulator ( $n = 63$ ) and non-hyperaccumulator ( $n = 273$ ) soils in  $\mu\text{g g}^{-1}$  or  $\text{mg g}^{-1}$  as indicated

Soil type	Al ( $\text{mg g}^{-1}$ )	Ca ( $\mu\text{g g}^{-1}$ )	Co ( $\mu\text{g g}^{-1}$ )	Cr ( $\mu\text{g g}^{-1}$ )	Fe ( $\text{mg g}^{-1}$ )
NON-HYP	18 [15]	1915 [220]	246 [15]	3907 [256]	155 [7]
HYP	21 [12]	4553 [721]	374 [30]	4510 [364]	147 [11]
<i>P</i> value	0.131	<0.001	<0.001	0.248	0.667
Soil type	Ca exch. ( $\mu\text{g g}^{-1}$ )	K exch. ( $\mu\text{g g}^{-1}$ )	Mg exch. ( $\mu\text{g g}^{-1}$ )	Ca CA ( $\mu\text{g g}^{-1}$ )	K CA ( $\mu\text{g g}^{-1}$ )
NON-HYP	230 [21]	33 [1.1]	500 [55]	114 [11]	23 [0.9]
HYP	1210 [171]	64 [7.6]	2075 [181]	643 [105]	50 [5.6]
<i>P</i> value	<0.001	<0.001	<0.001	<0.001	<0.001
Soil type	Mg CA ( $\mu\text{g g}^{-1}$ )	Ni ML-3 ( $\mu\text{g g}^{-1}$ )	Ni CA ( $\mu\text{g g}^{-1}$ )	Ni DTPA ( $\mu\text{g g}^{-1}$ )	Ni $\text{Sr}(\text{NO}_3)_2$ ( $\mu\text{g g}^{-1}$ )
NON-HYP	605 [56]	49 [4.3]	55 [4.6]	52 [3.6]	8 [0.6]
HYP	498 [33]	103 [7.7]	130 [15]	117 [9.9]	10 [1.4]
<i>P</i> value	0.366	<0.001	<0.001	<0.001	0.182
Soil type	pH				EC $\mu\text{S}$
NON-HYP	5.7 [0.1]				138 [6.2]
HYP	6.7 [0.1]				201 [17]
<i>P</i> value	<0.001				<0.001

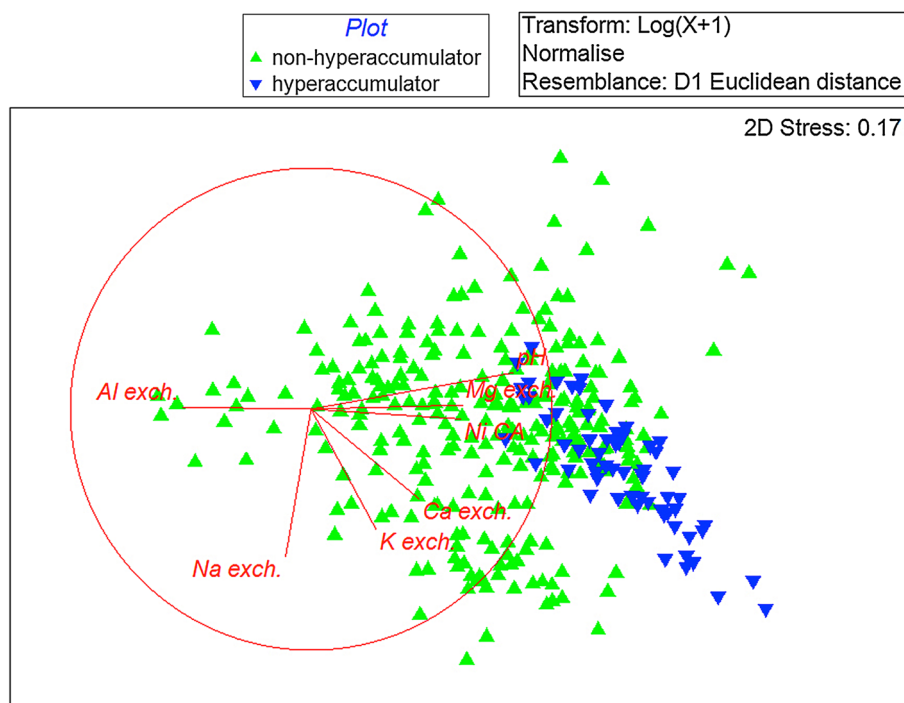
Total concentrations are the results of microwave-assisted digestion with  $\text{HNO}_3$  and  $\text{HCl}$ , 'ML-3' is Mehlich-3 extractable, 'DTPA' is DTPA-extractable, ' $\text{Sr}(\text{NO}_3)_2$ ' is extractable with dilute strontium nitrate solution and 'exch.' is exchangeable with silver-thioarea. *P* values are derived from a Student's *t* test with the two groups (HYP vs. NON-HYP)

non-hyperaccumulator soils are significantly different although hyperaccumulator soils are 'nested' within the broader non-hyperaccumulator soils.

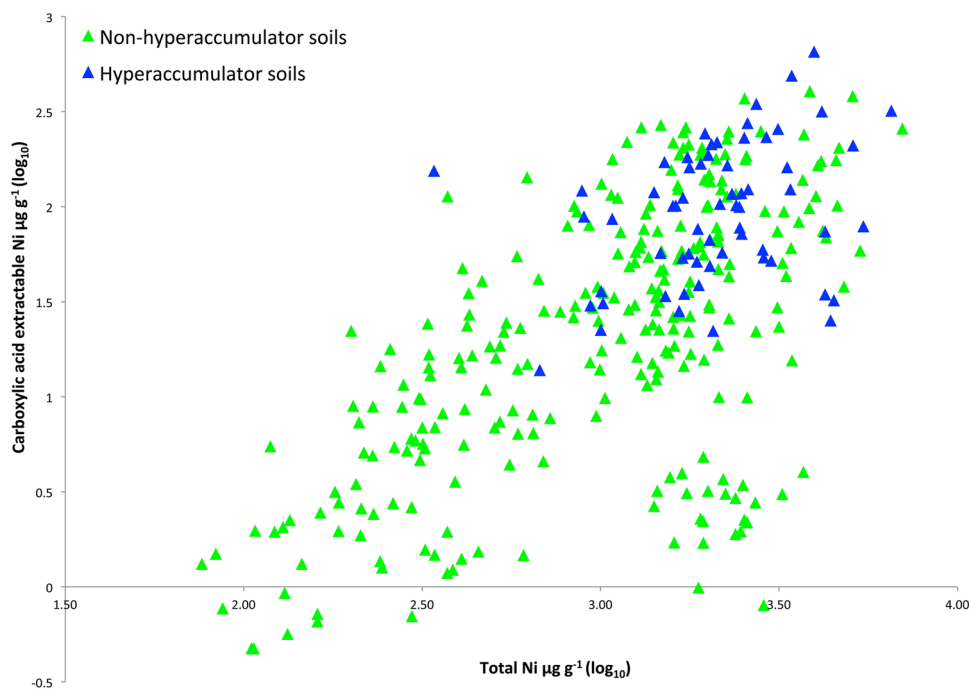
Total Fe and Cr concentrations were similar in both the hyperaccumulator and non-hyperaccumulator soils. Total Ni was nearly twofold as high in the hyperaccumulator soils, whereas phytoavailable Ni (DTPA, carboxylic acid, and Mehlich-3 extractable) were also significantly higher in the hyperaccumulator soils. The ratio total Ni: extractable Ni in DTPA, Mehlich-3 and carboxylic extracts (in hyperaccumulator soils) were similar (18–23) suggesting that these methods extract Ni from similar (labile) phases. Although the amounts of Ni extracted from non-hyperaccumulator soils were lower, the total Ni : extractable Ni ratios were only slightly higher (26–29). Figure 1 shows a PCA plot of soil chemical variables in hyperaccumulator and non-hyperaccumulator soils. Correlation coefficients between soil parameters (data not shown) showed significant ( $p < 0.01$ ) negative correlation between pH and exchangeable Al, and extractable Cr, Fe and exchangeable Ni, and a positive correlation between pH and Mg (total and exchangeable). Total Ni was positively correlated with exchangeable Ni (Fig. 2), whereas carboxylic acid extractable Ni was strongly correlated with extractable Mn.

Figure 3 shows the ( $\log_{10}$  transformed) carboxylic acid extractable Ni versus soil pH, which was positively correlated ( $r = 0.53$ ). The hyperaccumulator soils are colour-coded, and it is apparent from this figure that these soils occupied the section with the highest pH ( $>6.3$ ) and highest extractable Ni ( $>20 \mu\text{g g}^{-1}$ ) concentrations. Consequently, soil carboxylic acid extractable Ni versus soil exchangeable Al (in hyperaccumulator and non-hyperaccumulator soils) showed an opposite correlation than for pH (Fig. 4), which was expected due to the solubility properties of Al versus pH. These results are in contrast to Echevarria et al. (2006) who reported an inverse negative correlation ( $r^2 = 0.67$ ) between DTPA-extractable Ni and soil pH in a range of soils that included non-ultramafic soils. Although soil organic matter is known as an important Ni soil phase through the formation of organic-Ni-complexes (Nachtegaal and Sparks 2003; Li et al. 2003; McNear et al. 2007), the soil samples studied here were low in organic matter. The very low exchangeable Ni in the  $\text{Sr}(\text{NO}_3)_2$  extracts in hyperaccumulator soils ( $9.8 \mu\text{g g}^{-1}$ ), and the similar concentrations in non-hyperaccumulator soils ( $7.9 \mu\text{g g}^{-1}$ ), indicate that Ni was sorbed onto phases accessible to both DTPA and carboxylic acid extracts ( $117$  and  $130 \mu\text{g g}^{-1}$ , respectively) which were qualitatively and quantitatively different between hyperaccumulator and non-

**Fig. 1** Multi-factorial PCA plot of soil chemical variables (log transformed and normalised) in hyperaccumulator and non-hyperaccumulator soils. *exch* is exchangeable with silver-thiorea and CA are carboxylic acid extractable elements



**Fig. 2** Bivariate plot of soil total Ni ( $\log_{10} \mu\text{g g}^{-1}$ ) versus soil carboxylic acid extractable Ni ( $\log_{10} \mu\text{g g}^{-1}$ ) in hyperaccumulator and non-hyperaccumulator soils

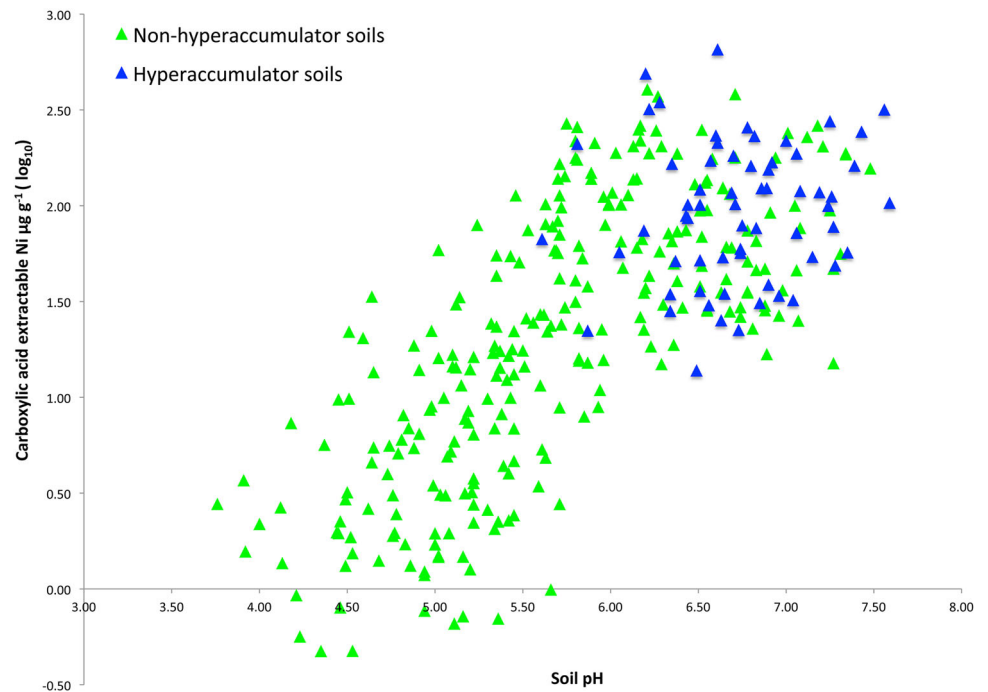


hyperaccumulator soils (see Table 1), most likely as a result of a different soil mineralogy.

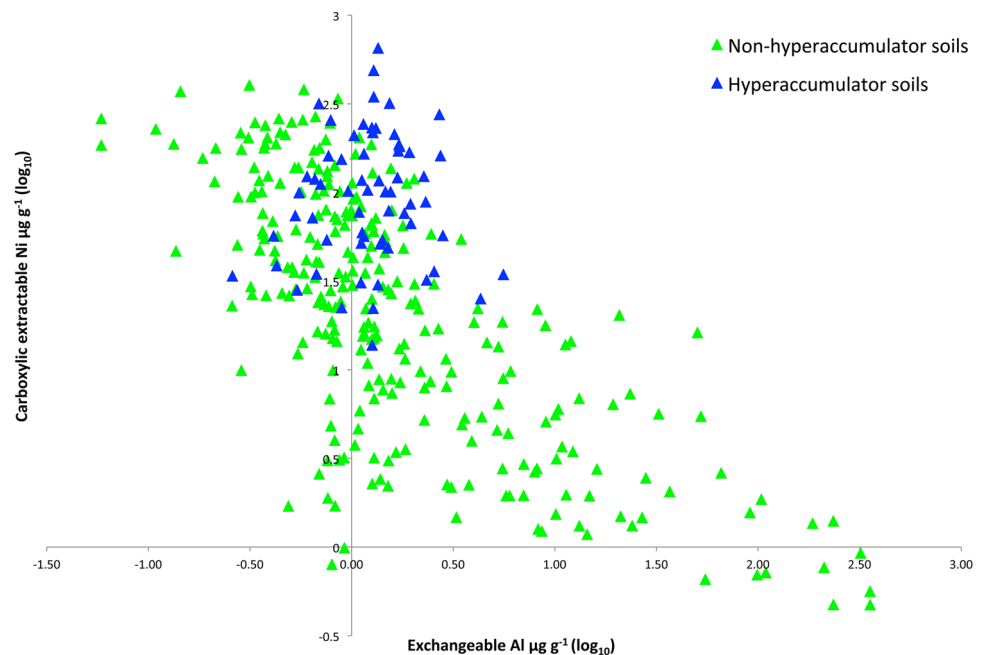
Carboxylic acid extractable Co gives the same response as Ni with extractable and total Mn. Clearly both Ni and Co are removed from the same Mn phases using this extraction, and this suggest that Mn-rich phases may control Ni

and Co availability in these soils. Although the hyperaccumulator soils contained significantly more total Co than non-hyperaccumulator soils (374 and 246  $\mu\text{g g}^{-1}$ , respectively), the exchangeable Co ( $\text{Sr}(\text{NO}_3)_2$  extract) was lower in the hyperaccumulator soils (0.5 and 1.9  $\mu\text{g g}^{-1}$ , respectively) and DTPA and carboxylic acid extractable Co

**Fig. 3** Bivariate plot of soil pH versus soil carboxylic acid extractable Ni ( $\log_{10} \mu\text{g g}^{-1}$ ) in hyperaccumulator and non-hyperaccumulator soils



**Fig. 4** Bivariate plot of soil carboxylic acid extractable Ni ( $\log_{10} \mu\text{g g}^{-1}$ ) versus soil exchangeable Al ( $\log_{10} \mu\text{g g}^{-1}$ ) in hyperaccumulator and non-hyperaccumulator soils



were not significantly different between hyperaccumulator and non-hyperaccumulator soils (Table 1).

### Sequential extraction and soil mineralogy

Minerals detected in the bedrock included antigorite, lizardite, chrysotile (serpentine minerals) and olivine/pyroxene solid-solution series (forsterite, augite). Nickel and Co

bearing phases were also identified (Ni silicate hydrate e.g. 'garnierite' and Co-Mg silicate). The soils (Table 2) were rich in serpentine minerals, such as antigorite and lizardite. Furthermore, they contained primary (diopside, fayalite, pargasite, enstatite) and secondary/weathering products (chrysotile, glagolevite). Complex Al-Mg silicates of the chlorite group (clinocllore, chamosite), and Fe-oxides (goethite and hematite) were also common.

**Table 2** Mineral phases as determined by XRD-analysis in selected hyperaccumulator rhizosphere soil samples (top table) and elemental concentrations in matching samples (bottom table)

Mineral	<i>Mischocarpus sundaicus</i> (Serinsim)	<i>Phyllanthus balgooyi</i> (Bukit Hampuan)	<i>Phyllanthus cf. securinegoides</i> (Nalumad)	<i>Rinorea bengalensis</i> (Nalumad)
Actinolite	–	–	+	–
Antigorite	+	–	+	+
Chamosite	–	–	+	–
Chrysotile	+	–	–	–
Clinochlore	–	–	+	+
Diopside	–	–	–	+
Enstatite	–	+	–	+
Fayalite	+	–	–	–
Forsterite	+	–	–	–
Gismondine	–	+	–	+
Glagolevite	–	–	–	+
Goethite	+	–	–	–
Hematite	+	–	–	–
Lizardite	–	–	–	+
Na–Al silicate	+	+	–	–
Pargasite	–	–	–	+
Quartz	–	+	–	–
Tremolite	–	–	+	–
Soil elemental concentrations ( $\mu\text{g g}^{-1}$ or $\text{mg g}^{-1}$ )				
Al*	6.2	35	20	20
Ca	540	702	1533	4693
Co	362	561	262	408
Cr	1983	6518	2491	5415
Cu	19	63	69	54
Fe*	148	151	130	91
K	144	39	25	142
Mg*	48	14	47	31
Mn*	4.4	7.3	3.6	5.5
Mo	4.5	4.8	4.9	4.2
Na	215	255	268	288
Ni	3401	1623	2863	2526
P	536	121	47	205
S	633	276	98	299
Zn	99	93	66	70

The + signs designates positive identification of mineral species. Total elemental concentrations in selected hyperaccumulator soil samples (in  $\mu\text{g g}^{-1}$  or  $\text{mg g}^{-1}$  for elements marked with an asterisk)

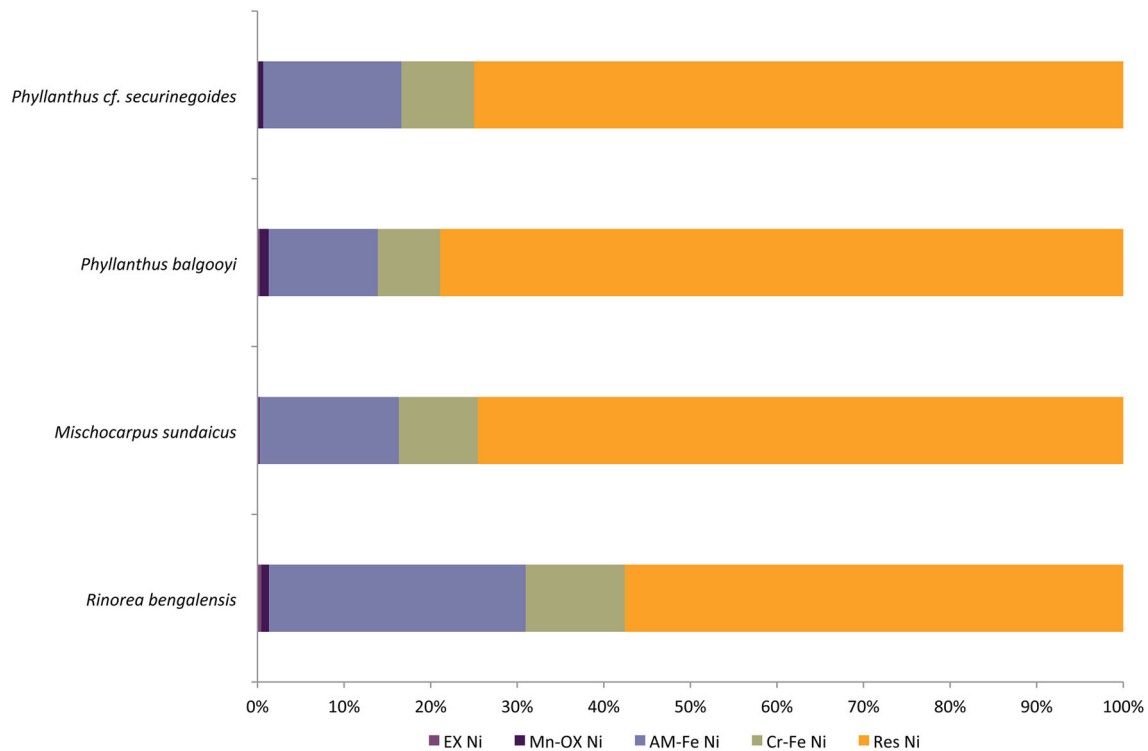
The Ni fractionation over mineral phases (Fig. 5) showed that exchangeable (EX) Ni concentrations are low, and that Mn-oxides (Mn-OX) were also a small fraction. The most important fraction for Ni were amorphous Fe-oxides (AM-Fe) that hosted up to 30 % of total concentrations present in the soil, which is known to be highly labile (Massoura et al. 2006). Crystalline Fe-oxides (CR-Fe) contained an additional <10 % Ni. The residual fraction accounted for 60–70 % of the total Ni. The large

amounts of Ni-scavenging Fe-oxides are clearly important for the Ni phytoavailability in these soils.

#### Soil samples in a distance gradient from a hyperaccumulator plant

Soil samples collected in the rhizosphere of the Ni hyperaccumulator *Phyllanthus cf. securinegoides* (2 m tall individual separated from other hyperaccumulators) in





**Fig. 5** Fractions of Ni bearing phases using a selective extraction scheme. The phases are: (1) water soluble and exchangeable (EX); (2) bound to Mn-oxides (Mn-OX); (3) bound to amorphous Fe-oxides (AM-Fe); (4) bound to crystalline Fe-oxides (CR-Fe); and (5) residual (RES)

increments of 5, 10, 50 and 100 cm from the main stem, showed no change in the soil pH. There were, however, much lower concentrations of extractable Co (and a slight decrease in total Co), lower total and extractable K, an increase in total and extractable Fe and Mn, and a decrease in total Ni further from the stem (Table 3). There was also a slight decrease of total Al (but not in extractable Al), and a decrease in total and extractable Ca further from the stem. A preliminary experiment with DGT's (Diffusive Gradient in Thin Films Technique) was carried out to assess Ni labile pools and resupply from mineral phases in this same gradient. The results (Table 4) showed that the most labile Ni ('R' value) was found closer to the plant (suggesting a Ni-flux towards the plant roots) but the pattern of DGT-induced fluxes was not clear due to the unexplained value at 10 cm. The overall results are similar to those obtained by Baker et al. (1992) for *Phyllanthus balgooyi*, a related species that also occurs in Sabah. Their results showed increased exchangeable K, Ca and Mg, and increased extractable Ni closer to the plant, but also a very moderate increase in pH. In either case, increased mineral weathering in the rhizosphere as a result of root exudates, as demonstrated

with another Ni hyperaccumulator (Wenzel et al. 2003), was not clearly evident here.

## Discussion

Nickel-DTPA has been shown to be correlated with medium-term isotopically-exchangeable Ni ('E-value'), from which hyperaccumulators (and non-accumulators) obtain Ni (Echevarria et al. 2006; Chardot et al. 2007). The low concentrations of exchangeable Ni (0.4 % of mean total soil Ni) in the hyperaccumulator soils were not remarkable. The carboxylic acid extractant, which consists of acetic, citric, and malic acid, could act as a proxy for Ni-complexes in ultramafic soils. Soil Ni sorption to smectite clay (montmorillonite) in the presence of low concentrations of citrate is reduced by 50–90 % (Poulsen and Hansen 2000; Marcussen et al. 2009) with the minimum sorption at pH 6.6. Further, Ni retention and the formation of Ni-LDH phases are reduced under the influence of citrate because of the formation of Al-citrate complexes thus limiting co-precipitation of Al with Ni hydroxide to form LDH-phases (Yamaguchi et al. 2002). Citrate and malate are the

**Table 3** Rhizosphere soil concentrations between the roots of *Phyllanthus cf. securinegoides* with the incremental distance from main stem indicated in cm (units as indicated)

Parameter	Extraction method	5 cm	10 cm	50 cm	100 cm
pH	1:2.5 H <sub>2</sub> O	6.5	6.4	6.5	6.4
EC	1:2.5 H <sub>2</sub> O (μS)	121	81	115	146
Al	Total (mg g <sup>-1</sup> )	19	19	25	29
Al	Carbox. extract (μg g <sup>-1</sup> )	706	824	648	728
Al	Sr(NO <sub>3</sub> ) <sub>2</sub> extract (μg g <sup>-1</sup> )	0.3	0.4	0.3	0.4
Ca	Total (μg g <sup>-1</sup> )	944	1261	1045	3343
Ca	Carbox. extract (μg g <sup>-1</sup> )	151	83	72	191
Ca	Sr(NO <sub>3</sub> ) <sub>2</sub> extract (μg g <sup>-1</sup> )	224	224	225	487
Co	Total (μg g <sup>-1</sup> )	246	248	341	306
Co	Carbox. extract (μg g <sup>-1</sup> )	3.5	6.7	14	28
Co	Sr(NO <sub>3</sub> ) <sub>2</sub> extract (μg g <sup>-1</sup> )	0.01	0.01	0.11	0.02
K	Total (μg g <sup>-1</sup> )	25	25	22	85
K	Carbox. extract (μg g <sup>-1</sup> )	3.3	4.9	3.4	20
K	Sr(NO <sub>3</sub> ) <sub>2</sub> extract (μg g <sup>-1</sup> )	4.7	6.4	4.5	30
Mg	Total (mg g <sup>-1</sup> )	54	62	37	41
Mg	Carbox. extract (μg g <sup>-1</sup> )	809	858	698	972
Mg	Sr(NO <sub>3</sub> ) <sub>2</sub> extract (μg g <sup>-1</sup> )	2041	1999	1881	1874
Mn	Total (μg g <sup>-1</sup> )	3417	3297	4816	4089
Mn	Carbox. extract (μg g <sup>-1</sup> )	49	77	116	249
Mn	Sr(NO <sub>3</sub> ) <sub>2</sub> extract (μg g <sup>-1</sup> )	3.8	2.4	4.4	0.8
Ni	Total (μg g <sup>-1</sup> )	3657	3658	2969	2131
Ni	Carbox. extract (μg g <sup>-1</sup> )	43	56	49	74
Ni	Sr(NO <sub>3</sub> ) <sub>2</sub> extract. (μg g <sup>-1</sup> )	9.3	7.6	8.8	2.5

Total concentrations were obtained with microwave-assisted digestion using HNO<sub>3</sub> and HCl, 'carbox.' means carboxylic acid extractable elements, 'DTPA' means DTPA-extractable elements, 'Sr(NO<sub>3</sub>)<sub>2</sub>' means extractable with strontium nitrate solution

**Table 4** DGT-induced Ni fluxes; solid phase buffering ratio ( $R_{diff}$ ) and effective concentrations (CE) of Ni in soils measured by DGT and simulated by 2D DFIS model, in a gradient from the root zone of *Phyllanthus cf. securinegoides*

Location (cm)	Particle concentration (Pc)	Soil porosity ( $\phi$ )	$R$	$R_{diff}$	$C_{DGT}$ (μg L <sup>-1</sup> )	$C_{SOL}$ (μg L <sup>-1</sup> )	$C_E$ (μg L <sup>-1</sup> )	DGT-induced fluxes (μg h m <sup>2</sup> <sup>-1</sup> )
5	1.67	0.61	0.97	0.12	285	293	2415	745
10	1.47	0.64	0.87	0.12	24	28	202	85
50	1.58	0.63	0.38	0.12	241	633	2011	621
100	1.7	0.61	0.13	0.13	29	224	234	96

$C_{DGT}$  Labile concentrations of Ni in situ measured by DGT device

$C_{SOL}$  Pore water concentrations of Ni

$R$  The ratio of the mean interfacial concentration ( $C_{DGT}$ ) and bulk solution concentration ( $C_{SOL}$ )

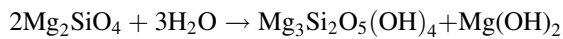
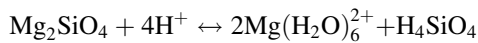
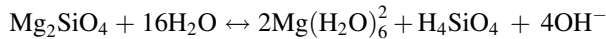
$C_E$  The potential elemental concentration that is effectively available from the solution-phase and solid-phase liable pool ( $C_E = C_{DGT}/R_{diff}$ ).  $R_{diff}$  is the ratio of the mean interfacial concentration due to resupply by diffusion ( $C_{diff}$ ) only to the initial or bulk concentration ( $C_{SOL}$ ) and is determined by the geometry of the DGT unit, deployment time and sediment tortuosities using the 2D DFIS model

Particle concentration (Pc) and Soil porosity ( $\phi$ s) are calculated using 2D DFIS model based on the moisture content during the DGT deployment

predominant Ni-binding ligands in tropical (Perrier et al. 2006; Callahan et al. 2006, 2008, 2012) and temperate (Tappero et al. 2006; Montargès-Pelletier et al. 2008; McNear et al. 2010) Ni hyperaccumulators, and the release of these carboxylic acids by hyperaccumulator roots could

also be highly effective in mobilizing of Ni for uptake. The relatively high concentrations of K, Ca and Mg in the hyperaccumulator soils are indicative of active mineral weathering and strong biogeochemical recycling. Dissolution of serpentine minerals releases OH<sup>-</sup> ions and hence

results in an increase in the soil pH (Chardot-Jacques et al. 2013), according to the following (very simplified reactions):



Weathering and dissolution of serpentine minerals thus results in a massive release of Mg (and consequently Ni included in the mineral matrix), followed by the formation of secondary silicates that can include Ni and Al layered double hydroxide (Ni–Al LDH) and Ni phyllosilicates (Scheckel and Sparks 2001; Basta et al. 2005). As alluded earlier, the amount of exchangeable Al is rate-limiting in the formation of Ni-LDH phases. The results of Fig. 4 appear to indicate formation of LDH phases because of a negative correlation in exchangeable Al and extractable Ni. The formation of relatively insoluble LDH phase complexes when exchangeable Al is high could account for Ni scavenging. A complicating factor, however, is that higher exchangeable Al in soils is linked to higher acidity (pH < 5.2), and because hyperaccumulators were found on more alkaline soils, exchangeable Al is inherently low under these conditions. The release of OH<sup>−</sup> combined with high Mg results in an increase in the soil pH and buffering capacity. The relationship between high Ni and high Ca in the studied hyperaccumulators is interesting as it has previously been reported from experimental work that low Ca increases Ni phytotoxicity, and high Ca (and Mg) ameliorate Ni-toxicity (Gabbrielli and Pandolfini 1984; Robertson 1985; Heikal et al. 1989; Chaney et al. 2008), through the reduction of ion activity (Becquer et al. 2010) and by competition for Ni adsorption on soil sorption sites. This could explain the high phytoavailability of Ni, and hence potential uptake, in these soils. Hydroponic experiments demonstrated that increasing Ni in the nutrient solution decreased Ca uptake in the hyperaccumulator *Berkheya coddii* (Robinson et al. 2003), indicating that Ca competes with Ni during uptake in the root. This is further exemplified by a positive correlation between foliar Ca and Ni in *Rinorea bengalensis* (Van der Ent and Mulligan 2015).

It is clear that hyperaccumulators deplete exchangeable (labile) pools of Ni in the soil, which affects the chemical equilibrium of Ni over labile and non-labile compartments in the soil (Wenzel et al. 2003; Centofanti et al. 2012). Experiments have shown that Ni hyperaccumulators extract less Ni from Ni-bearing chrysotile (low labile form) compared to Ni-bearing smectite (high labile form) (Montarges-Pelletier et al. 2008). This finds support in other experimental work that showed that Ni uptake in a hyperaccumulator plant is correlated with mineral Ni

solubility and the transpiration rate of the plant, with the most soluble minerals resulting in the highest plant Ni uptake. In contrast Ni<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>, Ni-phyllosilicate and Ni-acid-birnessite, which have low solubility, but are nevertheless highly taken up with high transpiration rates for hyperaccumulator plants (Centofanti et al. 2012). In addition, further experimental work showed that foliar Ni accumulation in a hyperaccumulator often increased as soil pH increased (Li et al. 2003; Kukier et al. 2004; Everhart et al. 2006) although there are some exceptions. This trend is opposed to the response of non-accumulator plants (L'Huillier and Edighoffer 1996; Kukier and Chaney 2004) and opposite to general metal availability responses to pH. This might be explained by (1) increased Ni binding to organic ligands, (2) enhanced pH-dependant sorption onto CEC; or (3) surface complexation to Fe-oxides which are all pools that can supply hyperaccumulators through reversible desorption because of the strong depletion of soluble Ni in the immediate vicinity of the root surface. The fact that Ni phytoavailability (labile pool) for the hyperaccumulator soils in this study was higher than for the non-hyperaccumulators, while concomitantly also having a higher pH, does not mean that Ni availability as such increases with increasing pH, but rather indicates different Ni-mineral forms in these soils. Experiments in which ultramafic soils were made either more acidic or more alkaline demonstrated unequivocally that the extractable and exchangeable fraction of soil Ni decreased with increasing pH (Crooke 1956; Bisessar 1989; Robinson et al. 1999; Kukier and Chaney 2001; Kukier et al. 2004; Everhart et al. 2006). This is also indicated by the negative correlation between pH and foliar Ni (unpublished data) in non-hyperaccumulator plants from the study area.

The behaviour of Ni hyperaccumulators might be explained in the context of the 'Excluder', 'Bioindicator' and 'Hyperaccumulator' responses to soil metal concentrations (Baker 1981; Van der Ent et al. 2013a). The Excluder remains with low shoot concentrations over a wide range of soil metal concentrations, the Bioindicator has a linear response in shoot concentrations in relation to soil metal concentrations, whereas Hyperaccumulators preferentially uptake metals from a wide range of soil concentrations. Below phytotoxicity thresholds, the rates of Ni uptake and translocation in a Ni hyperaccumulator and non-accumulator were shown to be similar, but above that threshold, hyperaccumulators quickly reach a plateau for Ni concentrations in their shoots (Krämer et al. 1997). It appears from Fig. 2 that the response of Ni hyperaccumulators to soil conditions is that of a 'threshold' with minimum concentrations of soil carboxylic extractable Ni around 20 µg g<sup>−1</sup> (or 630 µg g<sup>−1</sup> total Ni) and a minimum soil pH of approximately pH 6.3. Essentially, this suggests a sober mode of Ni accumulation that can occur when

sufficient phytoavailable Ni is present in the soil, and might be the result of a steady state of highly effective Ni uptake mechanisms on one hand (e.g. uptake, translocation) and continuous supply of rhizosphere Ni through release from Ni-bearing phases (which appears intrinsically high in the hyperaccumulator soils). It should be noted that foliar Ni and soil (extractable) Ni concentrations are only very weakly correlated (Van der Ent et al. 2015b) and this may be explained because above the soil Ni-supply threshold the foliar concentrations in Ni hyperaccumulators follow the flat 'saturation' end of a response curve.

The ecophysiological and biogeochemical mechanisms that tropical hyperaccumulator plants employ for achieving exceptional levels of Ni accumulation in their shoots are still largely unravelled. Two general (not mutually exclusive) hypotheses might be put forward to explain the extraordinary levels of accumulation of Ni in hyperaccumulator plants:

1. Hyperaccumulators excrete root exudates, such as carboxylic acids, perhaps in higher amounts than normal plants, thereby increasing Ni phytoavailability through intense rhizosphere mineral weathering;
2. Hyperaccumulators have extremely high Ni uptake efficiency, which, combined with the very large total surface area of the roots, severely deplete Ni ions in the soil moisture thereby stimulating re-supply of Ni from diffusion from labile Ni pools.

With regards to hypothesis (1), Chardot-Jacques et al. (2013) showed experimentally that a hyperaccumulator increased the rate of dissolution of a Ni-bearing serpentine mineral (with low labile Ni) by more than twofold, and this hyperaccumulator accumulated up to 88 % of the total amount of Ni released from the mineral phase. Other experimental work demonstrated that rhizosphere bacteria could increase Ni uptake in a hyperaccumulator by up to 32 % (Abou-Shanab et al. 2003). Furthermore, a field study by Wenzel et al. (2003) showed significantly higher soluble Ni in the rhizosphere soil of a hyperaccumulator compared to non-hyperaccumulator soils, which was attributed to higher concentrations of root exudates in the rhizosphere of the hyperaccumulator (causing organic ligand-promoted dissolution of Ni from mineral phases and displacement of Ni from the exchange complex). Wenzel et al. (2003), found a highly significant correlation between Ni and dissolved organic carbon (DOC) in the hyperaccumulator rhizosphere soil solution. But subsequent rhizobox experiments (Puschenreiter et al. 2005), although confirming increased soluble Ni, found only a weak correlation between Ni and DOC. Enhanced mineral dissolution in the rhizosphere of the hyperaccumulator was indicated in these studies by concomitantly (1) higher concentrations of soluble Ca, Mg and K, (2) correlation of these elements with Ni, and (3) a

higher soil pH. These findings closely match those of the present study, but crucially, the study by Wenzel et al. (2003) compared hyperaccumulator and non-hyperaccumulator rhizosphere soils in the same habitat (spatially separated by <math><1\text{ m}^2</math>), whereas in the current study the hyperaccumulator and non-hyperaccumulator soils that were compared were collected from an area >700 km<sup>2</sup>. Moreover, in contrast to Wenzel et al. (2003), the soils in the present study are not 'rhizosphere soil' and the tropical Ni hyperaccumulators studied here are all large trees, and not small herbs. Although it is clear from the previously mentioned studies that Ni hyperaccumulators can increase mineral dissolution in the rhizosphere, the present study shows that hyperaccumulators preferentially grow in soils that have highly active mineral weathering. Evidently, active mineral weathering of the bulk soil releases far more Ni than exudate-induced mineral weathering on the root-interface alone. Hence root exudates might not be important for increasing soluble Ni in soils with intrinsically high labile Ni due to active mineral weathering. Rather, root exudates might increase soluble Ni by mineral weathering in soils with moderate or low phytoavailable Ni. Any acidification by the exudation of citrate or other carboxylic acids in serpentine mineral rich soils will also be masked by continuous supply of OH<sup>-</sup> in the soil through mineral weathering of serpentine minerals (Chardot-Jacques et al. 2013).

With regards to hypothesis (2), experimental work has shown that non-accumulators and hyperaccumulators access the same soil labile Ni pool regardless of the amount of Ni uptake in the plants (Massoura et al. 2004). This argues against any significant rhizosphere effect of exudates. Direct evidence for a role of root exudates or root-induced acidification in relation to hyperaccumulation has not been found in experimental work (Bernal and McGrath 1994; Bernal et al. 1994; McGrath et al. 1997; Salt et al. 2000; Zhao et al. 2001; Li et al. 2003). Rather, the roots of hyperaccumulators may be considered highly effective 'Ni diffusion sinks'. Nickel diffusion depends largely on the speciation of Ni-bearing minerals in the soils, and the actual diffusion rate can be measured with Diffusive Gradients in Thin-films (DGT). The diffusion process of Ni from other Ni pools (under the same 'depletion gradient') is mainly controlled by (1) quality of the Ni pools e.g. chemical forms of Ni and the Ni-bearing mineral phases; (2) quantity/size of these different pools; (3) chemical weathering processes stimulating the release of Ni over the diffusion gradient; and (4) speed of re-supply as a result of all of these processes.

## Conclusions

The main aim of this study was to determine whether the soil chemistry of Ni hyperaccumulators in their natural habitat has certain characteristics that somehow determines their

occurrence. The results showed that Ni hyperaccumulators are absent from acidic soils (<pH 6.3) and consistently occur on soils with relatively high phytoavailable concentrations of Ca, Mg and Ni. The types of soils on which Ni hyperaccumulator plants occur are all derived from strongly serpentinised ultramafic bedrock on shallow young (moderately weathered) soils with pH > 6.3. In such soils (hypermagnesian Cambisols), the most important Ni-bearing phases for Ni are likely hydrous and crystalline ferrous oxides and smectite minerals in which Ni is either sorbed or included in the crystal lattice. Because Ni–Al layered double hydroxide (LDH) can form at pH > 6.25–6.5 (Elzinga and Sparks 2001; Shi et al. 2012), LDH might also be an important Ni phases in these soils. However, the sequential extraction method used in this study could not differentiate between such phases. Rather, it showed large amounts of neogene amorphous and crystalline Fe-oxides acting as ‘Ni-sinks’. The exchangeability on such complexes is the likely cause of high Ni phytoavailability in these soils. It is unknown whether the indirect mechanisms of re-supply of Ni by diffusion from non-labile pools over time with a continuous depletion of soluble Ni by uptake are enough to explain hyperaccumulation. This research showed that the specific Ni and cation chemistry of ultramafic soils is important for the distribution and occurrence of Ni hyperaccumulator plants. This does not, however, clarify whether hyperaccumulation depends on highly labile Ni pools associated with these soils, or on easily weathered non-labile pools. Anomalies in the anatomical features relating to the Casparian bands in the root ultra-structure of a Ni hyperaccumulator plant have been implicated in enhanced Ni uptake (Mesjasz-Przybyłowicz et al. 2007), whereas an exceptionally low reflection coefficient for Ni on the root interface was determined in another species (Coinchelin et al. 2012).

The correlation of high total Ni with the occurrence of populations of hyperaccumulators effectively renders hyperaccumulator plants as ‘indicators’ of surface Ni ore reserves in these lithosequences (see Fig. 5). Sites where hyperaccumulator plants were found often had an abundance in individuals with a range of different Ni hyperaccumulator species co-occurring together. This observation, combined with the soil chemical data presented in this study, is suggestive of a specific edaphic requirement of Ni hyperaccumulators for circum-neutral soils with high labile Ni.

Understanding of the uptake processes of Ni at the soil-root interface is critical for successfully developing Ni phytomining because it is fundamental to optimize ‘metal yield’. The speed and amount of addition of phytoavailable Ni from the non-phytoavailable pools due to depletion of soil Ni in phytomining is an important factor to consider in feasibility studies (Van der Ent et al. 2013b, 2015a). Furthermore, the specificity to particular soil chemistry also has consequences

for determining in advance which areas might host Ni hyperaccumulators in a mine lease, and for selecting those soils potentially suitable for future phytomining operations. As such, deep Fe-oxide rich limonite materials (Ferralsols) are unlikely to be suitable for phytomining, but younger Cambisols and ‘saprolite’ materials could be promising. Experimental work is needed in which tropical Ni hyperaccumulator species are grown in soils amended with natural or synthetic (ultramafic) Ni-bearing minerals combined with isotopic exchange kinetics (IEK) (Chardot et al. 2005; Echevarria et al. 1998, 2006) and/or the use of Ni stable isotopes (Estrade et al. 2015) to determine Ni uptake fluxes and rates of Ni depletion from mineral phases. A detailed understanding of these processes will provide critical insights in the limitations of the soils that may be suitable for phytomining, and ultimately permit for manipulations that increase phytomining yields in a given ‘metal crop’ by targeted soil amendments.

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**Author contributions** AVDE and MT conceived and designed the field study and analytical procedures. AVDE conducted the experiments. AVDE and GE analysed the data. AVDE, MT and GE wrote the manuscript. All authors read and approved the manuscript.

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