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Ecology of nickel hyperaccumulator plants from ultramafic soils in Sabah (Malaysia)

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Abstract Sabah (Malaysia) has one of the largest surface expressions of ultramafic rocks on Earth and in parallel hosts one of the most species-rich floras. Despite the extensive knowledge of the botanical diversity and the chemistry of these substrates, until recently the records for nickel (Ni) hyperaccumulator plants in the area have been scant. Recent intensive screening has resulted in 19 new records, adding to the 5 previously known from Sabah. The results of this study indicate that most Ni hyperaccumulator plants in Sabah are restricted to successional habitats (ridges, river banks, secondary vegetation) at elevations <1200 m a.s.l. Moreover, Ni hyperaccumulators are locally common both in terms of number of individuals and relative number of species. Nickel hyperaccumulation occurs most frequently in the Order Malpighiales (families Dichapetalaceae, Phyllanthaceae, Salicaceae, Violaceae), and is particularly common in the Phyllanthaceae (genera *Phyllanthus*, *Glochidion*). Comparison of soil chemistry with elements accumulated in hyperaccumulator foliage showed significant correlation between soil exchangeable Ca, K, P and the foliar concentrations of these elements. No direct relationship was found between soil Ni and foliar Ni, although foliar Ni was negatively correlated with soil pH. Nickel hyperaccumulation has been hypothesised to fulfil herbivory protection functions, but extensive herbivory-induced leaf damage on Ni hyperaccumulators in Sabah was common, and specialist (Ni-tolerant) insect herbivores were found on several species in this study. The identification of Ni hyperaccumulators is necessary to facilitate their conservation and potential future utilisation in Ni phytomining.

Keywords Allelopathy · Dimethylglyoxime · Elemental herbivory defense · Kinabalu Park

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Introduction

Ultramafic soils represent a category of substrates derived from ultramafic bedrock and are sparsely distributed around the world. Such soils are known for relatively high concentrations of potentially phytotoxic trace elements, including nickel (Ni), cobalt (Co) and chromium (Cr) while concomitantly having cation imbalances and general nutrient deficiencies (Brooks 1987; Proctor 2003). Of the trace elements enriched in ultramafic soils, Ni is a micronutrient that is essential for some plant species, and influences plant senescence, nitrogen metabolism, germination and plant disease resistance (Brown et al. 1987; Welch 1995). On ultramafic soils, however, this trace element can be phytotoxic and symptoms indicating excess can include leaf chlorosis and reduced growth (Brune and Dietz 1995; Kukier and Chaney 2001; Weng et al. 2003). Some plants restricted to ultramafic soils have evolved ecophysiological mechanisms to tolerate and accumulate Ni, and are termed Ni hyperaccumulators when having in excess of 1000 μg g^{-1} Ni in the foliage (Reeves 1992; Van der Ent et al. 2013a). The phenomenon is exceptionally rare and known in approximately 400 species worldwide in a range of different plant families. The Salicaceae,

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Buxaceae, Phyllanthaceae and Rubiaceae are the most common families for Ni hyperaccumulators in tropical regions (Reeves 2006). Nickel hyperaccumulators can be categorised into 'strict' and 'facultative' hyperaccumulators. Strict hyperaccumulators are exclusively confined to ultramafic soil and all populations of the particular species are hyperaccumulators. However, species that are 'facultative' hyperaccumulators have populations on ultramafic soils that are hyperaccumulators, and populations on other soils that are not (Pollard et al. 2014). An example of a facultative Ni hyperaccumulator which occurs in Sabah is *Rinorea bengalensis* (Violaceae), that has Ni concentrations of 1000–17,750 μg g^{-1} in the foliage of specimens of this species growing on ultramafic soils, and 1–300 μg g^{-1} in foliage of specimens of this species growing on non-ultramafic soils (Reeves 2003; Van der Ent et al. 2013a).

Hyperaccumulation is hypothesised to have evolved to interfere with other competing plant species ('elemental allelopathy'), or to protect against insect herbivores ('elemental herbivory defense'), although a variety of other explanations have also been suggested (Martens and Boyd 1994; Boyd and Jaffré 2001). The first hypothesis suggests that hyperaccumulators increase Ni concentrations in the soil area around the plant base ('phyto-enrichment') via leaf litter deposition as a result of leaf senescence and abscission which, as a result of toxicity effects, might reduce growth performance and germination of competing plant species (Boyd and Martens 1998). Given the inherent Ni-tolerance of hyperaccumulators, this might also provide advantages in survival of seedlings of the same hyperaccumulator species (or indeed, of different Ni hyperaccumulator species if these occur in the same habitat). The second hypothesis suggests that high foliar Ni concentrations protect against insect herbivores. As a consequence, Ni hyperaccumulators suffer less damage as a result of insect herbivory, and hence have competitive advantages. Further refinements of this model led to the formulation of the 'Defensive Enhancement Hypothesis', which proposes that after an initial defensive benefit resulting from relatively low initial foliar Ni concentrations, increased concentrations provided increased plant fitness, and led to a step-wise increase in foliar Ni accumulation (Boyd 2012). Furthermore, the 'Joint Effects Hypothesis' proposes that Ni accumulation in combination with organic chemicals (such as alkaloids) could have synergistic effects (Boyd 2012). In the context of 'elemental herbivory defense', foliar Ni accumulation has the distinct benefit of requiring limited energetic resources (although the uptake and transport physiology requires Ni complexing ligands such as citrate) because Ni is not produced but rather translocated from the soil and, contrary to organic molecules, Ni cannot be broken down or metabolised to avoid toxicity (Martens and Boyd, 1994; Boyd and Martens 1998). However, whereas the synthesis of organic toxic molecules by plants is relatively flexible in evolutionary terms, Ni accumulation is not (Cheruiyot et al. 2013).

The ultramafic soils of the Malaysian state of Sabah on the Island of Borneo are extensive, occupying a total area of about 3500 km^2 (Proctor et al. 1988) and is renowned for high species richness (Van der Ent et al. 2014). The flora of Sabah has an estimated 8000 vascular plant species (Wong 1992) with over 5000 plant species in the <1200 km^2 Kinabalu Park (Beaman 2005). Prior to this study, the following Ni hyperaccumulators were known to occur in Sabah: *Rinorea bengalensis* and *R. javanica* (Violaceae) (Brooks and Wither 1977; Brooks et al. 1977), *Phyllanthus balgooyi* (Phyllanthaceae) (Baker et al. 1992; Hoffmann et al. 2003), *Dichapetalum gelonioides* (Dichapetalaceae) (Baker et al. 1992), *Psychotria* cf. *gracilis* (Rubiaceae) (Reeves 2003) and *Shorea tenuiramulosa* (Dipterocarpaceae) (Proctor et al. 1989). The objective of this study was to screen the flora of ultramafic outcrops in Sabah, mainly Kinabalu Park, for the occurrence of (more) Ni hyperaccumulators. Further aims were to elucidate general phylogenetic patterns of Ni hyperaccumulation, habitat characteristics, overall plant-soil relationships and potential ecological interactions relating to herbivory and allelopathy.

Materials and methods

Study area and field collection

As part of a larger study on the relationships between plant diversity and soil chemistry of ultramafic outcrops at Mount Kinabalu and Mount Tambuyukon in Sabah, Malaysia, plants were screened for Ni hyperaccumulators (Van der Ent et al. 2013c). During the fieldwork on the ultramafic soils in Kinabalu Park (January 2011–September 2012), leaf samples were collected from all plants in plots (there were a total of 101 plots, ranging is size from 2000 to 250 m^2 and as many different plants as possible in the surrounding vegetation). In addition, plants were screened during fieldwork in the following Forest Reserves elsewhere in Sabah: Mount Tavai, Bidu-Bidu Hills, Mount Silam and Bukit Hampuan. Figure 1 shows a map of Sabah (Malaysia) with the study localities. In the field, the leaf samples were pressed against white test paper impregnated with the Ni-specific colorimetric reagent, dimethylglyoxime ('DMG'), which changes colour, to purple, upon contact with Ni. Approximately 5000 plant samples have been tested using this method. All samples that tested visually positive were re-collected (fully grown sun leaves, at least 2 m above the soil surface) by hand. Fresh plant leaves were put in paper bags to prevent decomposition

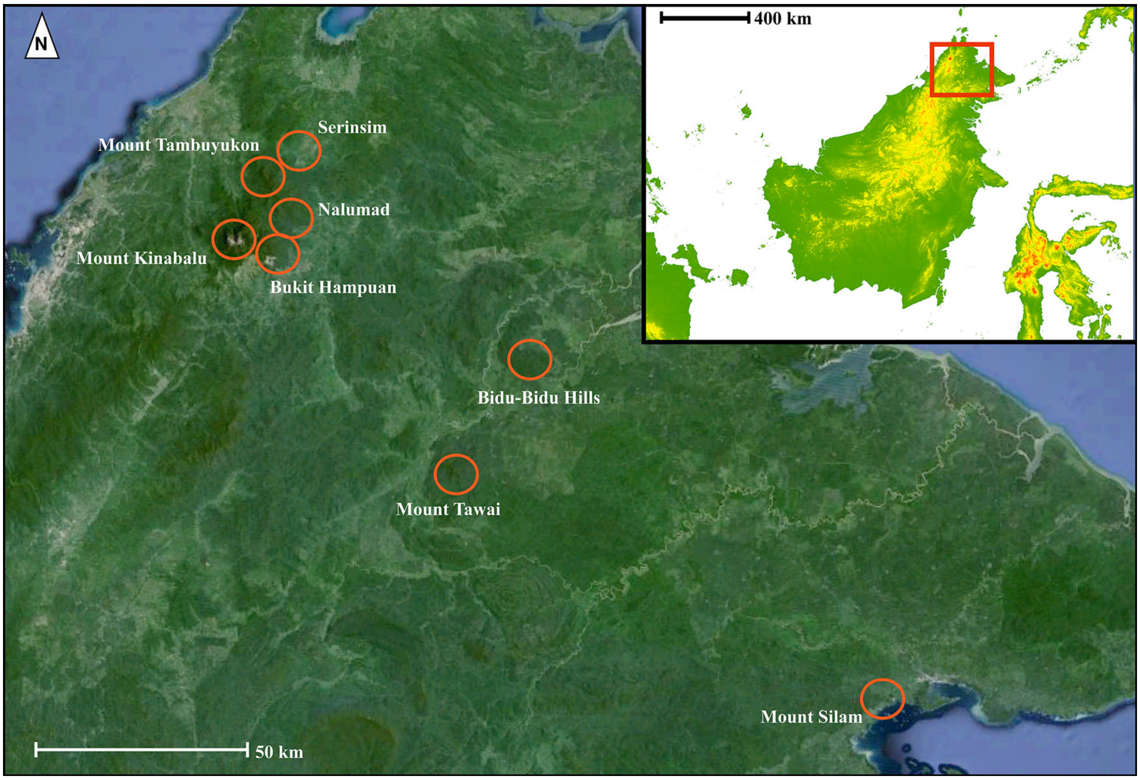

Fig. 1 Map of Sabah (Malaysia) with study localities. The *insert* shows the location of Sabah on the Island of Borneo. Satellite imagery is Landsat, from Google Earth with insert from ArcGIS (esri)

before transport to the field station. Leaves were thoroughly washed with demineralised water to remove potential dust contamination and then dried at 70 °C for 5 days in a dehydrating oven, packed for transport to Australia and gamma irradiated at Steritech Pty. Ltd. in Brisbane following Australian quarantine regulations. Soil samples (20–30 cm from base of the Ni hyperaccumulator plant, 10–20 cm deep) were also collected for analysis. Leaf litter samples were collected under Ni hyperaccumulator plants by carefully collecting partly decomposed leaves from a 1-m^2 area, ensuring that no mineral constituents were adhered to the collected material. In addition to new collections of potential Ni hyperaccumulators in the family Phyllanthaceae (specifically in the genera *Phyllanthus, Glochidion* and *Breynia*) collected from the field, examples from existing herbarium specimens held at the Sabah Parks Herbarium (SNP) Herbarium were also sourced. For each species of interest (220 specimens across 41 species), 50–100 mg samples were obtained and the original collection data associated with the specimens were recorded.

Chemical analyses of plant tissue samples

Foliar (and leaf litter) samples were crushed and ground, and a 300-mg subsample was digested in 4 mL concentrated nitric acid (70 %) and 1 mL hydrogen peroxide (30 %) in a microwave oven. The digest was diluted to 40 mL with TDI water before analysis with ICP-AES (Varian Vista Pro II). Elements included in the analysis were Al, Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, S and Zn. The potential for foliar contamination with soil particulates is a major risk for accurate analysis of foliar elemental composition. This risk is highest in samples of ground-herbs, and lesser so for trees, but it cannot be entirely avoided. Concomitantly high foliar concentrations of Fe (>2500 µg g^{-1}) and Cr (>50 µg g^{-1}) are an indication for soil contamination as these elements are major constituents of ultramafic soils.

Chemical analyses of soil samples

Soil samples (300 mg subsample) 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 for a 2-h programme and diluted with TDI water to 45 mL before analysis. The method followed Rayment and Higginson (1992) method 17B1 and results in 'pseudo-total' elemental concentrations. Soil pH and electrical conductivity (EC) were measured in a 1:2.5 soil:water mixture. Exchangeable Ni, Co, Cr and Mn were extracted in 0.1 M $Sr(NO_3)_2$ at a soil:solution ratio of 1:4 (10 g : 40 mL) and 2-h shaking time. Phytoavailable Ni, Co, Cr and Mn were extracted with diethylene triamine pentaacetic acid (DTPA) according to Lindsay and Norvell 1978, but with modifications from Bequer et al. (1995) (excluding TEA, buffered at pH 5.3). Another method for phytoavailable Ni, Co, Cr and Mn was also used, namely extraction with organic acids (acetic, malic and citrate acid in molar ratio of 1:2:2 at 0.01 M) at a soil:solution ratio of 1:4 (10 g : 40 mL) and 2-h shaking time. Exchangeable cations were extracted with silver-thiourea (Dohrmann 2006) over 16 h. All soil extractions were undertaken in 50-mL PP centrifuge tubes. Soil samples were weighed using a four-decimal balance and weights recorded for correction of the precise weights in the mass balance calculations. Samples were agitated for method-specific times using an end-over-end shaker at 60 rpm and subsequently centrifuged (10 min at 4000 rpm). The resultant supernatant was collected in 10-mL PP tubes. All soil samples were analysed with ICP-AES (Varian Vista Pro II) for Al, Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, S and Zn. The ICP-AES instrument was calibrated using a six-point multi-element standard prepared in each extraction solution.

Statistical analysis

The ranges and means of the foliar and soil elemental concentrations were calculated. Correlation coefficients between the soil and plant chemistry data were also calculated. These analyses were undertaken using the software packages STATISTICA Version 9.0 (StatSoft) and Excel for Mac version 2011 (Microsoft).

Results

Confirmation of Ni hyperaccumulator status and new discoveries

All previously known Ni hyperaccumulators from Sabah were located during the current field explorations of this study and chemical analysis of foliar samples re-confirmed their Ni hyperaccumulation status. Of these, *Phyllanthus balgooyi* is a widespread shrub often dominating open habitats on mountain ridges and along riverbanks, for example on Bukit Hampuan and in the Mount Tawai Forest Reserve. The climber *Psychotria* cf. *gracilis* (here identified as *P. sarmentosa* complex) is a common understory creeper of open forests. In contrast, the medium-sized trees *Rinorea bengalensis* and *R. javanica* (Violaceae) are comparatively rare. Another widespread, but rare, species, *Dichapetalum gelonioides* subsp. *sumatranum* and *D. gelonioides* subsp. *pilosum*, is a strong hyperaccumulator of zinc (Zn), but not of Ni on non-ultramafic soils, and vice versa on ultramafic soils. Finally, the tree *Shorea tenuiramulosa* (Dipterocarpaceae) is restricted to Mount Silam and the Meliau Range, and the only dipterocarp known to hyperaccumulate Ni (Proctor et al. 1989). In Table 1, newly discovered and confirmed Ni hyperaccumulators from Sabah are listed with their highest foliar Ni values. The strongest Ni hyperaccumulators are *Phyllanthus* cf. *securinegoides* (23,300 μg g^{-1}) and *Psychotria sarmentosa* complex (24,200 μg g^{-1}), whereas *Ptyssiglottis* cf. *fusca* (Acanthaceae) (1160 μg g^{-1}) and *Baccaurea lanceolata* (Phyllanthaceae) (1450 μg g^{-1}) are weak Ni hyperaccumulators, only just reaching the threshold foliar concentration that defines a Ni hyperaccumulator. Of some potential concern is the knowledge that fruits from *Bacaurea* species are frequently harvested and eaten. This is disconcerting in view of its Ni hyperaccumulation characteristic when growing on ultramafic soils, although fruits have not been analysed.

Habitats of nickel hyperaccumulators

Nearly all Ni hyperaccumulators recorded in Sabah grow in successional or open habitats, such as on natural landslides, burnt areas, along rivers and on the top of ridges. Furthermore, all Ni hyperaccumulators have been recorded from lowland areas up to the lower montane forest boundary, and no Ni hyperaccumulators have been recorded at altitudes >1200 m a.s.l. (despite extensive screening at higher altitudes). The growth forms of all known Ni hyperaccumulators in Sabah are small trees (*Rinorea bengalensis* up to a height of 23 m with a bole diameter of 58 cm is the largest) with *Psychotria sarmentosa* complex being the only exception (a creeper). The five main habitat types in which nickel hyperaccumulators have been recorded in Sabah are: (1) open shrub on hill ridges; (2) riparian zone and open forest along rivers; (3) landslide areas on serpentinite soils; (4) disturbed areas in lowland forest; and (5) seepage zones below peridotite rock cliffs. Figure 2 shows some of the Ni hyperaccumulator species from Sabah studied here. Their habitats are described in more detail below.

Open shrub on hill ridges

The main site that falls in this category is located in Nalumad. Here, a lowland (400 m a.s.l.) hill near the Mekadou River has been burnt as a result of an uncontrolled forest fire in 2008. Prior to burning, the site had been logged and most major trees were removed. At present, the site has a short and open shrub community (1–3 m) dominated by pioneer non-accumulating species such as *Macaranga kinabaluensis* (Euphorbiaceae). In this

Table 1 New and confirmed nickel hyperaccumulators from Sabah, Malaysia

Family	Species	Habitat	Distribution	Maximum foliar Ni (μg g^{-1})
Acanthaceae	*Ptyssiglottis* cf. *fusca*	Lowlands, mixed Dipterocarp forest	Sabah	1160
Dipterocarpaceae	*Shorea tenuiramulosa*	Lower montane forest	Sabah	1790
Meliaceae	*Walsura* cf. *pinnata*	Lowlands, mixed Dipterocarp forest	SE Asia	4580
Monimiaceae	*Kibara coriacea*	Lowlands, mixed Dipterocarp forest	SE Asia	5840
Phyllanthaceae	*Actephila* sp. nov.	Lowlands, secondary vegetation	Nalumad	11,520
Phyllanthaceae	*Aporosa chalarocarpa*	Lowlands, mixed Dipterocarp forest	SE Asia	1560
Phyllanthaceae	*Baccaurea lanceolata*	Lowlands, mixed Dipterocarp forest	SE Asia	1450
Phyllanthaceae	*Cleistanthus* sp. 1	Lowlands, secondary vegetation	Bidu-Bidu Hills	2110
Phyllanthaceae	*Glochidion brunneum*	Lowlands, mixed Dipterocarp forest	SE Asia	6200
Phyllanthaceae	*Glochidion* cf. *lanceisepalum*	Lowlands, mixed Dipterocarp forest	Serinsim	3270
Phyllanthaceae	*Glochidion* cf. *mindorense*	Lowlands, mixed Dipterocarp forest	SE Asia	2280
Phyllanthaceae	*Glochidion* cf. *rubrum*	Lowlands, mixed Dipterocarp forest	SE Asia	7000
Phyllanthaceae	*Glochidion* cf. *sericeum*	Lowlands, mixed Dipterocarp forest	Serinsim	2190
Phyllanthaceae	*Glochidion* sp. 'bambangan'	Lower montane forest, along rivers	Kinabalu Park	16,700
Phyllanthaceae	*Glochidion* sp. 'nalumad'	Lowlands, secondary vegetation	Nalumad	9000
Phyllanthaceae	*Phyllanthus balgooyi*	Lowlands, secondary vegetation	Sabah and Philippines	8610
Phyllanthaceae	*Phyllanthus* cf. *securinegoides*	Lowlands, secondary vegetation	Nalumad	23,300
Rubiaceae	*Psychotria sarmentosa* complex	Lowlands, secondary vegetation	Sabah	24,200
Salicaceae	*Flacourtia kinabaluensis*	Lowland forest, along rivers	Sabah	7280
Salicaceae	*Xylosma luzonensis*	Lowlands, secondary vegetation	SE Asia	5360
Sapindaceae	*Mischocarpus sundaicus*	Lowlands, mixed Dipterocarp forest	SE Asia	4425
Violaceae	*Rinorea bengalensis*	Lowlands, mixed Dipterocarp forest	SE Asia and Australia	12,800
Violaceae	*Rinorea javanica*	Lowlands, mixed Dipterocarp forest	SE Asia	9680
Violaceae	*Rinorea* sp. undet.	Lowlands, mixed Dipterocarp forest	Sabah	5830

habitat type, the Ni hyperaccumulator *Phyllanthus* cf. *securinegoides* is common with others including *Mischocarpus sundaicus* (Sapindaceae), *Rinorea javanica, Psychotria sarmentosa* complex*, Glochidion* cf. *brunneum* (Phyllanthaceae) and *Xylosma luzonensis* (Salicaceae) also present. The site is also the only known site for *Actephila* sp. nov. (Phyllanthaceae). The shrub *Phyllanthus balgooyi,* characteristically colonising rock crevices on the bare ridges, occurs in this habitat type at Nalumad and many other similar sites. Unusually, at nearby Bukit Hampuan, *Phyllanthus balgooyi* grows as a tree up to 9 m tall (and a bole diameter of 20 cm) in an open lower montane forest. A not dissimilar habitat at Mount Silam is a site where *Shorea tenuiramulosa* occurs.

Riparian zone and open forest along rivers

The main sites in this category are those along the Bambangan River, the Wuluh River, the Bangau-Bangau River and the Panataran River. Several Ni hyperaccumulators are associated with the riparian habitats at these sites: the tree *Flacourtia kinabaluensis* (Salicaceae) grows on the streambed, the shrub *Phyllanthus balgooyi* (Phyllanthaceae) grows inside the riverbed forming a fringe, and *Glochidion rubrum* (Phyllanthaceae) grows as a large tree on the alluvial clays. Besides the riparian habitat, a small valley near Bukit Hampuan has an open shrub community dominated by Ni hyperaccumulators, including the trees *Kibara coriacea* (Monimiaceae), *Walsura* cf. *pinnata* (Meliaceae), *Mischocarpus sundaicus,* the shrubs *Phyllanthus* cf. *securinegoides, Phyllanthus balgooyi* and *Xylosma luzonensis,* and the creeper *Psychotria sarmentosa* complex.

Landslide areas on serpentinite soils

The vegetation on serpentinite rocks is characterised by the (non-accumulating) trees *Ceuthostoma terminale* or *Gymnostoma sumatranum* (Casuarinaceae). Serpentinite outcrops are prone to landslides, and the pioneer vegetation in such habitats hosts *Xylosma luzonensis, Glochidion* species and *Psychotria sarmentosa* complex. The main sites in this category are at the Wuluh and Panataran Rivers in Kinabalu Park.

Fig. 2 Nickel hyperaccumulator species from Sabah studied in the present study

Disturbed areas in lowland forest

Open areas in lowland forest, such as at Serinsim, form the habitats of the trees *Glochidion* cf. *rubrum* and *G.* cf. *sericeum* and the herb *Ptyssiglottis* cf. *fusca*. These species, together with *Baccaurea lanceolata*, are the only Ni hyperaccumulators known to occur to date in tall lowland forest.

Seepage zones below peridotite rock cliffs

Seepages zones below rock cliffs near Serinsim and at Bambangan form the habitat of several Ni hyperaccumulators. At Serinsim, peridotite cliff faces are up to 70–80 m, *Phyllanthus balgooyi* is a common shrub growing on the rock face, whereas at the foot of these cliffs *Rinorea bengalensis, R. javanica*, *Mischocarpus sundaicus* (Sapindaceae) and *Flacourtia kinabaluensis* occur where seepage water drains out. A similar habitat is found at Bambangan (on Mount Kinabalu) where *Glochidion rubrum* grows below the rock face in the seepage zone.

Taxonomy and phylogeny of Ni hyperaccumulators

The main families in which Ni hyperaccumulators occur in Sabah are the Phyllanthaceae, Rubiaceae, Salicaceae and the Violaceae. This conforms to the global trends for Ni hyperaccumulators, with these families also being the most important in other tropical hotspots for Ni hyperaccumulators, such as in New Caledonia and Cuba (Reeves 2003). These families, except the Rubiaceae, are all in the Order Malpighiales (Angiosperm Clade Rosids). The records for Monimiaceae and Sapindaceae are the believed to be the first records for Ni hyperaccumulators in these families globally. The genera *Phyllanthus* and *Glochidion,* in the large family Phyllanthaceae (over 2000 species in 60 genera), have the highest diversity in the Malesian and Australian regions (Govaerts et al. 2000; Kawakita, 2010). The genus *Phyllanthus* has over 800 species globally, with major centres of diversity in New Caledonia (113 spp.), Madagascar (63 spp.), Cuba (50 spp.) and Venezuela (58 spp.) (Govaerts et al. 2000). Of the New Caledonian species, 14 are Ni hyperaccumulators (Kersten et al. 1979; Reeves 2003) and of the Cuban species, 19 are Ni hyperaccumulators (Reeves 2003). Approximately 12 species of this genus are known in Sabah and it is noteworthy that a number of the newly discovered Ni hyperaccumulators also represent undescribed plant species. This mainly reflects the complex taxonomy of Phyllanthaceae, which to date has not been revised in the 'Tree Flora of Sabah and Sarawak' or 'Flora Malesiana' (taxonomical revisions for the region). It is further worth noting that the majority of Ni-hyperaccumulating *Phyllanthus* and *Glochidion* species seem restricted to a single or a few ultramafic outcrops and are hence rare. The exception is *Phyllanthus balgooyi*, which is widespread and locally common, but the *P. balgooyi* species complex is taxonomically not completely understood (Hoffmann et al. 2003), and there might be several variants that warrant subspecies status, based on their morphologies and the distinct ecological niches they occupy (e.g. riparian zones versus rock ridges). This species was first discovered as a Ni hyperaccumulator in the Philippines by Baker et al. (1992) as *P.* '*palawanensis'* and subsequently described as a new species (*P. balgooyi*). Maximum foliar Ni was 16,230 μg g^{-1} in the Philippine material (Baker et al. 1992), whereas in this Sabah study, the maximum Ni concentration recorded was 8606 μg g^{-1}. Extremely high phloem tissue Ni concentrations (up to 88,580 μg g^{-1}) are another characteristic of this species (Hoffmann et al. 2003). Taxonomical difficulties also pertain to *Phyllanthus* cf. *securinegoides*, which is also part of a difficult section in the genus. This species was first recorded as a Ni hyperaccumulator from historic collections made in Palawan and Mindanao in the Philippines from which herbarium samples were analysed by Baker et al. (1992). This species was not known from Sabah or the island of Borneo, until collections were made as part of this study (if indeed it belongs to the same taxon). This species is rare and appears to be restricted in Sabah to a narrow band of ultramafic outcrops stretching between Bukit Hampuan and Nalumad. The Philippine material accumulated up to 34,750 μg g^{-1} Ni in the foliage (Baker et al. 1992) whereas the maximum found in Sabah in this study was 23,300 μg g^{-1}.

Since several new Ni hyperaccumulators in the family Phyllanthaceae were discovered during the field sampling, it seemed possible that this family could contain more Ni hyperaccumulators. Therefore, 220 specimens covering 41 species were sampled from existing herbarium specimens held at the SNP Herbarium. Analytical results of the elemental analysis of plant species in the Phyllanthaceae occurring in Kinabalu Park are given in Table 2. Several more species of *Phyllanthus* and *Glochidion* were found to be Ni hyperaccumulators. It should be noted that phylogenetic studies have shown that *Phyllanthus* is paraphyletic over *Glochidion* and *Breynia* and that the species in these genera be will transferred to *Phyllanthus* in a future revision for the region (Hoffmann et al. 2006; Kathriarachchi et al. 2006; Wagner and Lorence 2011). The collection data confirmed that all of specimens that hyperaccumulated Ni had been collected from ultramafic soils. In addition, there was a propensity for the Phyllanthaceae to accumulate not only Ni, but also other trace elements such as Co, Cr, Mn and Zn. Cobalt was accumulated to a significant level in *Aporosa chalarocarpa* (468 μg g^{-1}), while the *Glochidion* cf. *sericeum* specimens contained 442–1310 μg g^{-1} (and is therefore clearly a hyperaccumulator of this element). One specimen of *Baccaurea lanceolata* was unusual in

Table 2 Elemental concentrations (Co, Cr, Mn, Ni and Zn) in Phyllanthaceae

Species (Phyllanthaceae)	*N*	Co (μg g^{-1})		Cr (μg g^{-1})		Mn (μg g^{-1})		Ni (μg g^{-1})		Zn (μg g^{-1})	
		Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Actephila sp. nov.	5	22–95	65	2–2.9	2.5	238–933	566	1844–11,520	7500	153–227	183
Antidesma coriaceum	1		2		4		290		51		2
Aporosa chalarocarpa	1		468		62		1443		1560		35
Aporosa falcifera	1		18		104		13,500		34		34
Aporosa lucida	1		18		68		2765		27		27
Aporosa benthamiana	1		6		68		1382		21		21
Baccaurea lanceolata	1		179		143		436		1450		1451
Breynia sp. 1	6	1–30	9	<0.01–10	4	89–1391	495	1–19	9	15–129	52
Breynia sp. 2	1		49		7		1000		469		73
Breynia coronata	1		4		6		424		38		38
Cleistanthus ellipticus	1		6		2		1000		12		12
Cleistanthus gracilis	3	5–15	10	1–5	3	268–1005	531	5–20	12	5–9	7
Cleistanthus sp. 1	3	17–21	20	5–7	6	46–115	79	2047–2110	2068	13–16	14
Cleistanthus myrianthus	2	1–4	2	1–5	3	492–1792	1142	5–10	7	25–25	25
Glochidion angulatum	2	18–28	23	5–9	7	431–880	656	3–14	8	20–38	29
Glochidion arborescens	1		272		17		1112		1036		61
Glochidion borneense	5	4–76	21	3–30	10	56–1892	871	4–65	20	10–44	23
Glochidion brunneum	4	21–55	38	4–15	10	80–343	170	2175–6200	4480	11–29	22
Glochidion calospermum	2	7–19	13	<0.01–4	2	1531–3480	2510	4–4	4	15–173	94
Glochidion cf. *insigne*	2	3–3	3	2–4	3	20–54	37	15–51	33	29–34	32
Glochidion cf. *kunstlerianum*	1		73		15		1451		371		34
Glochidion glomeratum	3	7–23	23	5–21	11	1434–3535	2250	2–12	6	31–465	185
Glochidion kerengae	1		1		1		681		2		45
Glochidion laevigatum	8	2–6	3	<0.01–9	3	135–1016	417	3–169	27	19–277	98
Glochidion lanceilimbum	14	4–33	13	3–15	10	21–224	77	499–5010	2174	10–35	19
Glochidion littorale	2	8–8	8	4–4	4	381–804	593	4–7	6	52–137	95
Glochidion lutescens	5	1–3	2	<0.01–6	3	26–1588	586	13–24	17	16–45	27
Glochidion mindorense	9	4–41	16	3–42	15	31–209	88	611–7000	1777	11–33	17
Glochidion monostylum	7	<0.01–13	5	<0.01–15	7	11–2464	606	<0.01–38	11	1–159	52
Glochidion racemosa	1		6		4		1645		8		43
Glochidion rubrum	7	1–38	14	<0.01–8	5	40–1581	745	8–58	24	16–64	27
Glochidion cf. *sericeum*	4	442–1310	979	48–83	67	727–2541	1694	1036–2190	1786	21–103	42
Glochidion sp. 'bambangan'	22	3–138	30	4–48	17	13–458	147	1721–16 700	5354	20–155	62
Glochidion sp. 'marai parai'	6	19–188	88	5–16	12	370–2488	1024	57–761	469	15–131	38
Glochidion sp. 'nalumad'	3	10–204	95	24–45	33	108–291	172	4804–9000	7198	24–55	40

Table 2 continued

Species (Phyllanthaceae)	*N*	Co (μg g^{-1})		Cr (μg g^{-1})		Mn (μg g^{-1})		Ni (μg g^{-1})		Zn (μg g^{-1})	
		Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Glochidion sp. 'panantaran'	3	2–34	22	15–42	28	17–135	95	2271–3457	2893	19–22	21
Glochidion superbum	3	14–28	22	7–21	12	41–203	101	7–15	12	4–9	7
Glochidion obscurum	4	4–11	8	8–13	10	86–994	406	5–43	17	22–58	32
Glochidion singaporense	1		120		3		1224		64		33
Phyllanthus amarus	3	21–53	38	23–48	38	74–983	567	129–400	236	89–1475	571
Phyllanthus balgooyi	42	2–114	26	3–181	22	20–1359	239	309–8610	2467	4–413	65
Phyllanthus cf. *securinegoides*	25	2–197	39	1–114	18	23–461	145	2196–23,300	12,780	9–248	85
Phyllanthus kinabaluicus	1		109		77		433		702		11
Phyllanthus lamprophyllus	1		11		3		10		2		0
Phyllanthus pulcher	4	22–43	31	23–87	54	241–464	355	61–112	97	67–99	87
Phyllanthus reticulatus	3	3–9	5	6–12	9	16–66	34	1–27	15	11–26	17
Phyllanthus urinaria	3	8–15	12	11–23	17	105–1059	435	6–32	15	26–57	45
Phyllanthus sp. nov. 'serinsim'	2	148–169	158	43–126	84	427–557	492	457–563	510	16–136	76

Results are from microwave-assisted digestion with HNO_3 and H_2O_2 (ranges and means)

containing 179 μg g^{-1} Co, 143 μg g^{-1} Cr, 1451 μg g^{-1} Ni and 1450 μg g^{-1}Zn. Manganese concentrations were also relatively high, but varied widely. One species (*Aporosa falcifera*) appeared to be a Mn hyperaccumulator with 13,500 μg g^{-1} Mn, as the foliar threshold for Mn hyperaccumulation is >10,000 μg g^{-1} (Van der Ent et al. 2013a). *Aporosa chalarocarpa, Baccaurea lanceolata, Cleistanthus* sp. 1, *Glochidion arborescens*, *Glochidion* sp. 'bambangan', *G.* cf. *brunneum, G. lanceilimbum, G. mindorense*, *Glochidion* sp. 'nalumad', *G.* cf. *sericeum, Phyllanthus balgooyi,* and *P.* cf. *securinegoides* are Ni hyperaccumulators, but almost all species collected from ultramafic soils have higher than normal Ni concentrations in their tissues. As *Glochidion* sp. 'panantaran' probably represents the same species as *Glochidion* sp. 'nalumad' we do not consider this a new record (the infertile specimens were of poor quality making identification impossible). Some specimens collected from non-ultramafic soils have unusually high Zn concentrations, such as *Glochidion glomeratum* (185 μg g^{-1} Zn) and *Phyllanthus amarus* (571 μg g^{-1} Zn). The new Ni hyperaccumulation record for *Actephila* sp. nov. also represents a new genus holding a Ni hyperaccumulator in the Phyllanthaceae family, with up to 11,520 μg g^{-1} Ni. The relatively small family Dichapetalaceae, which is mainly distributed in Africa, has a few species in Southeast Asia of which subspecies of *D. gelonioides* are of particular interest with regard to metal accumulation. *D. gelonioides* subsp. *tuberculatum* and subsp. *pilosum* are strong Ni hyperaccumulators when occurring on ultramafic soils (up to 26,650 μg g^{-1} Ni), and strong Zn hyperaccumulators (up to 30,000 μg g^{-1} Zn) when occurring on non-ultramafic soils, whereas *D. gelonioides* subsp. *sumatranum* is a Zn hyperaccumulator (up to 15,660 μg g^{-1}), and does not accumulate Ni (Baker et al. 1992). In this research, only *D. gelonioides* subsp. *pilosum* was located and analysed. The specimens accumulated little Ni, but contained up to 4922 μg g^{-1} Zn, confirming the results of earlier studies.

The Rubiaceae, and the genus *Psychotria* in particular, is well represented globally with Ni hyperaccumulators including *Psychotria gabriellae* (formerly *P. douarrei*) from New Caledonia, *P. grandis* from Puerto Rico, *P. clementis, P. costivenia, P. glomerata, P. osseana* and *P. vanhermanii* from Cuba (Reeves et al. 1999; Reeves 2003). In Sabah, *Psychotria* cf. *gracilis* (here named as *P. sarmentosa* complex) has previously been recorded by Reeves (2003). Samples of this species were collected from various locations during the current study and confirmed its status as one of the strongest known Ni hyperaccumulators in the region. The taxonomy of this species is, however, difficult and probably comprises several closely related species and subspecies. High, though sub-hyperaccumulator, levels of Ni also occur in other members of the Rubiaceae, including *Timonius* cf. *eskerianus* (500 μg g^{-1} Ni).

The record for *Ptyssiglottis* cf. *fusca* (Ni up to 1160 μg g^{-1}) in the Acanthaceae is interesting because another member of that family, *Rostellularia adscendens* var. *hispida,* was recorded in Queensland, Australia, with concentrations up to 2190 μg g^{-1} (Reeves 2003), and *Phidiasia lindavii* from Cuba with concentrations up to 1853 μg g^{-1} (Reeves et al. 1999). The only *Buxus* species in Sabah, *Buxus rolfei* (Buxaceae) from a family with a large number of Ni hyperaccumulators in Cuba (17 Ni hyperaccumulators out of 37 species that occur in the country; Reeves et al. 1996), does not accumulate Ni. The Sapotaceae, which contains the famous Ni hyperaccumulator *Pycnandra acuminata* (formerly *Sebertia acuminata*) (from New Caledonia and renowned for its green nickel-rich sap; Jaffré et al. 1976), and the Ni hyperaccumulator *Planchonella oxyedra* from Indonesia (Wither and Brooks 1977), did not contain Ni hyperaccumulators in Sabah in the 18 species across five genera tested, with *Planchonella* sp. nov. from Nalumad being the highest with a Ni concentration of 160 μg g^{-1}. The record for *Dalbergia beccarii* recorded in this study with 2620 μg g^{-1} Ni is interesting, because another species in this genus (*D. melanoxylon*) and another member in this family (*Pearsonia metallifera*) are (hyper)accumulators of Ni in Zimbabwe (Cole 1971; Reeves 2003). The Ochnaceae has a Ni hyperaccumulator in *Brackenridgea palustris* (several subspecies) from Palawan and Sulawesi (Baker et al. 1992), and this species has also been recorded from Sabah. The Clusiaceae contains Ni hyperaccumulators in *Garcinia bakeriana, G. revoluta* and *G. ruscifolia* from Cuba (Reeves et al. 1999), but analyses in this study reveal no hyperaccumulators (29 species across five genera tested), the highest Ni concentrations being found in *Mesua paniculata* with 146 μg g^{-1}, *Kayea macrantha* with 125 μg g^{-1}, *Calophyllum soulattri* with 123 μg g^{-1} and *Garcinia bancana* with 105 μg g^{-1} Ni. Although Ni hyperaccumulators have been recorded in the Myrtaceae in Cuba and New Caledonia, no Ni hyperaccumulators were recorded in this family during this study, with the highest Ni concentrations found in a specimen of *Leptospermum recurvum* with 140 μg g^{-1}, *Syzygium* cf. *pterophera* with 115 μg g^{-1} and *Rhodamnia cinerea* with 108 μg g^{-1} Ni. Finally, although the genus *Chionanthus* (Oleaceae) has yielded a Ni hyperaccumulator species (*C. domingensis*) from Cuba, the two species in that genus tested in this study contained <6 μg g^{-1} Ni.

Soil chemistry and foliar concentrations in Ni hyperaccumulators

The results of the analysis of the soil chemistry in the rhizosphere of 12 Ni hyperaccumulators are given in Tables 3 and 4. These soils are characterised by circumneutral pH (mean pH 6.7), relatively high total (mean 2985 μg g^{-1}) and exchangeable Ca (mean 1417 μg g^{-1}), extremely high total Mg (mean 43.8 mg g^{-1}) and low exchangeable K (Table 3). Total Co (mean 387 μg g^{-1}), Cr (mean 3205 μg g^{-1}) and Mn (mean 3946 μg g^{-1}) are also high (Table 3). However, phytoavailable concentrations of these elements are low. Mean total soil Ni is high (1890 μg g^{-1}) and potentially phytoavailable Ni (mean 99, 127, 128 μg g^{-1} for Mehlich, DTPA and organic acid-extractable Ni, respectively) is also high. Despite the fact that the soils originate from different localities and have wide ranging properties, extractable Ni concentrations are similar (Table 4).

Foliar elemental concentrations in hyperaccumulators were compared with soil elemental concentrations (total and extractable/exchangeable) of the hyperaccumulator soils (of matched pairs). Table 4 shows summarised elemental concentrations in foliage and associated soils in a range of hyperaccumulators. On average, foliar Ni is three times higher than soil Ni (but reaches a factor of sixfold in *Phyllanthus* cf. *securinegoides*). While such levels of biomagnification are impressive, it should be noted that hyperaccumulator plants (particularly trees) are long lived and hence slowly accumulate Ni from the soil into their biomass over an extended period of time. Working with native hyperaccumulators in New Caledonia, Lee et al. (1977) found a positive relationship between foliar Ni and extractable Mn and Ni in the soil. However, in this study only a weak (but significant) correlation was found between foliar Ni and total soil Ni ($r = 0.20$), and correlations between foliar and extractable soil Ni were weaker. There are also (weak) correlations between foliar Ni and pH ($r = -0.28$) and foliar Ni and soil P ($r = 0.47$). Further correlations were found between foliar S and P and soil Mg ($r = -0.40$ and -0.27), foliar K and exchangeable Al, Ca and K ($r = 0.32$, 0.28 and 0.30, respectively) and foliar Al and pH ($r = -0.28$).

High-Ni leaf shedding and potential allelopathic effects

The field survey showed that most Ni hyperaccumulators were locally abundant in their habitats, both in terms of numbers of individuals and relative numbers of (hyperaccumulator) species. This suggests preferential habitats on certain soil types, but the high relative density of hyperaccumulators could also potentially locally induce allelopathic effects. It has been shown that leaves shed by the New Caledonian Ni hyperaccumulator tree (*Pycnandra acuminata*) increases the concentrations of Ni in the top soil directly under the canopy substantially (Boyd and Jaffré 2001) potentially constituting an allelopathic system that could give the hyperaccumulator competitive advantages. Recent work with Se hyperaccumulators have shown

Table 3 Main soil chemistry parameters in the rooting zone (major cations)

Species	Location	*N*	pH		EC (µS cm^{-1})		Ca (µg g^{-1})		Ca exch. (µg g^{-1})	
			Range	Mean	Range	Mean	Range	Mean	Range	Mean
Actephila sp. nov.	Nalumad	3	6.4–7.0	6.7	250–507	357	1152–6990	3164	1813–4500	2923
Flacourtia kinabaluensis	Serinsim, Wuluh	2	7.3–7.4	7.3	98–113	106	5312–6100	5709	225–306	266
Glochidion sp. undet.	Bukit Hampuan, Nalumad, Wuluh, Panataran, Bidu-Bidu, Bambangan	15	6.1–7.9	6.8	47–276	137	85–8050	3025	86–1935	579
Kibara coriacea	Nalumad	1		5.8		89		345		154
Mischocarpus sundaicus	Serinsim, Bukit Hampuan	2	6.6–6.9	6.8	135–273	204	998–1270	2270	186–1530	858
Phyllanthus balgooyi	Bukit Hampuan, Nalumad, Serinsim, Panataran, Bidu-Bidu	13	6.2–7.3	6.7	34–533	209	178–7770	2056	80–3316	775
Phyllanthus cf. *securinegoides*	Nalumad, Bukit Hampuan	9	5.6–7.3	6.6	87–359	212	90–7715	3745	450–4435	1596
Psychotria sarmentosa complex	Bukit Hampuan, Nalumad, Serinsim, Wuluh	5	5.9–7.2	6.6	44–248	129	177–1048	385	207–1477	653
Rinorea bengalensis	Bukit Hampuan, Nalumad, Serinsim	6	6.6–7.6	7.1	40–632	224	63–9240	2821	358–3458	1327
Rinorea javanica	Bukit Hampuan, Nalumad	3	6.5–6.8	6.7	217–408	283	616–8075	4671	2149–4200	2910
Walsura cf. *pinnata*	Bukit Hampuan, Nalumad	3	6.2–6.9	6.6	93–233	176	2082–5695	3395	341–2237	1374
Xylosma luzonensis	Bukit Hampuan, Nalumad	3	6.5–7.4	6.9	136–588	332	1533–5885	3512	1100–6950	3585

Species	Location	*N*	Mg (mg g^{-1})		Mg exch. (µg g^{-1})		K exch. (µg g^{-1})		P (µg g^{-1})	
			Range	Mean	Range	Mean	Range	Mean	Range	Mean
Actephila sp. nov.	Nalumad	3	24.1–40.9	30.9	1713–2830	2250	102–228	153	20–182	76
Flacourtia kinabaluensis	Serinsim, Wuluh	2	24.1–28.3	26.2	1156–2316	1736	16–50	33	91–111	101
Glochidion sp. undet.	Bukit Hampuan, Nalumad, Wuluh, Panataran, Bidu-Bidu, Bambangan	15	7.5–39.7	20.1	128–6180	2186	19–77	38	35–182	80
Kibara coriacea	Nalumad	1		98.8		305		36		142
Mischocarpus sundaicus	Serinsim, Bukit Hampuan	2	35.7–39.4	37.6	2880–5200	4039	72–85	79	75–75	75
Phyllanthus balgooyi	Bukit Hampuan, Nalumad, Serinsim, Panataran, Bidu-Bidu Hills	13	2.3–135.3	73.3	195–4780	2054	16–100	43	45–245	111
Phyllanthus cf. *securinegoides*	Nalumad, Bukit Hampuan	9	33.3–147.1	75.6	507–6120	2732	17–204	67	44–585	197
Psychotria sarmentosa complex	Bukit Hampuan, Nalumad, Serinsim, Wuluh	5	2.9–132.9	36.7	57–23,000	913	22–52	32	59–279	152
Rinorea bengalensis	Bukit Hampuan, Nalumad, Serinsim	6	2.0–53.9	24.5	708–4790	2188	21–307	123	45–536	202
Rinorea javanica	Bukit Hampuan, Nalumad	3	8.1–53.2	33.1	1705–3355	2319	63–108	83	85–185	137
Walsura cf. *pinnata*	Bukit Hampuan, Nalumad	3	21.6–28.3	24.7	675–2060	1522	35–71	50	31–109	72
Xylosma luzonensis	Bukit Hampuan, Nalumad	3	37.8–48.4	44.5	1623–2415	1905	43–264	144	47–132	86

Main soil chemistry parameters in the rooting zone (trace elements)

'Pseudo-total' microwave-assisted digestion with HNO_3 and HCl, *'exch.'* exchangeable with silver–thiourea, *'Pseudo-total'* microwave-assisted digestion with HNO_3 and HCl

Table 4 Ni concentrations in soil in the rooting zone

Family	Species	N	Foliar Ni ($\mu g\ g^{-1}$)		Total soil Ni ($\mu g\ g^{-1}$)		Soil ML-3 Ni ($\mu g\ g^{-1}$)	
			Range	Mean	Range	Mean	Range	Mean
Meliaceae	*Walsura* cf. *pinnata*	2	1870–4580	3226	676–1015	845	95–130	112
Monimiaceae	*Kibara coriacea*	1		4150		1510		94
Phyllanthaceae	*Actephila* sp. nov.	3	6795–11 520	9125	1000–2330	1786	109–157	132
Phyllanthaceae	*Glochidion* sp. undet.	13	882–9000	3748	185–2545	672	22–188	101
Phyllanthaceae	*Phyllanthus balgooyi*	10	1073–8290	3550	624–3415	1931	19–172	66
Phyllanthaceae	*Phyllanthus* cf. *securinegoides*	8	319–23 300	10,400	782–2490	1729	55–150	90
Rubiaceae	*Psychotria sarmentosa* complex	5	7205–20,600	12,790	1962–5100	3496	19–213	89
Salicaceae	*Flacourtia kinabaluensis*	2	1229–3990	2610	473–1215	844	57–133	95
Salicaceae	*Xylosma luzonensis*	3	1315–4970	3705	1717–2865	2344	80–112	100
Sapindaceae	*Mischocarpus sundaicus*	2	555–3120	1838	2135–3260	2697	70–138	104
Violaceae	*Rinorea bengalensis*	5	3730–8470	6130	887–4255	2220	19–221	110
Violaceae	*Rinorea javanica*	3	6090–9680	7385	1860–3960	2608	51–149	97
Family	Species	N	Soil DTPA Ni ($\mu g\ g^{-1}$)		Soil CA Ni ($\mu g\ g^{-1}$)		Soil $Sr(NO_3)_2$ Ni ($\mu g\ g^{-1}$)	
			Range	Mean	Range	Mean	Range	Mean
Meliaceae	*Walsura* cf. *pinnata*	2	94–166	130	101–169	135	4–11	8
Monomiaceae	*Kibara coriacea*	1		196		211		27
Phyllanthaceae	*Actephila* sp. nov.	3	171–226	199	89–219	160	4–7	6
Phyllanthaceae	*Glochidion* undet.	13	19–202	98	32–490	138	1–27	8
Phyllanthaceae	*Phyllanthus balgooyi*	10	17– 291	131	14–655	122	2–48	12
Phyllanthaceae	*Phyllanthus* cf. *securinegoides*	8	21–200	93	35–347	120	2–15	8
Rubiaceae	*Psychotria sarmentosa* complex	5	21–204	116	22–233	89	5–37	12
Salicaceae	*Flacourtia kinabaluensis*	2	44–157	100	57–112	84	1–5	3
Salicaceae	*Xylosma luzonensis*	3	13–169	115	52–183	132	2–22	10
Sapindaceae	*Mischocarpus sundaicus*	2	69–70	70	54–124	89	3–4	4
Violaceae	*Rinorea bengalensis*	5	21–442	136	25–318	148	3–16	8
Violaceae	*Rinorea javanica*	3	122–185	146	36–213	109	3–10	6

Foliar concentrations are from microwave-assisted digestion with HNO_3 and H_2O_2

'Pseudo-total' Ni microwave-assisted digestion with HNO_3 and HCl, *CA* carboxylic acid-extractable Ni, *'ML-3 Ni'* Mehlich-3-extractable Ni, *'$Sr(NO_3)_2$ Ni'* dilute strontium nitrate-extractable Ni, *'DTPA Ni.'* DTPA solution-extractable Ni

that such plants may reduce the growth of sensitive neighbouring plants while facilitating tolerant plants with the latter experiencing less herbivory (El Mehdawi et al. 2011a; El Mehdawi and Pilon-Smits 2011). These effects were linked to 'phyto-enrichment' (increase of Se in the surrounding soil) and hence causing elemental allelopathy (El Mehdawi et al. 2011b). In contrast, experimental work with a temperate Ni hyperaccumulator demonstrated that leaf shedding did not inhibit germination or growth of competing plants (Zhang et al. 2006).

Leaf litter samples collected at Nalumad under the canopies of *Phyllanthus* cf. *securinegoides* and *Rinorea bengalensis* contained 665 and 2128 $\mu g\ g^{-1}$ Ni, respectively, and exceed the concentrations in the sub-surface soil. Compared to the mean Ni concentration (74 $\mu g\ g^{-1}$) in leaf litter samples from 84 plots in Kinabalu Park (Van der Ent et al. unpublished), the leaf litter under these hyperaccumulators is extremely Ni rich. It is to be expected, however, that co-occurring plant species are also Ni tolerant. Nevertheless, the high leaf litter Ni could exert a selective force on some (generalist) plant species that may outcompete Ni hyperaccumulators. Furthermore, high Ni in the organic topsoil could have mutualistic effects on seedlings of both the Ni hyperaccumulator species that is the source of the leaf litter, and co-occurring Ni hyperaccumulator species. As such, Ni loading in the topsoil could benefit hyperaccumulator seedlings by having a ready supply of Ni to their shallow roots to aid herbivory protection in emerging leaves. Indeed, high densities of seedlings of *Rinorea bengalensis* at Nalumad, and *Phyllanthus balgooyi* at Bukit Hampuan, have been observed. These interactions might not explain why Ni hyperaccumulation has evolved, but are rather consequences of the immediate Ni hyperaccumulator environment. Leaf shedding could also act as a tolerance mechanism by translocation of Ni in abscised leaves (Martens and Boyd 1994). Although leaf litter Ni concentrations were high as outlined earlier, they were still lower than samples from living (attached) leaves on the plants, hence it would be an inefficient process. It should be noted that leaf litter decomposes very rapidly under the conditions of intense rainfall and the tropical humid climate, and any contained Ni probably leaches rapidly.

Interaction of Ni hyperaccumulators with epiphytes

The ecological relationships between epiphytes and hyperaccumulators is poorly understood, although Boyd et al. (2009) found that epiphytes (bryophytes) growing on Ni hyperaccumulator hosts contained greater levels of Ni than those growing on non-hyperaccumulator hosts in New Caledonia. Even more so, they concluded that bryophyte Ni concentrations often exceeded Ni hyperaccumulation thresholds (Boyd et al. 2009). Although it should be noted that bryophytes have no active vascular system for Ni uptake and translation and hence the definition of 'hyperaccumulation' should probably not apply to such non-vascular plants. Nevertheless, this seems to indicate that the suitability for epiphytes to grow on hyperaccumulators as hosts may depend on their capability to tolerate high Ni concentrations and this may in turn define the epiphyte community composition between Ni hyperaccumulator and non-hyperaccumulator hosts (Boyd et al. 2009). Most Ni hyperaccumulators in Sabah are small shrubs and hence do not carry epiphytes, but an exception are some particularly large specimens of *Phyllanthus balgooyi* on Bukit Hampuan, where ferns have colonised branches of these trees. Foliage of these ferns contained 251 μg g^{-1} Ni, whereas the bark of the host plant contained 358 μg g^{-1} Ni. This shows that the bark of some Ni hyperaccumulators is a high-Ni environment and that epiphytes can indeed take up Ni from the living or decomposing bark (or decomposing dropped leaves) on which they grow. As such, there is some selective force for Ni-tolerance in epiphytes growing on *Phyllanthus balgooyi* hosts, but it is unknown whether this might have any potential beneficial effects for the epiphyte ('enhanced herbivory protection').

Interaction of Ni hyperaccumulators with insect herbivores

The 'Elemental Herbivory Defense' hypothesis (Boyd and Martens 1998; Boyd 2009) suggests that high Ni loading in the leaves of Ni hyperaccumulators could result in reduced (insect) herbivory attack. However, field observations on

Fig. 3 Geometric moth (Erebidae: Erebinae tribe Poaphilini) feeding on the leaves of the Ni hyperaccumulator *Phyllanthus balgooyi* (*top*) and aphids, with ants, feeding on *Phyllanthus* cf. *securinegoides* (*bottom*)

Mount Bloomfield, Palawan (Philippines), showed that Ni hyperaccumulators did not experience less herbivory (Proctor et al. 2000), and the Ni hyperaccumulator *Shorea tenuiramulosa* suffered as much foliar herbivory damage as 'normal' plant species growing in the area on Mount Silam in Sabah (Proctor et al. 1989). It should be noted, however, that this species achieves only relatively low Ni accumulation (351–1787 μg g^{-1} recorded in this study), and the extremely high concentrations in, for example *Psychotria sarmentosa* complex (up to 24,200 μg g^{-1}) and *Phyllanthus* cf. *securinegoides* (up to 23,300 μg g^{-1}) will undoubtedly exert a stronger toxicity effect on insect herbivores. Nevertheless, extensive insect herbivory damage to all Ni hyperaccumulator species in Sabah was observed during this study. Specialist insect herbivores may feed on Ni hyperaccumulators without negative effects (Boyd 2009), and one geometric moth species (Erebidae: Erebinae tribe Poaphilini) has been found in this study to feed exclusively on the Ni hyperaccumulator *Phyllanthus balgooyi* (illustrated in Fig. 3, top). Such Ni-tolerant insects preferentially feed on Ni hyperaccumulators to increase their own body Ni content to deter their predators (Boyd 2009). Aphids have also been found feeding on *Phyllanthus* cf. *securinegoides* at Nalumad (also see Fig. 3, bottom). The extremely high Ni concentrations in the tissues of these hyperaccumulators necessitate exceptional Ni-tolerance by these insects. If not tolerant, other strategies that could be employed by herbivore insects to avoid Ni toxicity include diet dilution (feeding on low-Ni plant parts in addition to high-Ni plant parts), or avoidance (by selective feeding) (Martens and Boyd 1994; Boyd and Martens 1998; Boyd 2009).

Discussion

Laboratory analysis with ICP-AES confirmed the indicative results achieved from the initial testing in the field with dimethylglyoxime (DMG). Field testing with DMG paper therefore remains a reliable and quick method for Ni hyperaccumulator reconnaissance. The method is, however, fairly insensitive, and depends on Ni being present in the aqueous phase. Some plants, for example *Shorea tenuiramulosa,* did not react with DMG in the field, and only laboratory analysis showed significant Ni accumulation. This intriguing example is suggestive of the various biochemical forms in which Ni may be present in plants. Recent progress has been made with understanding Ni chemical forms in *Alyssum* ssp. (Tappero et al. 2007; McNear et al. 2010), and in some hyperaccumulators from New Caledonia, most notably *Pycnandra acuminata* (formerly *Sebertia acuminata*) (Perrier et al. 2004; Callahan et al. 2008), providing evidence for the important role of carboxylic acids such as citrate in Ni complexation. The wide range of plant families and life forms involved in Ni hyperaccumulation suggests that various different physiologies, and hence Ni chemical forms, might be present in hyperaccumulator plants. Such differences in chemical bonding of Ni in plant tissues might also contribute to lower or higher toxicity to insect herbivores.

Nickel hyperaccumulators in Sabah occur mainly in open, successional habitats, mainly on ridges, below rock faces and along rivers. Recent studies on soils associated with these hyperaccumulators have shown that the occurrence is correlated with soils with highly available Ni concentrations (Van der Ent et al. unpublished). These open, often bare ultramafic soils are also difficult for most plants to colonise and make plants colonising these habitats potentially more susceptible to insect herbivores. The local abundance of Ni hyperaccumulators might indicate possible advantages, including highly efficient nutrient sequestration, or foliar toxicity to reduce insect herbivory (‘elemental herbivory defense’) or competitive advantages over other plant species (‘elemental allelopathy’). Stepwise evolution of the Ni hyperaccumulator trait, starting with relatively low foliar metal concentrations and associated competitive advantages over other plants with normal foliar concentrations has been hypothesised to lead to hyperaccumulation (Boyd 2012; Cheruiyot et al. 2013). The question arising from this hypothesis is why some Ni hyperaccumulators reach extremely high Ni concentrations (for example, 23,300 μg g^{-1} in *Phyllanthus* cf. *securinegoides* or >100-fold higher than MLC for the generalist insect herbivore in Cheruiyot et al. 2013), which presumably comes at an energetic cost. The answer could lie in a biogeochemical ‘arms race’ with Ni-tolerant insects (Boyd 2004, 2009).

Several specialist Ni-tolerant insects have been discovered that feed on Ni hyperaccumulators: for example, the insects *Melanotrichus boydi* (Schwartz and Wall 2001) and *Chrysolina pardalina* (Mesjasz-Przybylowicz and Przybylowicz 2001). Such specialised insects have co-evolved with Ni hyperaccumulators and adapted to increasing foliar Ni concentrations that are lethal for generalist insect herbivores. The field observation of widespread herbivory damage on Ni hyperaccumulators in Sabah, and the local discovery of specialist herbivores suggest that Ni-tolerant insects are perhaps a relatively common feature in the ultramafic flora of Sabah. Though in order to test this, controlled experiments are needed in which herbivory damage between two closely related species (a Ni hyperaccumulator and a non-hyperaccumulator) is carefully compared. Apart from scientific interest in the co-evolution of Ni hyperaccumulator plants and specialist insects, this could have economic consequences, because as indicated by Boyd (1998), potential future sites for phytomining might already harbour specialist Ni-tolerant herbivores that could pose a threat to the phytomining crop. Moreover, metal transfer over trophic levels could have ecosystem health implications. As such either mammals (wild animals such as deer or orang-utan, or domestic animals such as cattle) or invertebrates (such as insects) feeding on high-nickel plants could be exposed to Ni (Peterson et al. 2003; Miranda et al. 2009).

On a global scale, Ni hyperaccumulation occurs in approximately 400 species (about 40 families). This widespread phylogenetic occurrence of Ni hyperaccumulation indicates that the trait has independently evolved multiple times (Pollard 2000; Krämer 2010). Globally, 30 % of the Ni hyperaccumulators (and 83 % of those in New Caledonia) belong to the COM clade (Orders Celastrales, Oxalidales, Malpighiales) of the Rosids (Jaffré et al. 2013). In Sabah, Ni hyperaccumulation occurs most frequently in the Order Malpighiales (mainly the families Dichapetalaceae, Phyllanthaceae, Salicaceae, Violaceae), and is particularly common in the Phyllanthaceae (genera *Phyllanthus*, *Glochidion*). The genus *Phyllanthus* has most

Ni hyperaccumulators worldwide, and is well represented in Sabah. In this genus, hybridization and introgression could be responsible for the frequent occurrence of the Ni hyperaccumulation trait, as virtually all species in this genus occurring on ultramafic soils in Sabah are Ni hyperaccumulators. Not infrequently, several *Phyllanthus* spp. co-occur in the same habitat. In this respect, the observation that certain *Phyllanthus* hybrids, for example *P. x pallidus* (*P. discolor x orbicularis*) from Cuba (Reeves et al. 1996) reach Ni hyperaccumulation far in excess of the parental species, is of particular interest.

Conclusion

With only approximately 10 % of the ultramafic flora of Sabah screened, it is expected that more Ni hyperaccumulators will be recorded in the near future, particularly in the order Malpighiales. The restriction of most hyperaccumulators species to ultramafic-derived soils, which can be mining targets, means that these species are both rare and threatened (Baker et al. 2010). It is therefore necessary that systematic screening and cataloguing of Ni hyperaccumulator plant species and other plants native to ultramafic ecosystems take place prior to any land clearing or Ni mining activities. Screening also supports strategies aimed at the preservation of hyperaccumulator germplasm, which is especially critical for local ecotype populations possessing enhanced hyperaccumulation traits. Unfortunately such screening is not part of any environmental impact assessment (EIA) undertaken on Ni mining leases in the region.

Although there are no current Ni mining activities in Sabah, there has been substantial loss of forest cover with $>7000\ km^2$ lost between 1990 and 2010 (Reynolds et al. 2011). Protected areas amount to approximately 8 % of the land surface in Sabah (Bryan et al. 2013). The recent establishment of the 'Hyperaccumulator Botanical Garden' in Sabah, under the auspices of Sabah Parks, serves as an example of ex situ preservation of hyperaccumulator germ plasm. Such preservation is essential if Ni hyperaccumulator species (and ecotype populations) are to be utilised in potential future Ni phytomining operations, aimed at growing such plants at agricultural scale to harvest Ni bio-ore (Van der Ent et al. 2013b). In the 'Hyperaccumulator Botanical Garden', living plants of all currently known Ni hyperaccumulator species from Sabah have been planted (10–20 plants for each species) in naturally occurring ultramafic soil.

Despite the richness of the ultramafic flora, very few studies have focussed on the occurrence of Ni hyperaccumulators in this region. As such, many important questions remain unanswered: for example, how Ni hyperaccumulators have evolved and whether this trait can be induced in facultative Ni hyperaccumulators such as *Rinorea bengalensis* by exposure to ultramafic soil? The richness not only in Ni hyperaccumulator species, but also in Ni-tolerant insects, indicates potentially rewarding avenues for future scientific research in Sabah.

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