

Coupling nickel chemical speciation and isotope ratios to decipher nickel dynamics in the Rinorea cf. bengalensis-soil system in Malaysian Borneo

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- 1 Coupling nickel chemical speciation and isotope ratios to decipher nickel dynamics in
- 2 the Rinorea cf. bengalensis-soil system in Malaysian Borneo
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17 Abstract

18 Aims. Rinorea cf. bengalensis is a Ni hyperaccumulator which occurs in Sabah (Malaysia), on Borneo Island,

- 19 that is able to accumulate considerable amounts of Ni and influences the Ni cycle in surface soil layers, both in
- 20 terms of Ni concentration and Ni isotopic composition. In this study, the biogeochemical processes underpinning
- 21 Ni isotopic fractionation in the soil-plant system and the mechanisms regulating Ni homeostasis in R. cf.
- 22 *bengalensis* plants were elucidated.
- 23 Methods. Two specimens of R. cf. bengalensis of different ages and associated surface soils were collected from
- 24 ultramafic soils in Sabah. Soil mineralogy, Ni concentrations, speciation and isotopic signatures were
- 25 subsequently determined in plant and soil samples.
- 26 Results. Nickel in R. cf. bengalensis leaves is mainly complexed with citrate. Soil Ni available fractions have
- 27 different δ^{60} Ni values depending on the Ni bearing phases. *Rinorea* cf. *bengalensis* specimens take up lighter Ni
- 28 isotopes and a pronounced isotopic fractionation within the plant is observed, especially in the young specimen.
- 29 Conclusions. The results suggest that the observed fractionation in the young plant can be attributable to kinetic
- 30 effects (lighter isotopes move faster), which become less evident in the older specimen, as Ni is redistributed and
- 31 homogenized through phloem loading and unloading processes.

32 <u>Keywords:</u> Ni, ultramafic soil, hyperaccumulator plants, isotopic fractionation, chemical speciation

33 Introduction

34 Ultramafic rocks are mantle rocks characterized by a specific composition with low silica, high contents of mafic 35 minerals (Fe and Mg oxides). Soils derived from the alteration of ultramafic rocks pose edaphic stresses for 36 vegetation, including a low Ca/Mg ratio, lack of essential nutrients such N, P, and K, and a high concentrations 37 of Mn, Ni, Cr and Co (Whittaker 1954; Bonifacio et al. 1997; Becquer et al. 2006). These soils are known for 38 their hosting adapted vegetation, which includes the occurrence of hyperaccumulator plants that can achieve 39 extraordinary levels of metal accumulation due to enhanced uptake and translocation mechanisms from the roots 40 to the shoots (Baker, 1981). On ultramafic soils developed from serpentinized ultramafic rocks (which represent 41 the most important terrestrial Ni reservoirs), numerous Ni hyperaccumulator plant species have been reported 42 over the past 40 years (Reeves et al. 2018a). Hyperaccumulators can attain exceptionally high concentrations of 43 Ni in their living tissues, with up to several weight percent Ni (e.g. 25 Wt% in latex or 16 Wt% in phloem sap) 44 (Jaffre et al. 1976; Baker and Brooks 1988; Reeves 2003; van der Ent et al. 2013; Mesjasz-Przybylowicz et al. 45 2015; Reeves et al. 2018b; Jaffré et al. 2018). Nickel hyperaccumulator plants are known to transfer most of the 46 Ni into their aerial parts (shoots) of the plant, instead of using root sequestration for metal detoxification (Baker, 47 1981). Via leaf litter deposition, hyperaccumulators can increase Ni concentrations of the soil in a surface area surrounding the plant (Boyd and Martens 1998). In tropical Southeast Asia, Rinorea cf. bengalensis (Violaceae) 48 49 is one of more than 40 different Ni hyperaccumulator species identified from the Malaysian state of Sabah on 50 Borneo Island (van der Ent et al. 2018b; 2019a). Rinorea cf. bengalensis is a large tree (up to 25m tall) that 51 contain up to 4.77 kg of Ni (van der Ent and Mulligan 2015). Substantial Ni enrichment under the canopy of R. 52 cf. bengalensis has been reported due to the degradation of leaf litter (van der Ent et al. 2015). However, it is still 53 an open question to what extent Ni is recycled in the natural habitat of R. cf. bengalensis through Ni uptake, leaf 54 shedding, re-uptake of Ni from decomposed leaf litter, and the Ni release in surface soil (Nkrumah et al. 2019b, 55 d).

The interest toward Ni cycling at the Earth's surface has rapidly increased during the last decades but only a few scientific studies have been published proposing the use of stable Ni isotopes to elucidate the biogeochemical cycle of Ni in the upper soil horizons and surface waters (Elliott and Steele 2017). The degree of Ni isotope fractionation between the different surface compartments are a practical approach to identify and characterise the Ni biogeochemical processes. In order to better understand Ni isotopic fractionation on a larger scale, in 61 catchments or soil-plant systems, the processes that fractionate Ni isotopes at the molecular scale need to be 62 elucidated. Recent studies contributed to this understanding by showing Ni isotopic fractionation due to sorption 63 onto mineral phases under laboratory controlled conditions (Wasylenki et al. 2015; Wang and Wasylenki 2017; 64 Gueguen et al. 2018; Spivak-Birndorf et al. 2018), and due to complexation with organic acids relevant for the 65 soil-plant system (Zelano et al. 2018). Nickel is involved in various biological reactions that might be implicated 66 in isotopic fractionation (Cameron et al. 2009) but, to date, only few works have been focused on the impact of 67 hyperaccumulator plant homeostasis on the Ni isotopic signature in surface soils (Deng et al. 2014; Estrade et al. 68 2015; Zelano et al. 2018; Ratié et al. 2019). However, isotopic fractionation of metals has been observed in 69 higher plants and has been interpreted as a consequence of physiological processes involved in maintaining 70 homeostasis, especially with regard to Fe (Guelke and von Blanckenburg 2007), Zn (Weiss et al. 2004; Tang et 71 al. 2012, 2016; Deng et al. 2014; Caldelas and Weiss 2017), Cd (Imseng et al. 2018, 2019) and Cu (Jouvin et al. 72 2012). For example, in the case of Zn, the preferential uptake at root level of heavier or lighter Zn isotopes has 73 been associated with the presence of high- and low-affinity transport systems, respectively (Weiss et al. 2004; 74 Caldelas and Weiss 2017). The type of transport system is dependent on the bioavailability of the metal, e.g. a 75 high level of available Zn favours lighter isotopes uptake through low affinity transports, while a lower 76 availability of the metal induces uptake of heavy isotopes through high affinity transporters (Tang et al. 2016). 77 For Zn, it has been suggested that further fractionation from roots to higher parts of the plant can take place 78 during the xylem loading process and subsequent transportation (Tang et al. 2016).

79 In the case of Ni, the physiological mechanisms regulating metal homeostasis are still largely unknown (Deng et 80 al., 2014), but few studies posit that Ni sorption and translocation are regulated by low-affinity transport systems, 81 and no high-affinity transport systems have been discovered hitherto in higher plants (Cataldo et al. 1978; Deng 82 et al. 2014, 2019). Studying Ni isotopic fractionation and the homeostasis of the hyperaccumulator Alyssum 83 murale grown in controlled hydroponic conditions, a preferential sorption of lighter isotopes from solution was 84 observed (Δ^{60} Ni_{plant-solution} = -0.9 to -0.63 ‰) (Deng et al. 2014). It is still unclear, however, how and at which 85 stage Ni isotopic fractionation exactly takes place. Studying plants collected in the temperate ultramafic area of 86 Albania, Estrade et al. (2015) reported an enrichment in heavier isotopes of Ni in the plants compared to the bulk 87 soil, Δ^{60} Ni_{whole plant-soil} up to 0.40 %. Nevertheless, the Ni taken up by plants was lighter than the bioavailable Ni, 88 extracted by diethylenetriaminepentaacetic acid (DTPA), and the difference $\Delta^{60}Ni_{available-rhizo}$ soil was up to 89 0.89 \. This result suggests that hyperaccumulating plants take up lighter isotopes from the heavier bioavailable 90 Ni pool in soils. However, a recent study on hyperaccumulators in the tropical ultramafic region of Brazil, did

91 not report on any significant Ni isotopic fractionation neither during Ni uptake, nor during Ni translocation 92 within the plants (Ratié et al. 2019). In our previous work, we have studied the remobilization of Ni by 93 simulating the effect of leaf litter degradation of A. murale and R. cf. bengalensis. Obtained results showed that, 94 in the case of R. cf. bengalensis, depending on the entity of the fractionation factor between roots and leaves, Ni 95 recycling can result in a lighter isotope input into the surface soil (Zelano et al. 2018). Nickel hyperaccumulators, 96 therefore, are responsible not only for "phyto-enrichment" of Ni concentration in surface soil, but they can also 97 influence its isotopic composition. However, a clear mechanistic understanding of the observed Ni isotopic 98 signatures in the soil-plant system and the links with the processes responsible for Ni isotopic fractionation have 99 not been attained thus far. Only few publications have attempted to decipher the metal dynamics in the soil-root-100 shoot continuum by combining stable isotope tool and speciation data (Aucour et al. 2015, 2017). In this work, 101 we developed an analytical strategy to follow Ni transfer from soil to plant shoots, considering the soil 102 characteristics and the vertical distribution of Ni in the different horizons. Furthermore, in order to better 103 understand the dynamics of Ni during the growth of a woody tropical hyperaccumulator tree, we investigated 104 two differently aged plants. As such, this study focuses on two related soil-plant systems of R. cf. bengalensis 105 naturally occurring on ultramafic soils, in the tropical rainforest of Kinabalu Park (Sabah, Borneo Island, 106 Malaysia) (van der Ent and Mulligan 2015; van der Ent et al. 2015, 2018c; 2020). Two differently sized/aged 107 plants were sampled on two distinct soils having two different stages of pedogenesis. On the basis of Ni 108 concentrations, Ni speciation and δ^{60} Ni in soils and plants, first results on the Ni dynamics in the soil-plant 109 system are provided and interpreted.

110 Materials and methods

All reagents were prepared using ultrapure water (18.2 M Ω cm). Nitric and hydrochloric acids were of suprapure grade. Standard solutions were supplied by TechLab (1000 μ g mL⁻¹, in a 2–5% HNO₃).

113

114 Plant material and soil collection

Two individuals of *R*. cf. *bengalensis* were sampled in the native equatorial rainforest of Sabah, (Borneo Island, Malaysia). The plants naturally developed on soils derived from serpentinized and non-serpentinized ultramafic bedrock (for a detailed overview of the characteristics of the local soils, see van der Ent et al. 2018a; 2019b). The first specimen, R_{150} , measuring 150 cm high, was collected on a deeply weathered Ferralsol near Serinsim (Nkrumah et al. 2018). The other individual, R_{600} , is a tree of approximatively 6 m tall, and was collected near Lompoyou Hill, on an eroded hypermagnesian Cambisol on strongly serpentinized bedrock (Nkrumah et al. 2018; van der Ent et al. 2018a). According to a recent study (Nkrumah et al. 2019a,b), the R_{150} and R_{600} trees are estimated to be less than 1 year and about 3 years old, respectively. Although such trees are common in this area of the world, trees of different age were only found in distinct areas during our one-week campaign. This study is, therefore, focused on two trees of different age, respectively grown on a Ferralsol and on a Cambisol.

125

The R_{150} plant was harvested in its entirety and was subsequently divided into roots, trunk (later subdivided in bark and wood samples) and leaves. For R_{600} , multiple leaf and bark samples were collected along the entire height of the plant, from 1–6 m. Two phloem tissue samples were extracted by stripping the inner bark from the wood core at 5 cm under and 5 m above soil level. In the young tree, the phloem tissue is thin and nearly impossible to sample (van der Ent and Mulligan 2015), therefore samples were collected only for R_{600} . Two root samples, considered to be surface roots, were also collected at about 5 cm and 15 cm under soil level. Deeper root samples could not be collected.

133

134 The surface soils, between 0 cm and 15 cm depth, were collected at the base of R_{150} and R_{600} and referred to as 135 SR₁₅₀ H1 (0-5 cm horizon), SR₁₅₀ H2 (5-15 cm horizon), and SR₆₀₀ H1 (0-5 cm horizon), SR₆₀₀ H2 (5-10 cm 136 horizon) and SR_{600} H3 (10–15 cm horizon) respectively. About 500 g for each sample were collected. Several 137 rain forest species, including R. cf. bengalensis, are very shallow rooting and most of the nutrient cycling 138 happens in the top layer of the soil (Vitousek 1984; Vitousek and Sanford 1986), so only superficial soil horizons 139 were collected. Soils and plant samples were air-dried immediately after field collection, at temperature below 140 40°C. This preparation mode was selected due to the sampling conditions and the sampling location was not 141 compatible with the use of liquid nitrogen to preserve biological samples in situ. This mode of preparation is not 142 usually recommended for XAS measurements, and the drying step may have influenced the chemical speciation 143 of Ni, replacing water molecules by other ligands. The breaking of cell walls and membranes during the drying 144 could have induced the dissociation of Ni complexes with low stability. However, XAS results obtained on 145 plants show that the Ni speciation was not drastically modified by drying and the results are consistent with 146 published data (van der Ent et al. 2017).

147

Due to their apparent difference in particle size distribution, soils were sieved to compare similar size fractions.
Each soil sample was, then, subdivided in two equivalent parts; one was kept and referred to as 'bulk sample',

whereas the second part was sieved into four fractions, *i.e.* >1.5 mm; 1.5 mm – 250 μ m; 250–50 μ m and 151 <50 μ m.

152 Element concentration

All soil and plant samples were dried in a dehydrating oven at 110°C overnight, and manually ground using an agate mortar. About 50–70 mg of ground plant material was weighed in Savilex® beakers and left overnight in 3–5 mL of concentrated (70%) HNO₃ at room temperature. Samples were, then, heated to 140°C on a hot plate to complete the acid digestion. When necessary, a second digestion step was performed, using 3 mL of Aqua Regia and 1–2 ml of HF (32%) to allow for complete sample dissolution. Solutions were finally dried at 110°C, recovered with 0.3 M HNO₃and measured with Inductively Coupled Plasma Mass Spectrometer (ICP-MS Quadrupole, X-Series Thermo Fisher Scientific).

160

Major and trace elements in soil samples were analysed by Service d'Analyse des Roches et des Minéraux (SARM), at Centre de Recherches Pétrographiques et Géochimiques, Nancy, France. The content of organic carbon in soils was measured using the EMIA-Analyser Carbon/Sulfur supplied by HORIBA, after decarbonation with HCl and filtration.

165

166 To estimate the available Ni concentration in soil samples, an aliquot of bulk soil was suspended in ultra-pure 167 water for 24h, in triplicate, using 100 mg soil and 40 mL of milli-Q water. The final suspension was centrifuged 168 for one hour at 52000 g and the resulting supernatant was filtered through 0.22 µm with cellulose syringe filters. 169 Nickel concentration was measured in solution and, as an approximation, will be indicated hereafter as 'available 170 Ni'. Reported results of available Ni concentration correspond to the average values of experimental triplicates. 171 The bioavailable Ni pool in soils is operationally determined by chemical extraction using organic ligands, e.g. 172 DTPA (Echevarria et al. 2006). By performing, however, a water extraction we exclude any potential isotopic 173 fractionation arising from the Ni-ligand complex formation.

174

The cation exchange capacity (CEC) of the bulk soils was determined using cobalt hexammine. Briefly, 30 mL of 0.05 N hexammine cobalt(III) chloride were added to 1 g of dry soil, shaken for two hours at 30 °C and centrifuged at 52000 g. Supernatants were filtered through 0.22 μ m and the residual concentration of hexammine analysed with UV spectrometry. This makes possible to determine the total CEC of the soil. Furthermore,

- 179 element concentrations (Na, Ca, K, Mg, Fe, Al, Si and Ni) in the supernatants were measured using a Thermo
- 180 Fischer ICAP 6500 Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES).

181 Nickel isotope measurements

182 Prior to Ni isotopic measurement, a two-step chromatography separation was performed to remove sample 183 matrix. The reported procedure is based on a previously developed protocol (Cameron et al. 2009; Gueguen et al. 184 2013) and modified as described by Estrade et al. (2015) and Deng et al. (2014). Briefly, soil and plant samples 185 were digested, as described above, to determine, Ni content. Depending on the measured Ni concentration 186 (verified after digestion using a Q-ICP-MS X series 2 Thermo-Scientific), a known volume of dissolved sample 187 was evaporated to give a mass of Ni between 1 and 2 μ g. After evaporation, the residue was dissolved again in 2 188 mL of 3 M HNO₃. In order to correct for any isotope composition variation potentially induced by the chemistry 189 process, and for an instrumental mass bias, the technique of a double-spike addition was adopted. For that, a 190 double-spike solution containing an equal amount of ⁶¹Ni and ⁶²Ni isotopes was added to samples. The volume 191 of double-spike solution to add was adapted for each sample to obtain a spike/sample ratio of 1.15. The mix was 192 left equilibrating overnight, evaporated on a hot plate at 110°C and dissolved in 1 mL of 6 M HCl. Solution was, 193 then, passed on the first chromatographic column filled up with 2 ml of the anion-exchange resin AG®1-X8 and 194 recovered. The resin, which is in chloride form, is able to retain Fe and Zn present in the sample matrix, while Ni 195 is eluted with the solution. Additional 15 mL of 6 M HCl were passed through the column to recover all Ni. The 196 final volume was successively evaporated on a hot plate at 110°C and dissolved again in 1 mL HCl 1 M. The 197 second chromatographic separation to complete the sample purification is based on the use of a Ni specific resin, 198 supplied by Triskem Inc., France. The used resin is able to retain Ni through chelation with dimethylglyoxime at 199 pH value 8–9. Before the second chromatographic separation, 0.2 mL of ammonium citrate ($(NH_4)_6C_6H_6O_7$) 200 were added to the samples, the pH was adjusted at 8-9 with 100-150 µL of ammonia, and samples were left 201 overnight for equilibration. Solution was loaded on the column and Ni retained by the resin was successively 202 recovered by 4 mL of 3 M HNO, and put to evaporate on a hot plat at 110°C. To eliminate any potential residue, 203 acid digestion with aqua regia were performed, before recovery the sample with 0.3 M HNO₃ for measurement. 204 Procedural blanks were prepared in each sample batch, resulting in 0.058–0.256 nmol of Ni content that has a 205 maximum value of 1.5 % of the total Ni, and a corresponding negligible influence on reported isotopic values, 206 within uncertainties.

207

208 The solutions were measured by Multi-Collector Inductively Coupled Plasma Mass Spectrometer (MC-ICP-MS, 209 Neptune plus, Thermo Scientific) in medium resolution mode and using an Apex HF desolvation introduction 210 system in a 0.3 M HNO₃ media. Ni masses (58, 60, 61, 62) were measured simultaneously as well as Cu isotopes 211 and one Fe isotope at masses 63, 65 and 57 respectively. The ⁵⁷Fe intensity was monitored continuously to 212 correct for any ⁵⁸Fe contribution to ⁵⁸Ni. The correction is effective only for a signal lower than 10 mV. When 213 the signal was higher than 10 mV, samples were processed and eluted again through the first column. A standard 214 bracketing method was used during sample measurements and δ^{60} Ni in samples were normalized to NIST-986. 215 Sample δ^{60} Ni values were calculated as:

216

217
218
$$\delta^{60} \text{Ni} = \left(\frac{{}^{60/58} \text{Ni}_{\text{sample}}}{{}^{60/58} \text{Ni}_{\text{NIST 986}}} - 1\right) \times 1000 \text{ in (\%)}$$
(1)

219

220 The analytical reproducibility was monitored by processing and measuring a Ni ICP-MS standard solution for 221 each measurement session. Since no value exist for this solution, the standard subjected and not-subjected to the 222 entire sample preparation procedure was measured and compare to itself, in order to control for isotopes 223 composition modification during the process. The obtained average value was δ^{60} Ni = -0.11 ± 0.05 ‰ (2 σ , 224 n = 25). The reference material BHVO-2 was processed and measured ten times in different measurement 225 sessions, obtaining δ^{60} Ni = 0.05 ± 0.04 ‰ (2 σ), in agreement with published data (Gall et al. 2013; Gueguen et 226 al. 2013; Estrade et al. 2015; Ratié et al. 2015). Estimated recovery from the measured intensities is always 227 higher than 90%. However, recovery is not a crucial parameter as the double spike addition technique was used. 228 In addition, in order to have a matrix match reference for plant samples, the Oak Leaves reference material V464 229 from the French National Institute for Agricultural Research (INRA) was also digested, purified and measured. The value obtained for this material is δ^{60} Ni= -0.02±0.05‰ (2 σ , N=6). 230

231

All samples were measured three times. The soil-water suspension samples to determine available Ni were prepared in triplicate. For those samples, the reported δ^{60} Ni values correspond to the average *X* of experimental triplicates *x_m*. The associated standard error (2 σ) is calculated as:

235

$$236 \qquad \sigma = \frac{sd}{\sqrt{N}},\tag{2}$$

237

where *sd* is the standard deviation of the sample mean, and N is the number of samples.

239

For bulk soil and plant material samples, no experimental triplicates are available and the reported 2σ represent the analytical error deriving from three measurements of the sample. The $2\sigma = 0.05$ ‰ obtained for the Ni ICP-MS standard (digested and measured 25 times) was applied to all sample results presenting analytical $2\sigma < 0.05$ ‰.

244

The Ni isotopic fractionation between two samples A and B, Δ^{60} Ni_{A-B}, was calculated as the difference between the isotopic signatures measured in A and B, as reported below:

247

248
$$\Delta^{60} \text{Ni}_{\text{A-B}} = \delta^{60} N i_A - \delta^{60} N i_{B}^{2+}$$
 (3)

249

250 The standard deviation σ associated to Δ^{60} Ni includes the error propagation and was calculated as:

251
$$y = a \pm b, \ \sigma y = \sqrt{(\sigma a)^2 + (\sigma b)^2}$$
 (4)

252

253 Soil mineralogical composition

The mineralogical composition of the samples was determined by X-ray diffraction (XRD, Bruker D8 Advance) with an X-ray tube producing Co K α radiation (1.78886 Å) operated at 35 kV and 45 mA. The XRD patterns were obtained by scanning crushed powders of the sieved fractions and of the bulk soil, from 3° to 64° 20 with a 0.035° 20 step and with a 3s counting time per step. The diffracted signal was measured using a LynxEye detector with an energy discrimination window adjusted to minimize the high fluorescence background signal coming from Fe to improve the peak-to-background ratio.

260

Soil mineralogy was also investigated with electron microscopy, and transmission electron images were obtained with a Philips CM20 transmission electron microscope (TEM), operating at 200 kV, coupled to an Energy-Dispersive X-ray Spectrometer (EDXS). For TEM imaging and microanalysis, a few milligrams of sample were re-suspended in ethanol using ultrasonication (10 min) and a drop of suspension was evaporated on a carboncoated copper grid. The EDX spectra were obtained in nanoprobe mode (20–30 nm) using a counting time of 40–70 seconds.

267 Chemical speciation of nickel (X-ray absorption spectroscopy)

268 X-ray absorption spectroscopy (XAS) experiments were performed at two beamlines, in fluorescence mode at 269 the SAMBA beamline (SOLEIL synchrotron, France), and in transmission mode at the XAFS beamline 270 (ELETTRA synchrotron, Italy). In both cases, Ni K-edge X-ray absorption spectra (both in the XANES and 271 EXAFS regions) were recorded at low temperature (using a liquid He cryostat 25 K and liquid N₂ cryostat, 77 K 272 respectively) with a Si(111) double crystal monochromator. A Ni foil was positioned after the sample and 273 collected simultaneously to each spectrum in order to have perfect energy calibration; the first inflection point of 274 metal Ni spectrum was calibrated at 8333 eV. Soil samples were scanned at SAMBA in fly scan mode from 8000 275 to 9500 at velocity of 5 eV/s, resulting in energy step of 0.5 eV, using an X-ray fluorescence detector (VORTEX, 276 Hitachi). For each soil sample, between 22 and 35 spectra were recorded. Dried leaf samples were measured at 277 the XAFS beamline in transmission detection mode, with three scans recorded in a step by step mode, with 5 eV 278 steps for the first 200 eV, 0.2 eV for the XANES range and with a k-constant step of 0.03 (Å⁻¹) in the EXAFS 279 range. In all cases, the samples were ground and pelletized before the XAS measurements. Reference spectra 280 were obtained on the SAMBA and XAFS beamlines from previous sessions, under the same acquisition 281 conditions. These standards included Ni aqueous solution with and without organic ligands, Ni incorporated into 282 goethite, Ni sorbed onto phyllosilicate (charged and non-charged minerals), Ni sorbed onto calcite, Ni sorbed 283 onto iron oxyhydroxide (goethite), and natural Ni containing minerals including chrysoprase, nimite, and Ni-284 bearing serpentine (clay mineral).

285

286 The XANES and EXAFS data were reduced using standard normalization procedures performed with the 287 ATHENA and ARTEMIS programmes (Ravel and Newville 2005). The spectra were background subtracted, 288 and normalized. A spline function was fit through the absorption envelope and subtracted from each spectrum. 289 To transform data from energy space E (eV) to wave vector space k ($Å^{-1}$), E₀ edge energy was chosen in the higher part of the edge step, at 8343 eV. The resulting χ function was weighted by k³ to avoid oscillations 290 291 damping at high χ values. The EXAFS signals obtained from plant and soil samples were fitted as linear 292 combinations of the standard EXAFS spectra collected on solutions and solid samples. The number of 293 components was set to a maximum of three on the basis of TEM and XRD observations. The fitting parameters were then selected on the basis of quality indicators (χ^2 , r-factor and reduced χ^2). Shell by shell fitting was also 294 295 performed using theoretical scattering paths calculated from the unit cell structure of distinct Ni-bearing phases 296 (Artemis code on the basis of FEFF6) (Ravel and Newville 2005; Rehr et al. 2010).

297

298 Results

299

300 Chemical and mineralogical composition of soils

301 The elemental concentrations were determined in the different horizons of soils associated with plants, SR_{150} and 302 SR_{600} , for the bulk samples and for the size fractions i) >1.5 mm, ii) 250–50 µm, and iii) <50 µm, of both H1 303 horizons (Table SI 1). The major element contents are typical for soils developed on ultramafic bedrock (van der 304 Ent et al. 2018a). The Fe content is relatively high, especially in SR_{150} , where it reaches up to 34 and 39.8 % 305 (Fe₂O₃%), while it ranges from 4.6 and 22.1 % in SR₆₀₀, with a clear increasing trend with depth in the latter 306 case. The Si content is rather similar in the surface horizons of SR₁₅₀ and SR₆₀₀, with up to 28.9 and 29.2 % (SiO₂ 307 %) respectively. However, this content tends to increase with depth for SR₆₀₀, reaching up to 36.9 and 35.4 % in 308 the H2 and H3 horizons respectively. The third major element discriminating the soils is Mg, which reaches up 309 to 1.6% (MgO %) in SR150 and which ranges between 10.9 and 13.4 % in the SR600 soil horizons. Organic carbon 310 is higher in RS₆₀₀ (11.84% in H1) than in RS₁₅₀ (7.44% in H1) and in both cases organic carbon decreases with 311 depth. The trace elements are slightly higher in SR_{150} than in SR_{600} In general, no marked changes within the soil 312 profile can be observed for all trace elements measured. These results are consistent with previously published 313 data of ultramafic rhizosphere soils, collected in the same area in Sabah, that constitute the habitat of R. cf. 314 bengalensis (van der Ent et al. 2015, 2017; 2020).

315

Nickel concentrations (Table 1) are higher in SR_{150} than in SR_{600} , reaching 113 µmol g⁻¹ and only 61 µmol g⁻¹, respectively. In both cases, Ni concentration remains rather constant in the different horizons, but decreases with soil size fraction (from fraction >1.5 mm to fraction <50 µm). Available Ni in bulk samples was estimated by measuring Ni concentration in the supernatant from soil-aqueous suspension. For $SR_{150}H1$ and -H2, the concentration of available Ni is about 0.35 µmol g⁻¹, while it is 0.40 µmol g⁻¹ in $SR_{600}H1$ and decreases in $SR_{600}H2$ and $SR_{600}H3$ to 0.19 µmol g⁻¹ and 0.09 µmol g⁻¹, respectively.

322

The highest cationic exchange capacity (CEC) in both bulk soil samples (Table SI 2) is observed in the H1 horizons (54.6 mEq per 100 g and 22.7 mEq per 100 g in $SR_{600}H1$ and $SR_{150}H1$, respectively). In both cases, CEC decreases with depth as a result of the decrease of organic matter. As a general trend, SR_{600} has a higher CEC than SR_{150} and the difference in CEC between the two soils, about 32 meq 100g⁻¹, mainly derives from the higher concentration of exchangeable Mg that reaches 42.4 mEq per 100 g in $SR_{600}H1$, but only 5.8 mEq per 328 100 g in $SR_{150}H1$. Exchangeable Fe was under the detection limit in all of the samples. Exchangeable Ni is 329 similar in both H1 horizons, but its relative contribution to total CEC is 6% in SR_{150} and is less than 1 % in SR_{600} . 330 Moreover, it slightly increases in $SR_{150}H2$, while it decreases in $SR_{600}H2$ -H3.

331

332 The mineralogy, as revealed by both XRD and TEM data, supports the discrimination of SR150 and SR600 soils 333 (Fig.1 and Fig. SI 1-2). SR₆₀₀ XRD patterns (in all horizons and size fractions) show the presence of 334 phyllosilicate phases, commonly encountered in ultramafic soils. The clay minerals are represented by chlorite, 335 talc and 7Å minerals (i.e. serpentine and kaolinite) and are associated with other silicates such as quartz, 336 feldspars and amphiboles, as well as Fe-oxyhydroxide (goethite). The mineralogy of SR₆₀₀ relates to the presence 337 of the serpentinized bedrock beneath and to a less advanced weathering of this soil. The XRD patterns have 338 intense diffraction peaks from amphibole and chlorite minerals (Fig. SI 2). For the second soil SR₁₅₀, quartz, talc 339 and goethite diffraction lines dominate XRD patterns. Those patterns are all drastically different from those 340 obtained for the SR₆₀₀ samples, with a smaller number of crystalline phases (Fig. SI 1), and the presence of 341 goethite is consistent with the higher Fe content in the SR150 soil samples. For both soils, XRD data do not reveal 342 any variation in the different horizons and size fractions. The XRD analysis of the clay fraction of SR₆₀₀ shows 343 the predominance of chlorite minerals, and additional 7Å minerals (from the serpentine or kaolinite groups).

344

345 The XRD analysis is sensitive to crystalline phases only and soils are commonly constituted of poorly crystalline 346 or amorphous phases. To enhance the mineralogical investigations, TEM images and combined spectra (EDX) 347 were acquired on the fine fraction (<50 µm) of the topsoil horizons from SR₁₅₀ and SR₆₀₀ (Figure 1). The TEM 348 micrographs for SR₁₅₀ reveal mainly talc and goethite particles. Associated EDX spectra show that in SR₁₅₀ Ni is 349 mainly associated with Fe-oxyhydroxides, including goethite. In SR₆₀₀, different types of clay minerals are 350 observed, as well as several poorly defined phases. For SR₁₅₀, goethite appears to be the predominant Fe-bearing 351 phase, while for SR₆₀₀, TEM analyses reveal the presence of amorphous Fe-oxyhydroxides, frequently 352 containing Ni (shown by EDX analysis). In the latter sample, Ni was detected in most of the investigated 353 particles, ranging from 0.3 to 0.9% (atomic percentage, EDX data) including silicate particles.

354

The XAS spectra and extracted EXAFS oscillations were used to determine the solid speciation of Ni in the soils (Fig. 2). The comparison of bulk and fine fractions in the two soils show that the solid speciation of Ni was similar for both size fractions. XANES spectra from SR₁₅₀ and SR₆₀₀ appear very similar, mainly due to the fact 358 that Ni has the same oxidation state (2+) and octahedral coordination. Some differences, however, can be 359 observed in the XANES region and on EXAFS oscillations that are consistent with mineralogy results (TEM-360 EDXS and XRD). Superimposition of XANES spectra reveals slight differences in the position of the main edge 361 and of the first oscillation (Fig. SI.3). Differences were also noticed in the EXAFS oscillations and associated 362 Fourier transforms (Fig.2). Fourier transform curves (imaginary and magnitude parts) show that the slight 363 differences between these two spectra come from the nature and distribution of atoms in the 2nd and further 364 coordination spheres (Fig.2). Shell by shell fitting shows that for both soil samples, six atoms of oxygen at 2.03-365 2.1 Å surround Ni atoms, and dominate the backscattering signal (Table 2).

366

367 Shell-by shell fitting (Table 2) also indicates the presence of Fe atoms at 3.07 and 3.54 Å in the case of SR_{150} , 368 supporting the hypothesis that a fraction of Ni is adsorbed onto Fe oxyhydroxides, or is inserted in the crystal 369 lattice of Fe oxyhydroxides. This conclusion is consistent with the higher concentration of Fe (*i.e.* goethite) in 370 this sample SR₁₅₀ (See table SI 1). For SR₆₀₀, FEFF fitting also shows the presence of low Z atoms at 3.26 Å, 371 supporting the hypothesis that a part of Ni is incorporated in the silicate structure. This result is also correlated 372 with the high proportion of phyllosilicates in sample SR₆₀₀ (XRD and TEM data). Linear combination fitting 373 (Table 2) reveals matches with reference spectra in the samples SR₆₀₀ and SR₁₅₀: Ni sorbed onto smectite (59 % 374 and 50% for SR₆₀₀ and SR₁₅₀ respectively), Ni sorbed onto goethite (16% and 50% for SR₆₀₀ and SR₁₅₀ 375 respectively, and Ni in serpentine (26% for SR_{600}) (Fig. 2).

376 Element concentrations and Ni chemical speciation in plant samples

377 Multi-elemental analyses were performed on different plant material of R_{150} and R_{600} which conform with 378 concentrations ranges previously reported for *R*. cf. *bengalensis* (van der Ent et al. 2017; 2020). Results and 379 detailed discussion are provided in Supplementary Information Table SI 3–4 and associated notes.

380

The R_{150} specimen was entirely collected and the mass of plant material was weighed. The whole dry plant weight was 91.96 g. The plant was then divided in leaf, root, bark and wood samples and, except for leaves, the quantification of their relative mass was difficult to achieve. However, an estimation of the relative mass of bark and wood was made, and on the basis of the corresponding Ni concentration, the total Ni content has been calculated to be 8018 µmol. The weighted average concentration in the whole plant was 87.20 ± 70.65 µmol of Ni per g of plant. The weighted standard deviation has been calculated as reported in equation 5:

387
$$\operatorname{sd}_{w} = \sqrt{\frac{\sum_{i=1}^{N} wi(xi-xm)^{2}}{\frac{(N-1)\sum_{i=1}^{N} wi}{N}}},$$
 (5)

388 where *wi* is the weight for the *ith* observation, *N* is the number of non-zero weights, and *xw* is the weighted mean 389 of the observations.

390

391 In line with data previously published for R. cf. bengalensis (van der Ent et al. 2017; 2020), Ni concentrations leaves are high, between 134 μ mol g⁻¹ and 221 μ mol g⁻¹ in R₁₅₀ and, with the exception of leaves at 80 cm height, 392 393 Ni slightly decreases from the bottom to the top of the plant (Table 4). In contrast, no clear trend can be observed 394 in the bark samples where Ni concentrations range between 43 µmol g⁻¹ and 98 µmol g⁻¹, decreasing from 20 cm 395 to 60 cm and increasing again from 60 cm to 90 cm. Nickel concentrations in the wood samples have an 396 increasing trend from the bottom to the top, from 6.68 µmol g⁻¹ to 70 µmol g⁻¹. A different trend is observed in 397 R₆₀₀, where Ni concentrations, both in leaf and bark samples, clearly decreases as a function of plant height: from 398 522 µmol g⁻¹ in older leaves at 2 m, to 156 µmol g⁻¹ in younger leaves at 6 m, and Ni concentrations in bark at 399 the same height decreases from 133 μ mol g⁻¹ to 53 μ mol g⁻¹. Two root samples per plant were collected at about 5 cm and 15 cm under the soil surface and Ni concentrations have similar range, 154 μ mol g⁻¹ and 81.7 μ mol g⁻¹ 400 401 in R_{150} , and 63.2 µmol g⁻¹ and 111 µmol g⁻¹ in R_{600} .

402

403 Nickel chemical speciation in R. cf. bengalensis leaves was investigated (Fig. 3, Table 3) and the XAS spectra 404 are rather similar evidencing that Ni speciation is predominated by Ni complexed with low molecular weight 405 carboxylic acids (malate and citrate). These results are consistent with those previously reported (van der Ent et 406 al. 2017), and suggest that Ni chemical speciation was not drastically changed by the drying step. XAS spectra 407 could not reveal any differences of chemical speciation of Ni in the investigated R. cf. bengalensis specimen. 408 Linear combination fitting suggests the contribution of hydrated cations Ni²⁺(H₂O)₆ at 33% and 31% for R₆₀₀ and 409 R150 respectively, the presence of Ni-malate complexes at 41% and 42% for R600 and R150 respectively, and Ni-410 citrate complexes at 26% and 27 % for R₆₀₀ and R₁₅₀ respectively.

411

412 The chemical speciation of Ni in *R*. cf. *bengalensis* leaves supports the idea that Ni is not strongly chelated by

413 specific organic molecules and is not considered as a contaminant or as xenobiotic by the plants. The apparent

414 weakly bound state of Ni makes it readily mobile, and Ni might be not permanently stored within the leaves.

415 XAS data do not reveal any differences between the R_{600} and R_{150} leaves, suggesting that Ni remains relatively

416 mobile, whatever the age of the tree.

417

418 Nickel isotopic composition in soils and plants

419 The δ^{60} Ni values in bulk soils (δ^{60} Ni_{bulk}) and in the available fraction (δ^{60} Ni_{available}) are shown in Table 1. The 420 bulk soil horizons of SR₁₅₀ and SR₆₀₀ have slightly different Ni isotopic signatures, δ^{60} Ni_{bulk RS150 H1} = -421 δ^{60} Ni_{bulk SR150 H2} = -0.16 ± 0.05 ‰, 0.17 ± 0.05 ‰, δ^{60} Ni_{bulk RS600 H1} = -0.01 ± 0.05 ‰, and 422 δ^{60} Ni_{bulk RS600 H2} = 0.01 ± 0.06 ‰, respectively. In both cases, δ^{60} Ni does change neither with depth, nor in the 423 <50 µm fractions of H1 compare to the bulk. The SR₁₅₀ H1 available Ni (δ^{60} Ni_{available RS150 H1}) is slightly enriched 424 in lighter Ni isotopes in comparison with the corresponding bulk soil SR₁₅₀ (δ^{60} Ni_{available RS150 H1} = -425 0.32 ± 0.09 ‰), obtaining Δ^{60} Ni _{bulk-available RS150 H1}= 0.15 ± 0.10 ‰. However, this result is not observed for the 426 lower horizon SR₁₅₀H2, where available Ni is similar to the bulk soil, δ^{60} Ni_{available RS150 H2}= -0.14± 0.05 ‰, and 427 Δ^{60} Ni_{bulk-available RS150 H2}= -0.02 ± 0.07 ‰. On the contrary, SR₆₀₀ available Ni has an enrichment in heavier Ni 428 isotopes, increasing with soil depth, δ^{60} Ni_{available RS600 H1} = 0.32 ± 0.05 ‰, δ^{60} Ni_{available RS600 H2} = 0.52 ± 0.05 ‰ 429 and δ^{60} Ni_{available RS600 H3} = 0.62 ± 0.05 ‰. The isotopic difference with the corresponding bulk soils were 430 calculated as Δ^{60} Ni_{bulk-available RS600 H1}= -0.33 ± 0.06 ‰ and Δ^{60} Ni_{bulk-available RS600 H2}= -0.51 ± 0.07 ‰.

431

The Ni isotopic composition of the two *R*. cf. *bengalensis* specimens R_{150} and R_{600} are reported on Table 4. The root samples from R_{150} plant at -5 cm and -15 cm under the surface level has $\delta^{60}Ni_{roots} = -0.35 \pm 0.05$ ‰ and $\delta^{60}Ni_{roots} = -0.42 \pm 0.05$ ‰, respectively. Similarly, the surface roots of R_{600} plant, both collected at -5 and -15 cm under surface level, has negative values, -0.58 ± 0.05 ‰ and -0.59 ± 0.06 ‰ respectively (Table 4).

436

437 In the aerial parts, δ^{60} Ni in leaves of R₁₅₀ is between -0.66 and -0.89 ‰, clearly showing a preferential 438 translocation of light Ni isotopes from the roots to the leaves (Tables 4 and 5) and, among the leaves, from the 439 bottom to the top, with the presence of lighter Ni isotopes in the younger apical leaves. Moreover, it can be seen 440 from Fig.4 that leaves, which constitute the Ni-enriched parts of the plants (table 4), have δ^{60} Ni values lighter 441 than the ligneous material, *i.e.* wood and bark. The wood and bark samples have relatively wide-ranging values. 442 However, with the exception of the wood sample at 100 cm, a preferential concentration of heavier Ni isotopes 443 can be observed from the bottom to the top of the plant. The δ^{60} Ni value of the whole plant R₁₅₀ was estimated by calculating the weighted mean of the δ^{60} Ni in the different parts of the plant, 444

resulting in a value of -0.65 ± 0.23 %. The weighted standard deviation was calculated as reported in eq 5.

446

447 The δ^{60} Ni values in R₆₀₀ leaves range between -0.19 ‰ and -0.33 ‰, but no systematic variation is observed 448 between the basal and apical leaves, collected from 2 to 6 m. Moreover, bark samples also have variable δ^{60} Ni 449 values, from -0.29 ‰ to 0.15 ‰, in some cases heavier and in others lighter than the leaves collected at the same 450 height, without any clear trend. The phloem tissue sample collected from beneath the bark at -5 cm has a similar 451 isotopic composition than root collected at the same level, δ^{60} Ni_{phloem} = -0.60 ± 0.08 ‰. The phloem tissue at 5 m 452 high has, on the contrary, a heavier Ni isotopic composition (δ^{60} Ni_{phloem} = -0.10 ± 0.06 ‰), than leaf and bark at 453 the same height, δ^{60} Ni_{leaf} = -0.30 ± 0.05 ‰ and δ^{60} Ni_{bark} = -0.13 ± 0.09 ‰, respectively. However, the 454 anomalously low Ni concentration in this sample collected at 5 m suggests a collection error and, therefore, it 455 will not be included in the further discussion.

456 **Discussion**

457

458 Nickel isotopic composition in soil

459 The studied soil samples have two distinct stages of pedogenesis, which are reflected not only by the overall 460 element concentrations and associated mineralogy, but also by their Ni isotopic composition. Weathering 461 processes are expected to influence δ^{60} Ni by depleting the heavier fraction of Ni in soil (Ratié et al. 2015). This hypothesis is consistent with the lighter Ni isotopic signature of SR₁₅₀ bulk (δ^{60} Ni = -0.17 ± 0.05) compare to 462 463 SR_{600} bulk ($\delta^{60}Ni = -0.01 \pm 0.05$). The SR_{150} is a Ferralsol, collected near Serinsim region, and can be 464 considered to have reached steady state equilibrium, as in Ferralsols all the alterable minerals have been 465 weathered and secondary minerals carry most of the Ni in this soil. This assumption is supported by the soil 466 mineralogy, characterized by a quasi-absence of phyllosilicates, and by the Ni solid speciation, showing that Ni 467 is mainly bound to poorly crystalline Fe oxyhydroxides. Moreover, no difference is detected between the two 468 first SR₁₅₀ soil horizons H1 and H2, which have similar total and available Ni concentrations, and similar 469 isotopic composition. The available Ni from H1 is slightly enriched in light isotopes (Δ^{60} Ni_{bulk}available = 0.15±0.10 ‰), while no fractionation is observed for H2 (Δ^{60} Ni_{bulk-available} = -0.03 ±0.07 ‰). Such a 470 471 stable isotopic composition of Δ^{60} Ni_{bulk-available} ~ 0, can be the result of the loss of the heavier water soluble Ni 472 from the soil, due to full weathering of the primary minerals, achieved after thousands of years. In contrast, the 473 soil SR₆₀₀ is a partially weathered hypermagnesian Cambisol and the soil isotopic composition suggests the 474 contribution of other processes than in SR150. Primary and secondary phyllosilicates, in which Ni is often present 475 in structural positions and/or adsorbed onto the surface forming outer-sphere complexes (Chardot et al. 2007; 476 Raous et al. 2013; Bani et al. 2014), are the main crystalline phases. To a lesser extent, Ni is also associated to amorphous Fe oxides (Fig.1). Both total Ni concentration and δ^{60} Ni values in bulk soils remain constant in the 477 478 three horizons of SR₆₀₀, i.e. H1, H2 and H3. However, from H1 to H3, available Ni concentration decreases with 479 depth and has a clear enrichment of heavy isotopes, with δ^{60} Ni values increasing from 0.32 ‰ to 0.62 ‰ from 480 H1 to H3, respectively. These values are directly correlated with a decreasing of exchangeable Ni (see CEC 481 values in Table SI 2), a decreasing of organic C, and an increasing of Fe content. Performing water extraction 482 provides an estimation of the available Ni mainly involved in weak bonds with mineral surface groups or organic 483 matter. Such a contribution to the available pool of Ni in surface horizon of ultramafic soils has already been 484 noted (Zelano et al. 2015). The contribution of organic matter (OM) to available Ni is, therefore, likely more 485 important in H1 and H2 than in H3, and it can be hypothesized that the increasing positive values of δ^{60} Ni_{available} 486 in H2 and H3, are due to a lower proportion of weakly bound Ni in those horizons compared to SR_{600} H1. In the 487 light of the results, it can be assumed that the lighter pool of available Ni in SR₆₀₀H1 is the result of combined 488 processes, such as the prolonged Ni uptake by plant and the leaf litter degradation, during which Ni is released to 489 surface soil, in primis to H1. Reported results have shown that Ni isotopic composition of the whole plant R₁₅₀ is 490 lighter than the available Ni in soil (Table 5), suggesting that R. cf. bengalensis takes up lighter Ni isotopes. This 491 hypothesis can be reasonably extrapolated to the case of R_{600} , for which the calculation of a mass balance was 492 not possible, but in which all plant material have an isotopic signature lighter than the available Ni in soil. In a 493 previous study it has been shown that during leaf litter degradation process, about the 80 % of Ni released has 494 the same δ^{60} Ni value of the original leaves, and that after a few days, a release of light isotopes occurs (Zelano et 495 al. 2018). Therefore, the enrichment of lighter isotopes in the whole plant likely produces a release of lighter Ni 496 isotopes during leaf litter degradation to topsoil, H1, compared to the deeper horizons, H2 and H3. However, this 497 contribution might also vary depending on the potential isotopic fractionation between leaves and roots.

498

499 Nickel fractionation within plants

From the isotopic composition of both specimen R_{150} and R_{600} , it can be concluded that *R*. cf. *bengalensis* preferentially takes up the lighter Ni isotopes from the available Ni fraction in soil through absorption by root cells, as reported in Table 5. This result is consistent with previously published data for Ni hyperaccumulators grown in hydroponics, where it was also shown that the entity of the fractionation was due to a competition effect, induced by the concomitant presence of other metals, *e.g.* Zn (Deng et al. 2014). In the case of Zn 505 accumulation in wheat, a non-hyperaccumulating plant, it has been reported that the variation of Zn supply can 506 be a source of different trends of Zn isotopic fractionation in the soil-plant system. The lower availability of Zn 507 could enhance the release of organic ligands, such as phytosiderophores by the plant (Arnold et al. 2010), and 508 thus the Zn uptake from soil, with subsequent enrichment of the plant in heavy isotopes (Wiggenhauser et al. 509 2018). However, hyperaccumulators often have more specific processes of uptake and translocation of metals 510 than non-accumulating plants. For example, the Zn hyperaccumulator N. caerulescens has the ability to use both 511 high- and low-affinity transport systems depending on Zn availability, and this behaviour probably induces 512 different trends of Zn isotopic fractionation in the plants (Deng et al. 2014). In our case, the investigated R. cf. 513 bengalensis grew on ultramafic soils, and neither the scarcity of Ni nor the competition effect with other metals 514 can be considered as relevant factors determining Ni isotopic fractionation in the soil-plant system. In 515 hyperaccumulating plants, it has been assumed that Ni is taken up through a symplastic pathway, and that Ni 516 isotopic composition in whole plant derives from the uptake mechanisms of root cell membrane (Deng et al. 517 2014), promoting the uptake of lighter isotopes. Different pathways of fractionation within the plant parts can, 518 then, take place, but they are far from being fully understood. It has been recently reported that in wheat plants, 519 important Zn isotopic fractionation within plant parts can be attributed to the absorption of heavy isotopes to cell 520 walls in the apoplastic space (Wiggenhauser et al. 2018). However, through experiments based on the use of 521 radioactive ⁶³Ni, it was reported that Ni is transferred from roots to shoots via xylem, and also that its mobility is 522 greater than that of Zn, Mn, Co and Cd (Page and Feller 2005). These observations suggest that in 523 hyperaccumulators, Ni undergoes a very efficient translocation process that might not be the only source of 524 isotopic fractionation, or at most, it is responsible for the smaller isotopic fractionation observed for Ni compared 525 to e.g. Zn (Deng et al. 2014; Wiggenhauser et al. 2018) and Cd (Wiggenhauser et al. 2016). Whereas some 526 publications report demonstrations or assumptions about the translocation mechanisms of Ni (Kerkeb and 527 Krämer 2003; Alves et al. 2011), nothing is known about this mechanism for R. cf. bengalensis. In this study, 528 reported XAFS spectra on R. cf. bengalensis (Fig.3, Table 3) show that Ni in leaves is unequivocally present as a 529 complex with low molecular weight carboxylic acids (*i.e.* malate and citrate), confirming results reported by van 530 der Ent et al. (2017).

531

532 In the case of Zn and Cd, the isotopic fractionation in wheat shoots has been attributed to their different affinity 533 for functional groups of organic ligands (Wiggenhauser et al. 2018), or different chemical speciation. The 534 hypothesis about the possible influence of complexation with organic ligands on Ni isotopic fractionation was previously investigated (Zelano et al. 2018). However, a rather low potential of fractionation induced by complexation reaction between Ni and citric acid, oxalic acid and purified humic substances was reported with a maximum value of Δ^{60} Ni_{bond-free} < 0.2 ‰. Such a small fractionation can be excluded as a source of Δ^{60} Ni within plant material, discarding the hypothesis raised up for Zn fractionation in wheat shoots. The observed Δ^{60} Ni values within the two investigated specimen of *R*. cf. *bengalensis* cannot be fully explained by a complexation reaction or by different affinity towards organic molecules.

541

542 Despite only two specimens have been investigated in this work, one possible interpretation for the observed 543 Δ^{60} Ni might come from the different age of the two R. cf. bengalensis plants. Even though they grew on different 544 soils, both plants take up lighter Ni isotopes and the reported results suggest a correlation between the plant age 545 and the observed Δ^{60} Ni values. Both specimens have the highest concentration of Ni in leaves, confirming that 546 Ni is never stored in roots, but it is rapidly transferred to them, as it expected for Ni hyperaccumulators that do 547 not require defence mechanisms against high Ni concentration in soil. In the early stage of growth of the plant 548 (R150), 80 % of the total amount of Ni is mobilized and more than 70 % is concentrated in leaves. This transfer 549 produces a remarkable isotopic fractionation between leaves and roots, from which a preferentially translocation 550 of lighter isotopes, Δ^{60} Ni_{root-leaf} up to 0.51 ± 0.07 ‰, can be observed (Table 5). At the same time, an enrichment 551 of heavier isotopes in the ligneous parts of the plant is present. As the total amount of Ni in leaves is definitively 552 higher than in wood and bark, these results strongly suggest that lighter isotopes are transferred first, going from 553 roots to leaves. Data reported in Fig. 4 suggest, moreover, that the fractionation is correlated to the rate of Ni 554 translocation (lighter isotopes move faster) as an important fractionation is also observed as a function of height, 555 between younger (apical) and older (basal) parts of the plant. This implies that as soon as new leaves appear, Ni 556 is mobilized through leaves, and lighter isotopes arrive first. This trend is no longer observed in the older 557 specimen (R_{600}) , where the isotopic fractionation between basal and apical leaves is not evident anymore. This 558 result is surprising as Ni concentration still shows a clear trend between the apical and basal leaves, higher in 559 basal, and lower in apical ones. Moreover, the isotopic fractionation between leaves and other plant material is 560 less pronounced in the older specimen than in the younger plant (see Fig.4). This observation suggests that the 561 fractionation observed in R_{150} is not necessarily attributable to different transport or retention mechanisms, as it 562 is hypothesized for other metals, e.g. Cd (Wiggenhauser et al. 2016). If the fractionation were due to transport or 563 retention mechanisms, it would be accentuated in the older plant, but the opposite result is observed. In addition,

564 in R_{600} , all plant material has lighter isotopes than soil available Ni, but all aerial parts (leaves, bark, wood) are 565 enriched in heavier isotopes compared to roots.

566

567 Taken together, the available data suggest that during the first period of growth, when the plant starts to take up 568 Ni, the transfer of light isotopes from roots to leaves can be due to kinetic effects. Once the amount of Ni 569 accumulated in the plant has increased (see Ni concentration in R_{600} , Table 4), the Ni already present in the plant 570 circulates through the phloem tissue into all plant parts. Successive loading and unloading cycles would have, 571 therefore, the effect to homogenize the isotopic fractionation among leaves, and between leaves and the other 572 plant components, erasing the difference between new and old leaves. In old specimens, the consequent 573 increasing number of cycles that Ni makes within the plant through the circulation of phloem sap from top to 574 bottom, and inversely, would reduce the isotopic fractionation. The observed data in R_{600} are probably the results 575 of several additive processes and support the idea that in the hyperaccumulator R. cf. bengalensis, Ni is involved 576 in plant homeostasis as any other cation. However, to confirm this hypothesis, supplementary data should be 577 collected on a wider population of trees of R. cf. bengalensis, collected in their natural habitat and at different 578 stage of growth.

579 Conclusions

580 This study aimed to unravel the biogeochemical processes resulting in Ni isotopic fractionation in two different 581 soil-plant systems associated with the Ni hyperaccumulator plant R. cf. bengalensis. The investigated soils have 582 distinct stages of pedogenesis, which are both highlighted by the mineralogical composition and the isotopic 583 signature of the available pool of Ni. The Ferralsol SR₁₅₀ reflects a stable system, which has reached a steady-584 state equilibrium, subsequent to the complete weathering of primary minerals and for which Ni is mainly 585 associated to poorly crystalline Fe-oxyhydroxides. The isotopic signature has no variation, neither in soil bulk 586 surface horizons, nor in the available pool of Ni. In the partially weathered hypermagnesian Cambisol SR_{600} , Ni 587 is associated not only to Fe-oxyhydroxides, but also to the identified phyllosilicates, and even though the Ni 588 isotopic composition of bulk soils does not vary, an enrichment in heavy isotopes is observed with depth for the 589 Ni available fraction. In both soil systems, the two investigated specimens of the hyperaccumulator R. cf. 590 bengalensis accumulate lighter Ni isotopes from the soil available Ni pool. The Ni isotopic composition in the 591 different parts of the plants were investigated and correlated to the plant height and age. Despite the limited 592 number of investigated samples, the study highlights the potential effect of plant age on Ni isotopic fractionation

593 in hyperaccumulator plants. A translocation of lighter isotopes of Ni from roots to leaves was observed in the 594 young plant, Δ^{60} Ni_{root-leaf} up to 0.51 ± 0.07 ‰, and isotopic fractionation was also unravelled between the apical 595 and basal leaves, Δ^{60} Ni_{basal-apical} up to 0.23 ± 0.07 ‰ (Table 5). However, this effect was not detected in the older 596 plant, where no isotopic fractionation was observed as a function of plant height, and where the Ni isotopic 597 fractionation seems to be mask by successive cycles of Ni circulation through the plant. These results suggest 598 that once Ni is taken up from the soil by roots, it is rapidly transferred to leaves, without being permanently 599 stored there. Instead, it has an active and continuous cycle within the plant through phloem-mediated 600 redistribution. Once the plant has accumulated a substantial quantity of Ni, the isotopic fractionation induced by 601 Ni translocation among aerial plant material is masked by an isotopic homogenization, and the observed isotopic 602 signature is the result of consecutive internal translocation cycles. However, it is still not clear why the roots of 603 the older specimen have a lighter isotopic signature than in aerial parts.

604

The acquired results suggest that it is inordinately difficult to discriminate between specific, individual processes leading to Ni isotopic fractionation during Ni sorption and translocation within the plant, especially for hyperaccumuating plants. The comparison of two plants with a different stage of growth seems to highlight the role of plant's age on Ni isotopic fractionation. However, to better constrain Ni homeostasis and the contribution of *R*. cf. *bengalensis* to Ni isotopic signature in surface soil, more specimens should be investigated and compared, both in the early and in the advanced stages of growth.

611

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Table 1. Nickel concentration, μ mol g⁻¹, and isotopic composition δ^{60} Ni, in: i) bulk soil horizons collected at the bottom of *R*. cf. *bengalensis* R₁₅₀ and R₆₀₀; reported results are the average of three measurements for each sample with the corresponding instrumental standard deviation σ . The standard deviation obtained for the Ni ICP- MS standard solution (δ^{60} Ni = -0.11 ± 0.05 ‰) was applied to all sample results presenting analytical error $2\sigma < 0.05$ ‰. ii) Concentration and isotopic composition of Ni in solution after 24h of soil suspension in ultrapure water. Results are the average of triplicate samples and σ values were calculated as described in eq. 2, $\sigma = \frac{sd}{\sqrt{N}}$, where *sd* is the standard deviation of the sample mean, and N is the number of samples. The reference material BHVO-2 was processed and measured ten times, obtaining δ^{60} Ni = 0.05 ± 0.04 ‰ (2 σ).

Sample	B	ılk soil	Ni							
SR ₁₅₀ horizons	[Ni], µmol g ⁻¹	σ	δ^{60} Ni	2σ	[Ni], µmol g ⁻¹	σ	δ ⁶⁰ Ni	2σ	$\Delta^{60} Ni_{bulk}$ available	2σ
H1 Bulk	95.3	0.44	-0.17	0.05	0.35	0.02	-0.32	0.01	0.15	0.10
H2 Bulk	113	0.93	-0.16	0.05	0.34	0.0.02	-0.14	0.01	-0.02	0.07
H1 >1.5mm	85.8	0.86	/			/			1	
H1 250 - 50µm	77.6	0.78	/			/			/	
$H1 < 50 \mu m$	84.5	0.85	-0.17	0.05						
Sample	B	ılk soil	Ni			Available	Ni			
Sample SR ₆₀₀ horizons	Bi [Ni], µmol g ⁻¹	alk soil σ	Ni δ ⁶⁰ Ni	2σ	[Ni], µmol g ⁻¹	Available σ	Ni δ ⁶⁰ Ni	2σ	$\Delta^{60} { m Ni}_{ m bulk}$ available	2σ
Sample SR ₆₀₀ horizons H1 Bulk	Bi [Ni], μmol g ⁻¹ 61.0	alk soil σ 0.56	Ni δ ⁶⁰ Ni -0.01	2σ 0.05	[Ni], μmol g ⁻¹ 0.40	Available σ 0.02	Ni δ ⁶⁰ Ni 0.32	2σ 0.03	Δ^{60} Ni _{bulk} . available -0.33	2σ 0.06
Sample SR ₆₀₀ horizons H1 Bulk H2 Bulk	Bi [Ni], μmol g ⁻¹ 61.0 58.7	ulk soil σ 0.56 0.32	Ni δ ⁶⁰ Ni -0.01 0.01	2σ 0.05 0.06	[Ni], µmol g ⁻¹ 0.40 0.19	Available σ 0.02 0.05	Ni δ^{60} Ni 0.32 0.52	2σ 0.03 0.03	$\frac{\Delta^{60}\text{Ni}_{\text{bulk}}}{-0.33}$ -0.51	2σ 0.06 0.07
Sample SR ₆₀₀ horizons H1 Bulk H2 Bulk H3 Bulk	Bi [Ni], μmol g ⁻¹ 61.0 58.7 60.3	alk soil σ 0.56 0.32 0.35	Ni δ ⁶⁰ Ni -0.01 0.01 /	2σ 0.05 0.06	[Ni], μmol g ⁻¹ 0.40 0.19 0.09	Available σ 0.02 0.05 0.01	Ni δ^{60} Ni 0.32 0.52 0.62	2σ 0.03 0.03 0.02	$\frac{\Delta^{60}\text{Ni}_{\text{bulk}}}{-0.33}$ -0.51	2σ 0.06 0.07
Sample SR ₆₀₀ horizons H1 Bulk H2 Bulk H3 Bulk	Bi [Ni], μmol g ⁻¹ 61.0 58.7 60.3	ulk soil σ 0.56 0.32 0.35	<u>Ni</u> δ ⁶⁰ Ni -0.01 0.01 /	2σ 0.05 0.06	[Ni], μmol g ⁻¹ 0.40 0.19 0.09	Available σ 0.02 0.05 0.01	Ni δ^{60} Ni 0.32 0.52 0.62	2σ 0.03 0.03 0.02	$\frac{\Delta^{60}\text{Ni}_{\text{bulk}}}{-0.33}$ -0.51	2σ 0.06 0.07
Sample SR ₆₀₀ horizons H1 Bulk H2 Bulk H3 Bulk H1 >1.5mm	Bi [Ni], μmol g ⁻¹ 61.0 58.7 60.3 56.2	σ 0.56 0.32 0.35	Ni δ ⁶⁰ Ni -0.01 0.01 / /	2σ 0.05 0.06	[Ni], μmol g ⁻¹ 0.40 0.19 0.09	Available σ 0.02 0.05 0.01	Ni δ^{60} Ni 0.32 0.52 0.62	2σ 0.03 0.03 0.02	$\Delta^{60}\text{Ni}_{\text{bulk}}$ available -0.33 -0.51 /	2σ 0.06 0.07
Sample SR ₆₀₀ horizons H1 Bulk H2 Bulk H3 Bulk H1 >1.5mm H1 250 - 50µm	Bi [Ni], μmol g ⁻¹ 61.0 58.7 60.3 56.2 50.8	σ 0.56 0.32 0.35 0.56 0.51	Ni δ ⁶⁰ Ni -0.01 0.01 / / /	2σ 0.05 0.06	[Ni], µmol g ⁻¹ 0.40 0.19 0.09	Available σ 0.02 0.05 0.01	Ni δ^{60} Ni 0.32 0.52 0.62	2σ 0.03 0.03 0.02	$\Delta^{60}\text{Ni}_{\text{bulk.}}$ available -0.33 -0.51 /	2σ 0.06 0.07

Table 2. XAS data on SR₁₅₀ and SR₆₀₀ bulk soils, H1 horizons. 1-Summary of the local structural parameters derived from EXAFS data analysis (shell by shell fitting). The Ni EXAFS data were Fourier transformed over the k range Δk and fitted over the ΔR range to four single scattering paths involving O, Fe or Si atoms. The amplitude reduction factor S₀² was fixed to 0.9 as well as the energy shift on the basis of reference spectra analysis. The number of atoms of the first coordination shell was fixed to 6, but the number of atoms of other coordination shells was released. The distance R and disorder Δ^2 were varied for all the coordination shells. 2- Linear combination fitting was obtained on the fitting range Δk using as a maximum three components: Ni sorbed onto a purified clay in position of exchangeable cation, Ni sorbed onto goethite and Ni as structural cation in serpentine mineral.

				1-Structural	parameters				
	Fitting range ∆k(Å ⁻¹)	Fitting range ∆R (Å)	shell	Number of atoms	R(Å)	$\Omega_{i}(A_{i})$	ΔE (eV)	S ₀ ²	R factor
			Ni-O	6	2.030±0.01	0.00418±0.001			
	2 2 10	114	Ni-Fe	1.3±0.5	3.066±0.01	0.00423±0.001	5 70	0.00	0.00015
SR ₁₅₀	2.2-10	1.1-4	Ni-Fe	1.2±0.7	3.54 ±0.02	0.00618±0.0012	-3.79	0.90	0.00013
			Ni-O	2.6±0.6	3.82 ±0.02	0.00411±0.001			
			Ni-O	6	2.058 ±0.01	0.00454±0.001			
	2 5-10 4	1 1-4	Ni-Fe	0.7±0.6	3.118 ±0.01	0.0049±0.001	-4.12	0.90	0.000038
SR ₆₀₀	2.3-10.4	1.1-4	Ni-Si	1.6±0.6	3.261 ±0.02	0.0051 ± 0.0015	-7.12	0.90	0.000038
			Ni-O	3±0.6	3.92 ±0.02	0.0050±0.0009			
			2-Line	ear combination	n fitting param	eters			
	Fitting range ∆k(Å ⁻¹)	Ni sorbed onto Clay	Ni-Goethite	Ni- Serpentine					R factor
SR ₁₅₀	2-10	50.3+5	49.7±5						0.098
SR ₆₀₀	2-10	59 ±4	16±2	26±3					0.054

Table 3. XAS data on *R. bengalensis* leaves - Linear combination fitting was obtained on the fitting range Δk using as a maximum three components from a library of reference spectra obtained from organic complexes of Ni as well as from Ni aqueous solution. Ni-malate, Ni-histidine and Ni-citrate solutions were prepared at pH 6 with a metal/ligand ratio of 7.

Linear combination fitting parameters												
	Fitting range $\Delta k(A^{-1})$ Ni aqueous Ni-malate Ni-histidine Ni-citrate R factor											
R ₁₅₀	R_{150} 2-10.5 31±4 42±8 27±4 0.026											
R ₆₀₀	2-10.5	33±3	41±6		26±4	0.020						

Table 4. Plant mass (g), Ni concentration (μ mol g⁻¹) and isotopic composition (δ^{60} Ni) of *R. bengalensis* R₁₅₀ and Ni concentration (μ mol g⁻¹) and δ^{60} Ni values of R₆₀₀ specimens are reported, in blue and green, respectively. All results are the average of three measurements for each sample and the relative standard deviations correspond to the instrumental error. When the 2 σ associated to δ^{60} Ni values are < 0.05 ‰, the value of 2 σ = 0.05 ‰ obtained for Oak Leaves reference material V464 (δ^{60} Ni = -0.02 ± 0.05 ‰, N=6) is used. The Ni concentration and the δ^{60} Ni of the whole plant have been calculated as the weighted average values. The corresponding 2 σ values are calculated according to equation 5.

R ₁₅₀	Height	Mass, g	[Ni], µmol g ⁻¹	σ	δ ⁶⁰ Ni	2σ	R ₆₀₀	Height	[Ni], µmol g ⁻¹	σ	δ ⁶⁰ Ni	2σ	
	50cm	6	171	1.10	-0.66	0.05		2 m	522	7.84	-0.33	0.04	
	60 cm	7.6	156	0.62	-0.75	0.05		3 m	349	5.50	-0.19	0.05	
Leaf	80 cm	4.7	221	1.60	-0.78	0.05	Leaf	4 m	309	5.94	-0.20	0.04	
	110 cm	8.5	134	2.40	-0.82	0.05		5 m	220	6.85	-0.30	0.02	
	150cm	9.6	138	2.72	-0.89	0.05		6 m	156	1.17	-0.25	0.08	\setminus
								- 5 cm	9.56	0.04	0.12	0.05	
	20 cm	0.5	68.7	0.78	-0.29	0.05		1 m	133	1.23	-0.06	0.04	
	25cm	0.5	62.2	0.49	-0.22	0.05		2 m	149	2.37	-0.19	0.03	
Bark	50 cm	0.5	58.0	0.37	-0.40	0.10	Bark	3 m	108	1.13	-0.21	0.05	
	60cm	0.5	43.0	0.46	-0.26	0.05		4 m	105	1.49	-0.29	0.07	
	80 cm	0.5	67.5	1.07	-0.16	0.05		5 m	92.8	0.96	-0.13	0.09	
	100 cm	0.5	98.6	0.73	-0.15	0.09		6 m	52.6	0.38	0.15	0.05	
	20 cm	12.4	6.68	0.04	-0.29	0.13	Distances	-5 cm	545	7.05	-0.60	0.08	
	25cm	1.81	9.39	0.12	-0.32	0.05	Phloem	5 m	11.7	0.17	-0.10	0.06	
Wood	50 cm	18.9	15.6	0.15	-0.13	0.07							
wood	60cm	2.35	11.9	0.11	-0.17	0.05							
	80 cm	1.34	28.1	0.38	-0.05	0.05							
	100 cm	0.76	69.9	0.62	-0.50	0.05							
Deet	-5 cm	10	81.7	0.95	-0.35	0.05	Deet	-5 cm	63.2	0.24	-0.58	0.05	
KOOL	-15 cm	5	155	1.93	-0.42	0.03	KUUL	-15 cm	111	0.40	-0.59	0.06	
Whole plant		91.96	87.20	70.65	-0.65	0.23							

Table 5. Nickel isotopic fractionation Δ^{60} Ni, calculated between bulk soil and average δ^{60} Ni in roots, Δ^{60} Ni_{bulk-root}, between available Ni and roots, Δ^{60} Ni_{available-root}, and between roots and leaves, Δ^{60} Ni_{root-leaf}. Reported 2 σ values were calculated applying the error propagation formulas reported in equation 4.

SR ₁₅₀	$\Delta^{60} Ni_{bulk \; soil-root}$	2σ	$\Delta^{60} Ni_{available}$ - root	2σ	Plant height	$\Delta^{60} Ni_{root\text{-leaf}}$	2σ
H1	0.21	0.12	0.06	0.12	50cm	0.28	0.08
H2	0.22	0.09	0.24	0.09	60 cm	0.37	0.08
					80 cm	0.40	0.08
					110 cm	0.44	0.08
					150 cm	0.51	0.07
SR ₆₀₀	$\Delta^{60} Ni_{bulk}$ -root	2σ	$\Delta^{60} \mathrm{Ni}_{\mathrm{available}}$ - root	2σ	Plant height	$\Delta^{60} Ni_{root\text{-leaf}}$	2σ
H1	0.58	0.08	0.91	0.09	2 m	-0.26	0.08
H2	0.60	0.09	1.11	0.08	3 m	-0.40	0.08
H3			1.21	0.07	4 m	-0.39	0.08
					5 m	-0.29	0.07
					6 m	-0.34	0.11



Fig.1 XRD patterns obtained on bulk soils, H1 horizons of SR_{150} and SR_{600} (A). TEM micrographs of the fine fraction <50 μ m of SR_{150} (B) and SR_{600} (C) soils, H1 horizons.



Fig.2 EXAFS oscillations obtained at Ni-K edge, Linear combination fitting (left graphs) and theoretical fitting (or shell by shell fitting, right graphs), for SR_{150} (up) and SR_{600} (down). Fitting parameters are reported in Table 2.



Fig.3 EXAFS oscillations at Ni K edge of *R*.cf. *bengalensis* leaves from R_{150} and R_{600} specimen. Oscillations are fitted as a combination of reference spectra (see Table 3 for fitting parameters).



Fig.4 Nickel isotopic composition, δ^{60} Ni, in leaf, root, bark and wood samples of *R*. cf. *bengalensis* specimen R₁₅₀, and in leaf, bark and phloem tissue samples of the R₆₀₀ specimen. All results are the average of three measurements for each sample and the relative standard deviations correspond to the instrumental error. When the 2σ associated to δ^{60} Ni values are < 0.05 ‰, the value of $2\sigma = 0.05$ ‰ obtained for Oak Leaves reference material V464 (δ^{60} Ni = -0.02 ± 0.05 ‰, N=6) is used.

Table SI 1. Elemental composition of SR_{150} and SR_{600} soil samples in µmol kg⁻¹ for trace elements, oxide percentages for major elements and percentage of organic carbon. The range of standard deviation associated to reported results is between 5% and 20% for trace elements (5% for Ni), between 2% and 10% for major and minor elements, and 2% for organic carbon, as reported by the Service d'Analyse des Roches et des Minéraux, at Centre de Recherches Pétrographiques et Géochimiques, Nancy, France.

$\mu mol~kg^{\text{-1}}$ and %	Cu	Pb	Sc	Co	Cr	Zn	%SiO2	%Al ₂ O ₃	%Fe ₂ O ₃	%MnO	%MgO	%CaO	%Na ₂ O	%K ₂ O	%TiO ₂	%C _{org}
SR150 H1 bulk	591	24.71	663	7.46	283	3.59	28.9	4.36	34.0	0.47	1.43	0.49	< d.l.	0.10	0.15	7.44
SR_{150} H2 bulk	556	23.36	773	8.89	364	3.66	30.5	5.15	39.8	0.52	1.61	0.14	< d.l.	0.07	0.18	2.91
SR150 H1 >1.5mm	517	24.07	686	7.67	302	3.62	30.2	4.53	35.1	0.48	1.46	0.41	< d.l.	0.09	0.16	/
SR150 H1 250 - 50µm	818	33.18	630	6.91	313	3.80	26.7	4.20	31.5	0.43	1.42	0.62	< d.l.	0.10	0.14	/
$SR_{150}H1 < 50 \mu m$	1037	24.40	598	5.46	206	3.36	34.6	3.80	29.9	0.35	1.16	0.55	< d.l.	0.10	0.14	/
SR600 H1 bulk	573	12.79	424	4.65	276	3.95	29.2	4.96	14.6	0.35	10.77	1.06	0.21	0.04	0.20	11.84
SR600 H2 bulk	510	13.48	566	6.74	358	4.89	36.9	6.58	19.3	0.51	13.21	1.09	0.28	< d.1.	0.24	3.01
SR600 H3 bulk	548	13.70	596	8.39	381	4.70	35.4	7.02	22.1	0.64	13.41	0.97	0.29	< d.1.	0.23	1.64
SR ₆₀₀ H1 >1.5mm	586	11.57	463	6.12	296	3.83	31.2	5.40	16.3	0.49	11.48	1.03	0.21	0.04	0.20	/
SR600 H1 250 - 50µm	902	28.74	370	2.97	245	3.32	26.5	4.40	11.3	0.24	9.61	1.26	0.22	0.05	0.20	/
$SR_{600} \; H1 \; < 50 \mu m$	1695	41.18	380	2.84	278	3.82	35.8	4.55	10.8	0.24	10.25	1.33	0.28	0.06	0.16	/

Soil horizon	Na ⁺	K^+	Ca ²⁺	Mg^{2+}	Fe ³⁺	Al^{3+}	Si^{4+}	Ni ²⁺	Total CEC
SR150 H1	0.08	0.79	14.4	5.8	<d.l.< td=""><td><d.1.< td=""><td>1.24</td><td>0.30</td><td>22.7</td></d.1.<></td></d.l.<>	<d.1.< td=""><td>1.24</td><td>0.30</td><td>22.7</td></d.1.<>	1.24	0.30	22.7
$SR_{150}H2$	0.03	0.24	3.66	2.08	<d.l.< td=""><td>0.07</td><td>1.15</td><td>0.49</td><td>7.72</td></d.l.<>	0.07	1.15	0.49	7.72
SR ₆₀₀ H1	0.09	0.59	10.0	42.4	<d.1.< td=""><td><d.1.< td=""><td>1.09</td><td>0.38</td><td>54.6</td></d.1.<></td></d.1.<>	<d.1.< td=""><td>1.09</td><td>0.38</td><td>54.6</td></d.1.<>	1.09	0.38	54.6
SR ₆₀₀ H2	0.05	0.17	3.22	20.1	<d.l.< td=""><td><d.l.< td=""><td>1.15</td><td>0.23</td><td>24.9</td></d.l.<></td></d.l.<>	<d.l.< td=""><td>1.15</td><td>0.23</td><td>24.9</td></d.l.<>	1.15	0.23	24.9
SR ₆₀₀ H3	0.05	0.06	1.21	16.1	<d.1.< td=""><td>0.05</td><td>1.17</td><td>0.12</td><td>18.7</td></d.1.<>	0.05	1.17	0.12	18.7

Table SI 2. Exchangeable cations and total soil cation exchange capacity (CEC mEq per 100 g of soil), determined in bulk samples of different horizons of SR_{150} and SR_{600} .

Tree	Haisht	Ma	V	Ca	Ma	Ea	NI:	7	C.	C···	Ca
part	Height	Mg	К	Ca	Mn	Fe	IN1	Zn	Cr	Cu	Co
	50 cm	132±0.9	483±3.8	749±22.4	12.7±0.14	10.6±0.16	171±1.10	2.56±0.01	0.1±0.01	0.07 ± 0.01	1.78±0.03
	60 cm	131±0.24	459±2.0	785±21.3	12.5±0.06	13.3±13.6	156±0.62	2.83±0.03	0.2±0.01	0.07 ± 0.01	1.74±0.01
Leaf	80 cm	85±0.73	516±1.4	621±19.7	10.1±0.02	8.37±0.37	221±16.0	6.73±0.07	0.1±0.01	0.06±0.01	1.59±0.01
	110 cm	139±0.60	366±3.5	691±22	14.8±0.12	10.6±0.85	134±2.40	3.03±0.05	0.1±0.01	0.09±0.01	1.68±0.03
	150 cm	172±1.78	546±6.3	651±31.0	14.8±0.31	9.08±1.85	138±2.75	3.61±0.05	0.1±0.01	0.09 ± 0.01	1.47±0.04
	20 cm	41.5±0.17	244±1.2	1697±38.4	2.0±0.03	19.9±1.16	68.7±0.78	6.76±0.10	0.2±0.01	0.06 ± 0.01	0.18±0.0
	25 cm	41.1±0.12	203±1.2	2093±10.4	2.3±0.01	27.2±0.83	62.1±0.49	7.42 ± 0.06	0.2±0.0	0.09±0.0	0.19±0.0
Daula	40 cm	62.9±0.13	221±2.4	1669±41.6	4.4±0.06	17.6±1.48	58±0.37	3.54 ± 0.07	0.0 ± 0.0	0.11±0.01	0.27±0.01
вагк	60 cm	46.1±0.26	243±1.0	1519±32.4	5.4±0.06	14.5±1.24	43.0±0.46	2.38±0.01	0.0	0.12±0.01	0.24±0.01
	80 cm	71.2±0.03	344±3.3	1404±48.6	3.0±0.04	18±2.15	67.5±1.07	3.69±0.07	0.7±0.64	0.15±0.01	0.23±0.01
	90 cm	107±1.28	507±2.3	1364±25.4	2.01±0.02	15.5±1.42	98.6±0.73	4.43±0.01	0.0 ± 0	0.13±0.73	0.3±0.01
	20 cm	22.6±0.15	128±2.0	333±20.9	0.2±0.01	4.47 ± 0.58	6.7±0.04	0.25	<d.1.< td=""><td>0.02±0</td><td>0.02±0.0</td></d.1.<>	0.02±0	0.02±0.0
	25 cm	22.9±0.13	154±2.8	454±26.1	0.3±0.01	7.48±0.62	9.4±0.12	0.36±0.01	<d.1.< td=""><td>0.05±0.01</td><td>0.03±0</td></d.1.<>	0.05±0.01	0.03±0
Wood	40 cm	34.4 ± 0.08	224±37.3	666±32.9	0.4±0.02	8.96±1.02	15.6±0.15	0.59±0.01	<d.1.< td=""><td>0.08 ± 0.01</td><td>0.05±0</td></d.1.<>	0.08 ± 0.01	0.05±0
wood	60 cm	36.1±0.37	205±3.2	673±20.2	0.4±0.01	8.14±0.80	11.9±0.11	0.89 ± 0.02	<d.1.< td=""><td>$0.04{\pm}0.01$</td><td>0.04±0</td></d.1.<>	$0.04{\pm}0.01$	0.04±0
	80 cm	47.9±0.66	305±1.7	623±18.3	0.5±0.01	8.16±0.86	28.1±0.38	0.88 ± 0.01	<d.1.< td=""><td>0.20±0.02</td><td>0.07±0.0</td></d.1.<>	0.20±0.02	0.07±0.0
	100 cm	65.7±0.30	412±3.3	629±34.1	0.8±0.02	$10.0{\pm}1.08$	69.9±0.62	1.67 ± 0.02	<d.1.< td=""><td>$0.20{\pm}0.02$</td><td>0.16±0.0</td></d.1.<>	$0.20{\pm}0.02$	0.16±0.0
						-				-	
Poot	-5 cm	99.1±1.25	237±2.7	963±31.7	4.5±0.04	44.2±1.66	154±1.93	2.07 ± 0.03	0.8±0.01	0.06±0.0	0.38±0.0
ROOL	-15 cm	85.7±0.66	155±1.4	414±16.4	3.2±0.05	169±2.78	81.7±0.95	2.37 ± 0.02	4.1±0.35	0.07 ± 0.0	0.47±0.01
1.1 1						•				•	

Table SI 3. Element concentrations (μ mol g⁻¹) in leaf, bark, wood and root samples of *R. bengalensis* R₁₅₀ plant. Results are the average of three measurements for each samples. Standard deviation correspond to the triplicate of the measurements.

Tree part	Height	Mg	К	Са	Mn	Fe	Ni	Zn	Cr	Cu	Со
	2m	149±0.75	684±10.7	995±36.8	1.98 ± 0.03	9.99 ± 1.18	522±7.83	3.76±0	0.06 ± 0.01	0.05 ± 0.01	$0.32{\pm}0.01$
	3m	183±1.42	463±3.37	923±22.1	1.83±0.02	8.93±0.44	349±5.50	2.15±0	0.09±0.01	0.05±0.0	0.19±0.01
Leaf	4m	144±1.77	552±13.7	854±28.3	1.86 ± 0.03	8.95±0.80	309±5.94	1.31 ± 0.01	0.07 ± 0.00	0.06±0.0	0.20±0.0
	5m	209±3.22	686±21.5	1180±221	1.86±0.11	20.2±7.51	220±6.85	2.13±0.01	0.21±0.15	<d.l.< td=""><td>0.12±0.02</td></d.l.<>	0.12±0.02
	6m	97±0.93	436±26.5	873±151	2.15±0.02	13.7±2.02	156±1.17	1.15 ± 0.01	0.21±0.05	<d.l.< td=""><td>0.17 ± 0.01</td></d.l.<>	0.17 ± 0.01
				•					•	•	
	1m	36±0.22	278±3.95	1565±35.7	0.53±0.01	14.2±0.45	133±1.23	1.94±0.0	0.02 ± 0.00	0.06±0.01	0.03±0
	2m	33±0.37	305±4.98	1544±25.2	0.66 ± 0.02	14.1±0.68	149±2.37	1.60±0.0	$0.02{\pm}0.0$	0.05 ± 0.01	0.04±0
Pork	3m	33±0.18	287±4.81	1486±35.1	0.71 ± 0.01	14.2±1.42	108±1.13	0.66±0.0	0.01±0.0	0.07 ± 0.01	0.04±0
Daik	4m	33±0.37	450±4.62	1553±14.8	1.49±0.01	14.0±0.5	105±1.49	0.87 ± 0.0	0.01±0.0	0.07 ± 0.0	0.08±0
	5m	50±0.47	293±6.26	1770±39.2	2.21±0.02	16.4±0.9	93±0.96	1.40 ± 0.0	0.01±0.0	0.08±0.0	0.10±0
	6m	78±0.30	155±1.22	742±23.3	0.49±0.0	6.8±0.5	53±0.38	0.78±0.0	0.01±0.0	0.03±0.02	0.02±0
Dhloom	-5 cm	127.1±0.74	510±5.22	1488±35.1	10.6±0.09	16.33±1.09	545±7.05	19.4±0.02	0.26±0.01	0±0	0.6±0
rmoem	5 m	71±0.76	344±10.4	580±79.0	$0.39{\pm}0.04$	3.50±2.97	12±0.24	0.09 ± 0.00	0.01 ± 0.01	0.07±0.03	0.02±0
				•					•	•	
Root	-5 cm	91.2±1.09	228±3.40	301±12.9	1.37 ± 0.01	22.3±0.38	63.2±0.24	3.03±0.01	1.50±0.07	0.11±0.0	0.09±0
KUUI	-15 cm	186±0.61	188±10.4	447±232	2.60±0.08	41.9±8.77	111±0.4	5.57±0.00	4.35±0.07	0.22±0.08	0.17±0.01

Table SI 4. Element concentrations (μ mol g⁻¹) in leaf, bark, and phloem samples of *R. bengalensis* R₆₀₀ plant. Results are the average of three measurements for each samples. Standard deviation correspond to the triplicate of the measurements.

Notes

The concentrations of Ca and K in the plant material are relatively high compared to the amount of exchangeable cations in the corresponding soils. In both plants, the highest Ca concentration is in the bark, where it reaches 2000 μ mol g⁻¹ and 1700 μ mol g⁻¹ in R₁₅₀ and R₆₀₀, respectively. The highest K concentrations is recorded in the leaves, 546 μ mol g⁻¹ in R₁₅₀ and 686 μ mol g⁻¹ R₆₀₀. Despite the fact that exchangeable Mg in the soil is 10-fold higher in SR₆₀₀ than in SR₁₅₀, Mg concentrations are similar in both plants, *e.g.* ~150 μ mol g⁻¹ in the leaves. Other elemental concentrations, such as Fe, Mn, Cu and Zn are in the ranges previously reported for *R. bengalensis*(van der Ent et al., 2017).

Some clear trends are found in R_{150} , the Mg and K concentrations in the bark and wood increase with height, from the bottom to the top. This trend is also observed for Mg in bark of R_{600} , but no trend is observed for K. For the trace elements no clear trends is found. Cobalt is more concentrated in R_{150} leaves than in R_{600} leaves, although Co contents in SR₁₅₀ and SR₆₀₀ soils are in the same range of order.



Figure SI 1. XRD patterns obtained on SR_{150} bulk soil, horizon H1 (0-5 cm) and H2 (5-15 cm). The pattern demonstrates the predominance of goethite and quartz, associated to one phyllosilicate (talc), no clay minerals or other silicate could be evidenced.



Figure SI 2. XRD patterns obtained on SR_{600} bulk soil, horizon H1 (0-5 cm), H2 (5-10 cm) and H3 (10-15 cm). The patterns evidence a predominance of phyllosilicates, chlorite, talc and 7Å phyllosilicate (serpentine or kaolinite). Primary minerals such as amphibole and feldspars were also detected. Quartz and goethite appear as minor phases.



Figure SI 3. XANES spectra at Ni-K edge from SR₁₅₀ and SR₆₀₀ soils (surface horizon H1, bulk soil samples).

References

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