Evidence for nickel mobilisation in rhizosphere soils of Ni hyperaccumulator *Odontarrhena serpyllifolia*

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Abstract

Purpose Rhizosphere processes are known to modify uptake of elements from soil, but limited information is available for hyperaccumulators. We investigated labile Ni fractions and their kinetics of replenishment in the rhizospheres of the Ni-hyperaccumulator *Odontarrhena serpyllifolia*, the Ni-excluder *Holcus lanatus* and in bulk soils collected at the same serpentine outcrop.

Methods Labile Ni fractions in rhizosphere and bulk soil were evaluated using conventional extractions and also by the Diffusive Gradients in Thin Films (DGT) technique. DGT data were used to predict the kinetics of Ni resupply using DIFS modelling. Chemical imaging of Ni distribution

along roots using DGT coupled with laser-ablation was conducted.

Results Labile Ni forms were higher in both rhizosphere than in bulk soils, together with an increase in dissolved organic C, cation exchange capacity and the Ca/Mg ratio. Ni fractionation indicated a shift towards less stable Ni fractions in the rhizosphere, particularly in the hyperaccumulator.

DIFS modelling showed that the rhizosphere of the excluder was able to sustain the initially lower soluble Ni concentration through replenishment from the solid phase, while Ni resupply in the rhizosphere of the hyperaccumulator was not sufficient to maintain the initially high concentrations of soluble Ni. However,

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the amount of DGT-labile Ni was higher in the rhizosphere of the hyperaccumulator compared to the excluder in all deployment times.

Conclusion Our data suggest that compounds derived from root activity, in particular DOC, are important controls of Ni availability to plants growing on serpentine soil.

Keywords Serpentine soil · DGT · Phytomining · Ni kinetics · Ni replenishment · High resolution imaging

Introduction

The unusual ability of Ni-hyperaccumulators to accumulate Ni in their harvestable tissues makes these plants particularly useful for phytomining (Brooks et al. 1998; Chaney et al. 2007). Phytomining offers the opportunity to recover valuable metals, such as Ni, from metal-enriched biomass of hyperaccumulators. Ni extraction efficiency from this bio-ore has been shown to be high (up to 25% Ni in plant ashes) since these ashes, present lower quantities than conventional ores of Fe and Mg, which are considered impurities and difficult metal recovery increasing its costs (Chaney et al. 2007; Zhang et al. 2014). Moreover, crops of hyperaccumulators can be grown in low-grade ores (usually lower than 3%) which could not be exploited using conventional mining extraction techniques (van der Ent et al. 2015). For phytomining to be economically viable it is necessary to maximise the Ni uptake efficiency of hyperaccumulators and their biomass yield. Agronomic practices such as bioaugmentation with beneficial microorganisms or soil fertilisation regimes have been shown to significantly increase biomass production of metal hyperaccumulators (Abou-Shanab et al. 2006; Bani et al. 2015; Cabello-Conejo et al. 2014). Since metal sustained availability in the substrate greatly influences the success of phytomining, in-depth studies of rhizosphere processes determining metal availability and replenishment could help to further improve soil management practises aimed at increasing Ni availability. To date, mostly non-specific rhizosphere mechanisms have been proposed to explain metal uptake by these plants, and several studies have reported that hyperaccumulators access the same metal pools as non-accumulating plants (Echevarria et al. 2006; Hammer et al. 2006; Hutchinson et al. 2000; Massoura et al. 2004; Salt et al. 2000). Massoura et al. (2004)

studied an excluder plant species (*Triticum aestivum* L. cv. Sponson), an indicator species (*Trifolium pratense* cv. Violetta) and three populations of the Ni-hyperaccumulator *Alyssum murale* (Waldst. & Kit.) (now *Odontarrhena chalcidica*) to determine if the labile Ni pool in the soil varied among the plant species. Results showed that, for a given soil, the labile pools were similar for all three plant species and that they all accessed the same Ni exchangeable pool regardless of their Ni uptake capacity. Other studies such as that of Fitz et al. (2003) found sustained As solubility in the rhizosphere of the As-hyperaccumulator *Pteris vitatta* L. even though the labile As pool and resupply rates were decreased with time. These authors suggested that changes in the resupply kinetics of the soil in response to root activities and plant uptake rates could be a critical factor determining uptake rates in phytoextraction crops.

The labile metal concentration estimated through batch extractions (equilibrium-based) is often poorly correlated with plant metal uptake, making it difficult to elucidate the effects of hyperaccumulators on soil metal availability; this can be explained by the poor mechanistic meaning of batch extraction as metal uptake is not only controlled by the concentration in the soil solution but also by the transport towards the plant root and the rate of resupply from the soil solid phase (Degryse et al. 2009). Diffusive gradient in thin films (DGT) was developed by Davison and Zhang (1994) and removes metal from soil solution inducing a resupply of ions from the solid phase simulating a plant root (Ernstberger et al. 2005; Lehto et al. 2006a).

For the case of diffusion-limited metal uptake, metal availability determined by DGT has been shown to correlate better with plant metal concentrations than other estimations of labile metal fractions (Degryse et al. 2009; Puschenreiter et al. 2013). DGT also measures the extent of the depletion caused by the resin gel through the R ratio (Ernstberger et al. 2005; Harper et al. 1998). The R ratio is affected by the size of the labile solute fraction associated to the solid phase and by the time of the solid phase to resupply to the soil solution the element depleted by the gel uptake (response time). DIFS (DGT Induced Fluxes in Sediments) models the dependence of R on two main parameters named as K_D , (distribution ratio of metals between the solid phase and the soil solution), and as T_C , (rate of exchange between both pools) (Sochaczewski et al. 2007). By fitting the development of R over time, DIFS can be used to estimate K_D and T_C .

DGT together with laser-ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) also allows chemical imaging of labile metals distribution (Kreuzeder et al. 2013; Santner et al. 2015). High-resolution imaging of DGT-measured metal fluxes showed increased fluxes of Cd and Zn around the roots of the Cd/Zn-accumulator *Salix* × *smithiana* Willd. (*S. caprea* L. × *S. viminalis* L., clone BOKU 03 CZ-001) (Hoefer et al. 2015). However, this technique has not yet been used to assess Ni fluxes in Ni-hyperaccumulating plant species.

The aim of this work was to compare plant-induced effects on Ni availability and distribution of Ni fractions between the Ni-hyperaccumulator *Odontarrhena serpyllifolia* (Desf.) Jord. & Fourr. and the Ni-excluder *Holcus lanatus* L.. Both plant species can be found growing at the same serpentine outcrop in the NW of the Iberian Peninsula and present a clear different strategy to survive with the high Ni concentrations at this site. The Ni availability of non-vegetated and rhizosphere soils was assessed using conventional extractants and the DGT technique. Ni fractionation was estimated using a sequential extraction procedure, while Ni kinetics and the soil replenishment capacity were assessed through the C_{DGT}/C_{soln} ratio and DIFS modelling. Chemical imaging of labile Ni distribution along the root axis was also studied through DGT in both plant species growing in serpentine soil in rhizotrons.

Material and methods

Study site description and sample collection

Plants and bulk soils were collected in the serpentinic area of Barazón (NW Spain), which belongs to one of the main allochthonous ophiolitic units in the Iberian Peninsula. The site is included in Serra do Careón and covers an area of approximately 65 km² which is considered a Special Area of Conservation (SAC) and is included within the Natura 2000 Network (EC 2000). The soils show abundant rocky outcrops with limited edaphic evolution, and the most frequent soils are Mollic Leptosols and Haplic Phaeozems (IUSS 2015). Barazón has a European humid-temperate climate (Carballeira et al. 1983) with a mean annual temperature of 12.1 °C and mean annual precipitation of 1257 mm (mean data for the last 10 years (2008–2017), data obtained from the Meteorological Observation and

Prediction Unit of Galicia (MeteoGalicia)). The whole plant and root system (including the root ball) of five to six individuals of the Ni-hyperaccumulator *Odontarrhena serpyllifolia* (previously known as *Alyssum serpyllifolium* subsp. *lusitanicum* Dudley and P. Silva synonym *A. pintodasilvae*) and of the non-accumulator *Holcus lanatus* L. were collected. The Ni-hyperaccumulating populations of *O. serpyllifolia* are distributed through three ultramafic areas of the Iberian Peninsula (NE Portugal, NW Spain and S Spain). Rhizosphere soil was operationally defined as the soil attached to roots after gentle crushing of the root ball and shaking the root system. To the extent possible, root debris included in the collected rhizosphere soil were removed using tweezers or by sieving. In addition, five surface soil samples (0–15 cm) were collected at each site from bare patches where no plants were growing (bulk soil). Bulk and rhizosphere soils were sieved through a 2-mm stainless steel sieve. For each plant species, the rhizosphere soil samples were pooled together and thoroughly mixed to form one single composite sample.

Plant and soil analysis

Plant material was separated into shoots and roots, washed with pressured tap water followed by deionised water (conductivity 5.0 µS cm⁻¹, resistivity 15 MΩ.cm), oven-dried at 45 °C, weighed and ground using a Polymix PX-MFC 90 D mill (Kinematica™). Plant tissues (approximately 0.1 g) were digested in a 2:1 concentrated HNO_3 :HCl mixture on a hot plate at 160 °C. The Ca, Mg, K, P, Fe, Co, Cu, Mn and Ni concentrations were measured by inductively coupled plasma optical emission spectrometry (ICP-OES, Varian Vista Pro, Australia). Data were expressed on the basis of dry weight of plant material. Three (or five in the case of DGT analysis) technical replicates of air-dried soil were then analysed for each composite sample. Soil pH was measured in H_2O and 0.1 M KCl (1:2.5 w/v). Available P was determined by Olsen's NaHCO_3 method (Olsen et al. 1954). Cation exchange capacity (CEC) was estimated using 1 M NH_4Cl as the sum of the exchangeable cations Ca, Mg, Al, Na and K. The Ca/Mg ratio was calculated using the exchangeable values (cmol_c kg⁻¹) of Ca and Mg. Dissolved organic C (DOC) was determined in 1:2.5 (w/v) soil: H_2O extracts after 2 h of shaking (adapted from Jones and Willett (2006) 1 h shaking 1:5 (w/v) soil: H_2O ratio). Samples

were centrifuged at $3750 \times g$ for ten minutes, filtered at $0.22 \mu\text{m}$ (PES Syringe Filters, SFPE-22E-100 25 mm diameter, sterile) and acidified with HNO_3 (70%). DOC concentration was determined using a Vario TOC Cube (Elementar Analysensysteme GmbH, Hanau, Germany).

Nickel fractionation was determined according to the method of Zeien and Brümmer (1989) (Fig. 1). A more detailed description of the protocol used is presented in Online Resource 1 (ESM 1). The Ni concentration was analysed in all the extracts by ICP-OES. Due to analytical problems in the performance of F5 extraction, both Fe oxides fractions (F5 + F6) were considered as one unique fraction: Ni bound to Fe oxides (both amorphous and crystalline oxides).

Extractable soil metal fractions were assessed by two single extractions: (a) 0.01 M $\text{Sr}(\text{NO}_3)_2$ (Everhart et al. 2006) 1:4 w/v, 2 h shaking and (b) 0.05 M $\text{Ca}(\text{NO}_3)_2$ (McLaren et al. 2007) 1:4 w/v, 2 h shaking) and labile Ni was estimated by DGT (detailed in the following section).

* Due to problems in the fractionation performance F5 and F6 were considered together as only one fraction.

Fig. 1 Summary for the nickel fractionation method (adapted from Zeien and Brümmer (1989))

Further, extractable fractions of Ca, K, Mg, Mn, Fe, Co and Al were also measured in the same soil extracts ($\text{Sr}(\text{NO}_3)_2$, $\text{Ca}(\text{NO}_3)_2$ as well as in all the fractions of the sequential extraction (although only data from the NH_4NO_3 -extractable fraction is presented).

Prior to analysis of pseudo-total element fraction, soils were ground using an agate mortar (Retsch RM 200). Total C and N were analysed by combustion with a CHN analyser (Model CHN-1000, LECO Corp., St Joseph, MI). Soils (0.2 g) were digested in aqua regia (a 3:1 mixture of concentrated HNO_3 :HCl) and Ni concentrations were determined by ICP-OES. Detailed information of soil digestion is included in ESM 1. Blanks and soil reference materials (ultramafic rock SO-3, Canadian Certified Reference Materials Project) were also included in each digestion batch to ensure quality control.

All extraction mixtures were centrifuged for 15 min at $3750 \times g$. The soil extracts and the corresponding blanks were filtered through cellulose paper (Filter-Laboratory 1242, 90-mm diameter, 7–9 μm pore) and analysed by ICP-OES.

Diffusive gradient in thin films (DGT) measurements

Due to the small amount of rhizospheric soil available, a reduction in the recommended amount of soil used for the DGT deployments (following the approach by Muhammad et al. (2012)) had to be carried out.

Fresh soil composite samples used for DGT deployments were kept at 4°C for one week until analysis. Soil slurries were prepared by mixing field-moist and sieved (2-mm mesh) rhizosphere soil with deionised water up to the soils' maximum water holding capacity (MWHC). Five replicates were analysed for each soil and four deployment times were conducted (24, 72, 168 and 264 h). Sets of replicate slurries from each soil were prepared 48 h before DGT deployments to allow for equilibration. Soil solution was sampled at time 0 from the soil slurry (using an aliquot not deployed by DGT) as follows: slurries were transferred to plastic tubes and centrifuged at $3750 \times g$ for 10 min. The supernatant was removed, filtered using $0.22 \mu\text{m}$ filter paper (PES Syringe Filters, SFPE-22E-100 25 mm diameter, sterile), acidified with 1 M HNO_3 , and the soluble concentrations of Al, Ca, Co, Fe, Mn and Ni (C_{soln}) were determined by ICP-OES.

DGT samplers were assembled by placing together a 0.4-mm Chelex gel disc, overlain with a 0.8-mm

polyacrylamide diffusive gel (DGT Research Ltd., UK) and a 0.45 µm membrane filter (Supor 450, Pall Corporation, New York, USA). A constant mass (about 3 g) of soil slurry from each soil (bulk soil and rhizosphere) was placed onto the DGT-probe window, ensuring the absence of bubbles between the slurry and the sample window. The soils were then left on the DGT devices for four different times (24, 72, 168 and 264 h) in a water-saturated atmosphere at 25 °C in an air tight-plastic container. Upon retrieval, the DGT devices were washed with deionised water to remove soil particles, disassembled and the resin gels were then eluted with 10 mL of 1 M HNO₃ in closed microvials. The concentration of Al, Ca, Co, Fe, Mn and Ni in the eluent was measured by ICP-OES.

The accumulated Ni mass in the chelating resin (M , µg) was calculated as follows (eq. 1)

$$M = C_e(V_n + V_{rg})/f_e \quad (1)$$

C_e	metal concentration in the elution extract of the resin gel (in mg L ⁻¹)
V_n	volume of 1 M HNO ₃ where the chelating resin is eluted (10 mL)
V_{rg}	volume of chelating resin (0.18 mL)
f_e	correction factor that compensates for the fraction of metals that cannot be released from the chelating (0.8 for Ni and Mn and 0.7 for Fe)

From the accumulated mass of metal (M) the Flux (F , µg cm⁻² h⁻¹) and the DGT-labile metal concentration C_{DGT} (µg cm⁻³) were calculated as follows (eq. 2 and eq. 3):

$$F = M/At \quad (2)$$

M	accumulated mass (µg)
A	exposed gel area (cm ²)
t	deployment time (s)

C_{DGT} is the time-averaged concentration at the DGT interface.

$$C_{DGT} = (M\Delta g/ADt) \quad (3)$$

M	accumulated mass (µg)
Δg	diffusion layer thickness (diffusion gel + filter) (cm)
A	exposed gel area (cm ²)

D	diffusion coefficient of metal in the diffusive gel (cm ² s ⁻¹)
T	Deployment time (s)

C_{DGT} was used to calculate the R ratio, which represents the ratio of the mean metal concentration at the DGT surface and the bulk soil solution metal concentration (C_{soln}) (eq. 4) and is a measure of resupply from the solid phase.

$$R = C_{DGT}/C_{Soln} \quad (4)$$

DIFS modelling

The 2D DIFS software (Lehto et al. 2008; Sochaczewski et al. 2007) was used to assess the kinetics of Ni re-supply to both plant species. Briefly, the model considers the soil to have two separate pools of trace metals: metal dissolved in the soil solution (C , mol cm⁻³) and labile, solid phase metal (C_s , mol g⁻¹). The dynamics of exchange between these two pools are governed by first order kinetics, which are described by the rate constants k_f and k_b (representing the adsorption and desorption rate constants, respectively) (see Lehto et al. 2008; Sochaczewski et al. 2007). These two rate constants are components of the equation for determining the response time (T_C) that represents the characteristic time for the perturbed system (after deployment of DGT device) to reach 63% of the equilibrium. The ratio of the sorbed (C_s) to dissolved concentration (C) at equilibrium is defined as the K_D (cm³ g⁻¹) (Jannasch et al. 1988; Sochaczewski et al. 2007). The DGT calculated ratio R is determined by K_D and T_C . In conclusion, 2D DIFS provides an automated, iterative method that determines K_D and T_C for a series of experimentally determined R .

In this study, K_D and T_C were determined for each soil by comparing the experimentally determined R values to R values given by the model using different simulated deployment subjected to different K_D and T_C values (Ernstberger et al. 2002, 2005). The fit between the modelled and experimentally determined R values was tested visually following the approach by Ernstberger et al. (2005). The small set of data points and the small amount of soil used in the deployments only allowed for qualitative comparison between the K_D and T_C values from different soils. The sensitivity of the model system was tested by changing the fitted K_D and T_C values by 30–50%. To quantitatively express how

well the R data fitted the estimated K_D and T_C the following error function (E) was used (eq. 5).

$$E = 100 \cdot \sqrt{\sum_{i=1}^N \left(R_e(t_i) - R_s(t_i) \right)^2} \quad (5)$$

Where $R_e(t_i)$ is the experimentally derived R value and $R_s(t_i)$ is the simulated value for time t_i .

An E value <8 fits data very well according to Lehto et al. (2008).

Calculated and estimated parameters

- R , M and F were calculated as described above.

- K_D ($\text{cm}^3 \text{g}^{-1}$ distribution coefficient): Ratio of the sorbed (estimated using F1 of the fractionation scheme) to dissolved concentration (C_s). The sorbed concentration represents readily exchangeable, rather than total, material associated with the solid phase.

- P_c (g cm^{-3} particle concentration): Ratio of the mass of particles to volume of pore water in a given volume of soil, calculated using the porosity (ϕ) and the bulk density of the particulate matter (d_p (g cm^{-3})), using (eq. 6):

$$P_c = d_p(1-\phi)/\phi \quad (6)$$

- ϕ (porosity) was calculated assuming a standard particle density of 2.65 g cm^{-3} (standard value for quartz).

$$\phi = 1 - d_p/2.65 \quad (8)$$

- D_0 ($\text{cm}^2 \text{s}^{-1}$, diffusion coefficient in water).

- D_d ($\text{cm}^2 \text{s}^{-1}$, diffusion coefficient in the diffusive layer): it was assumed as $D_d = D_0(\theta^2 = 1)$ (Davison and Zhang 1994).

- D_s ($\text{cm}^2 \text{s}^{-1}$ diffusion coefficient in the soil): it has been found that diffusion coefficients in soils are related to the self-diffusion coefficients (D_0) by tortuosity 2 (eq. 9)

$$D_s = D_0/\theta^2 \quad (9)$$

- θ^2 : Tortuosity is not used as a parameter in DIFS, instead D_s is specified directly. Boudreau (1997) gives a number of proposed relationships that relate tortuosity to the more readily measured porosity.

A summary of the key parameters and variables used for the 2D DIFS is presented in the Online Resource 2.

High-resolution imaging of Ni fluxes along single plant roots

DGT was also used to measure the two-dimensional distribution of Ni fluxes along the root axis of either *O. serpyllifolia* and *H. lanatus*. Plants were grown for two months in rhizotrons filled with Ni-rich serpentine soil (< 2 mm) collected at the serpentine outcrop of Barazón. Soils were fertilized with 150 kg N ha^{-1} (in the form of NH_4NO_3) and mixed with 10% of sand (w/w) to reduce compaction before planting. Rhizotrons are flat plastic growth containers where roots develop preferentially along the front plate since plants are grown at an inclination. The rhizotrons used in this study had inner dimensions of $40 \times 10 \times 1.5 \text{ cm}$ (height x width x depth). To prevent the front plate from sticking to soil and plant roots, a 0.05 mm thick polytetrafluoroethylene (PTFE) foil was fixed before closing the rhizotrons. The soil was saturated to 60% WHC. After transplanting the plants, the rhizotrons were wrapped in aluminum foil to avoid algal growth. The experiment was carried out in a growth room at 30–40% relative humidity (day/night cycle: 16/8 h) and soil water content was kept at approx. 60% WHC throughout the experiment through regular additions of water. For chemical imaging, polyacrylamide diffuse gradients in thin film (DGT) gels containing suspended particulate reagent iminodiacetate (SPRIDA) resin (CETAC, Nebraska, USA) were prepared as described previously (Warnken et al. 2004; Hoefer et al. 2015; Valentinuzzi et al. 2015). DGT gel strips (approx. $2 \times 4 \text{ cm}$) were deployed in the region of interest during 24 h. A $10\text{-}\mu\text{m}$ thick polycarbonate filter membrane (Nucleopore, Whatman, Maidstone, UK) was used as a diffusive layer between the soil and DGT gel. The gels were retrieved, rinsed and dried using a gel dryer and fixed onto glass plates prior to laser ablation inductively-coupled plasma mass spectrometric (LA-ICP-MS) analysis; using a UP 193-FX208 (ESI, NWR Division, CA, US) laser ablation system coupled to a NexION 300D ICP-MS (Perkin-Elmer, MA, USA).

Preparation of standards and gel analysis were carried out as previously described (Hoefer et al. 2015; Valentinuzzi et al. 2015). Resin gel discs were assembled with a diffusion layer and a membrane filter (as for soil analysis) and immersed into well stirred 10 mM NaNO_3 solutions containing Ni (as $\text{Ni}(\text{NO}_3)_2$) at

concentrations of 6.3, 62.8, 627.6, 1882.8 $\mu\text{g L}^{-1}$. Two replicates of each standard were prepared: one gel was dried and analysed by LA-ICP-MS, and the other gel was eluted in 10 mL of 1 M HNO_3 , and the solutions were then analysed by ICP-MS. The eluent concentration was used to calculate the mass of metal bound per unit area of resin gel.

Statistical analysis

Significant effects of plant species on soil properties, plant ionome and plant metal accumulation were determined by the t-Student test or one-way ANOVA followed by the “post-hoc” Tukey test. All statistical analyses were performed with SPSS 21.0 software (SPSS Inc., Chicago, IL).

Results

Soil physicochemical properties and plant ionome

Soil physicochemical characteristics were typical of serpentine soils; a pH close to neutrality (pH 6.8), low total C and N content (1.34% and 0.13%, respectively), low CEC (6.65 $\text{cmol}_\text{c} \text{kg}^{-1}$) principally dominated by Mg (5.74 $\text{cmol}_\text{c} \text{kg}^{-1}$), Ca/Mg ratio of 0.15, low available P (4.3 mg kg^{-1}) and K (22 mg kg^{-1}), and high pseudo-total Ni concentration (3607 mg kg^{-1}) (data not shown). The shoot ionome of both plant species is presented in Table 1. Mean Ni concentrations in shoots were 9 g kg^{-1}

Table 1 Concentrations of macro- and micro-nutrients in the shoots of *Odontarrhena serpyllifolia* and *Holcus lanatus*

	<i>O. serpyllifolia</i>	<i>H. lanatus</i>
(g kg^{-1})		
Ca	25.4±0.8	0.7±0.3
K	9.4±1.1	6.5±1.0
Mg	11.4±0.1	1.1±0.1
(mg kg^{-1})		
Ni	9108±893	13±3
Co	19.4±1.5	0.4±0.0
Cu	6.1±0.9	4.2±0.3
Fe	255±36	116±20
Mn	232±41	52±5
P	952±131	207±40

for the hyperaccumulator and 0.01 g kg^{-1} for the excluder. The Ni-hyperaccumulator also presented significantly higher shoot concentrations of macronutrients, such as Ca (up to 40-fold higher), Mg (10-fold), P (5-fold) and K (1.5-fold), than the excluder. Similarly, concentrations of Co, Cu, Fe and Mn were higher in the shoots of the hyperaccumulator than in the excluder. The Ca/Mg ratio in the hyperaccumulator reached values of close to 2, while this ratio was consistently <1 in the grass species.

Characteristics of rhizosphere and bulk soils

Figure 2 shows the properties of the two rhizosphere soils compared to the bulk soil. Soil pH was slightly higher in the rhizosphere of the hyperaccumulator, and significantly lower in the rhizosphere of the excluder ($p < 0.05$), compared to bulk soil. Significantly higher concentrations of DOC were found in rhizosphere soil of both species compared to bulk soil. Moreover, DOC was significantly higher in the rhizosphere of the hyperaccumulator than of the excluder ($p < 0.05$). Both CEC and the Ca/Mg ratio were also significantly higher in the rhizosphere of the hyperaccumulator (than in either bulk soil or the rhizosphere of the excluder) ($p < 0.05$). Available P (P Olsen) was slightly but not significantly higher in the rhizosphere of *O. serpyllifolia* ($p > 0.05$) than either bulk or rhizosphere of *H. lanatus*.

Extractable (and DGT-labile) Ni in bulk soils was always lower than in the rhizosphere soils. Plant species-specific differences were observed in both, $\text{Sr}(\text{NO}_3)_2$ - and $\text{Ca}(\text{NO}_3)_2$ -extractable Ni, and in Ni_{DGT} values (Fig. 2). For the simple extractions, extractable concentrations of Ni were approximately 2-fold lower in bulk soil than in rhizosphere soils. Both $\text{Sr}(\text{NO}_3)_2$ - and $\text{Ca}(\text{NO}_3)_2$ -extractable Ni concentrations were slightly, but significantly, higher in the rhizosphere of the Ni-hyperaccumulator than in that of the excluder (32.2 mg kg^{-1} $\text{Ca}(\text{NO}_3)_2$ -extractable Ni and 23.3 mg kg^{-1} $\text{Sr}(\text{NO}_3)_2$ -extractable Ni in the rhizosphere of the hyperaccumulator compared to 23.3 mg kg^{-1} $\text{Ca}(\text{NO}_3)_2$ -extractable Ni and 21.3 mg kg^{-1} $\text{Sr}(\text{NO}_3)_2$ -extractable Ni in the rhizosphere of the excluder). More pronounced differences were observed using DGT: Ni_{DGT} was between 2- and 5-fold higher in rhizosphere soils than in bulk soil. Moreover, Ni_{DGT} in the rhizosphere of the hyperaccumulator was up to 2.5-fold higher than in the

Fig. 2 Properties of the non-vegetated (Bulk soil) and *Odontarrhena serpyllifolia* and *Holcus lanatus* rhizosphere soils and Ni availability (assessed through single extractions ($\text{Ca}(\text{NO}_3)_2$

and $\text{Sr}(\text{NO}_3)_2$) and diffusive gradient in thin films (DGT)). Different letters indicate significant differences between soils ($p \leq 0.05$)

rhizosphere of the excluder. $\text{Sr}(\text{NO}_3)_2$ - and $\text{Ca}(\text{NO}_3)_2$ -extractable Ni and DGT-labile Ni were positively correlated with DOC ($R^2 = 0.8$ – 0.9 and 0.9 respectively).

Figure 3a shows the Ni fractionation in bulk and rhizosphere soils. In all cases, the residual (silicate-bound) fraction (F7) was the largest metal pool (representing between 62 and 66% of the pseudo-total Ni), and this was followed by Ni bound to Fe oxides (F5 + F6) (representing 23 to 25% of pseudo-total Ni). Among the remaining fractions, Ni was mainly associated with Mn oxides in all the three soils (F3; 5 to 6% of pseudo-total Ni), or in the case of the hyperaccumulator, also organic matter-bound (4%). Figure 3b shows the change (decrease/increase) in Ni concentrations of each fraction compared to the bulk soil. Both plant species significantly increased F1 (water soluble+exchangeable fraction) (from $27.8 \text{ mg Ni kg}^{-1}$ in bulk soils to 55.4 mg

Ni kg^{-1} and $56.8 \text{ mg Ni kg}^{-1}$ in the rhizosphere of *O. serpyllifolia* and *H. lanatus*, respectively) ($p \leq 0.05$). Again, both plant species significantly increased the readily available Ni fraction (F2), but this was more pronounced in the hyperaccumulator: the F2 Ni fraction increased from 23.0 mg kg^{-1} in bulk soil to 82.2 mg kg^{-1} and 56.8 mg kg^{-1} in the rhizospheres of *O. serpyllifolia* and *H. lanatus*, respectively. Moreover, the hyperaccumulator significantly increased Ni bound to organic matter (F4) (from 69.1 mg kg^{-1} to 157.0 mg kg^{-1}).

Concentrations of other major elements are presented in Table 2 ($\text{Ca}(\text{NO}_3)_2$ -, $\text{Sr}(\text{NO}_3)_2$ - and NH_4NO_3 (F1)-extractable elements) and Table 3 (DGT-labile elements). In general, enrichment in most elements was observed in the rhizosphere of the hyperaccumulator compared to bulk or to the excluder, except for Mg

Fig. 3 Soil Ni fractionation in bulk and rhizosphere soils (a) and the mean decrease/increase (%) of each fraction in the rhizosphere soils relative to the bulk soil (b). The depletion/increase was calculated by subtracting the Ni concentration in each fraction (in mg kg^{-1}) in the rhizosphere soil from the corresponding concentration in the bulk soil. A significant decrease/increase compared to bulk soil is denoted by an asterisk and a significant difference between plants is denoted with “≠” ($p \leq 0.05$)

which was higher in the rhizosphere of *H. lanatus* (Table 3). NH_4NO_3 -extractable (F1) Ca concentrations were up to 3-fold higher in the rhizosphere of the hyperaccumulator than in bulk soil, and up to 2.5-fold

higher than in the rhizosphere of the excluder. Similarly, extractable K (extracted with $\text{Ca}(\text{NO}_3)_2$, $\text{Sr}(\text{NO}_3)_2$ and NH_4NO_3 (F1)) was significantly higher in the hyperaccumulator: $\text{Ca}(\text{NO}_3)_2$ -extractable concentrations of this nutrient were up to 5-fold higher than in bulk soils or the rhizosphere of the excluder. The highest concentrations of extractable Co, Mn, Fe (only in the case of NH_4NO_3 -extractable concentrations) and Al were also recorded in the rhizosphere of the hyperaccumulator, although this increase was only significant for Mn. The C_{DGT} of, Ni, Mn, Co and Al, were significantly higher in the hyperaccumulator rhizosphere soil compared to either bulk soil or the rhizosphere of the excluder (Table 4). In contrast, C_{DGT} Fe was significantly higher in the rhizosphere of the excluder. This was the case after 24 h of deployment, and the same trend was generally maintained until the longest deployment time. After 24 h deployment, DGT-labile Ni (C_{DGT}) in the rhizosphere of the hyperaccumulator was up to 5-fold higher than in bulk soil and 2.5-fold higher than in the rhizosphere of the excluder. Similarly, in the rhizosphere soil of the hyperaccumulator, DGT-labile Mn, Co and Al concentrations were up 24-, 28- and 10-fold higher than in bulk soil, and 2-, 1.2-, and 1.4-fold higher than in the excluder rhizosphere. On the other hand, DGT-labile Fe was 2.2-fold higher in the rhizosphere of the excluder than in that of the hyperaccumulator. For Ni, Mn and Co the same effects were observed at longer deployment times (Table 4). DGT-labile Ni was positively correlated to DGT-labile Mn, Co and Al ($R^2 = 0.8, 0.6$ and 0.7 , respectively).

Soil Ni replenishment kinetics

Figure 4a shows the accumulated mass of Ni on the resin gel (mol cm^{-2}) plotted against deployment time. In all soils the accumulated Ni mass increased with increasing deployment time. This increase was more pronounced in rhizosphere soils than in bulk soil, and in particular, in the rhizosphere of the hyperaccumulator. The accumulated Ni mass in the hyperaccumulator rhizosphere soil was significantly higher at all deployment times (increasing from $6.25\text{E-}08 \text{ mol Ni cm}^{-2}$ after 24 h to $2.32\text{E-}07 \text{ mol Ni cm}^{-2}$ after 264 h) than in both the excluder rhizosphere soil (increasing from $2.34\text{E-}08 \text{ mol Ni cm}^{-2}$ to $1.70\text{E-}07 \text{ mol Ni cm}^{-2}$) and the bulk soil (increasing from $1.30\text{E-}08 \text{ mol Ni cm}^{-2}$ to $8.19\text{E-}08 \text{ mol Ni cm}^{-2}$).

Table 2 Concentration of labile Ca, K, Mg, Mn, Fe, Co, Al determined through conventional soil extractions. Different letters indicate significant differences between soils ($p < 0.05$)

		Ca	K	Mg	Mn	Fe	Co	Al
		mg kg ⁻¹ (mean ± SE)						
Ca(NO ₃) ₂	Bulk	nd	15.2 ± 2.1 b	665.6 ± 6.4 c	1.1 ± 0.1 b	9.7 ± 2.6 a	0.06 ± 0.01 a	3.5 ± 2.0 a
	<i>O. serpyllifolia</i>	nd	70.2 ± 0.9 a	776.5 ± 18.0 b	2.0 ± 0.2 a	5.1 ± 0.5 a	0.08 ± 0.02 a	3.0 ± 0.3 a
	<i>H. lanatus</i>	nd	15.4 ± 0.2 b	958.5 ± 5.1 a	0.8 ± 0.1 b	7.8 ± 2.4 a	0.04 ± 0.01 a	1.9 ± 0.4 a
Sr(NO ₃) ₂	Bulk	220.9 ± 4.7 c	18.6 ± 2.0 b	639.8 ± 14.4 c	1.1 ± 0.1 b	7.3 ± 1.0 a	0.09 ± 0.01 a	4.4 ± 0.8 a
	<i>O. serpyllifolia</i>	708.1 ± 11.2 a	75.7 ± 2.7 a	715.9 ± 12.2 b	1.7 ± 0.1 a	6.8 ± 1.1 a	0.09 ± 0.01 a	4.8 ± 0.6 a
	<i>H. lanatus</i>	393.3 ± 27.6 b	18.4 ± 1.0 b	906.3 ± 5.9 a	0.9 ± 0.2 b	8.1 ± 0.5 a	0.10 ± 0.00 a	3.6 ± 0.8 a
NH ₄ NO ₃	Bulk	372.8 ± 118.0 b	44.4 ± 2.8 c	1034.7 ± 18.2 c	5.6 ± 1.4 b	4.9 ± 1.6 a	0.40 ± 0.03 a	4.1 ± 1.3 a
	<i>O. serpyllifolia</i>	1135.2 ± 205.0 a	162.6 ± 4.2 a	1209.2 ± 56.6 b	14.9 ± 1.5 a	9.4 ± 3.4 a	0.68 ± 0.11 a	6.6 ± 0.7 a
	<i>H. lanatus</i>	449.7 ± 3.2 b	74.3 ± 4.9 b	1582.2 ± 29.8 a	16.9 ± 0.7 a	4.9 ± 0.3 a	0.60 ± 0.16 a	3.8 ± 0.5 a

nd, not determined

Figure 4b shows the change in the R ratio (C_{DGT}/C_{soln}) with deployment time. *H. lanatus* rhizosphere soil showed significantly higher R ratios than either *O. serpyllifolia* rhizosphere or bulk soil. R ratios were 0.74, 0.59 and 0.13 after 24 h in *H. lanatus*, *O. serpyllifolia* or bulk soil, respectively. R ratios of the bulk soil were always significantly lower than those of either rhizosphere soil ($p < 0.05$). In rhizosphere soils, there was a progressive depletion with increasing deployment time (from 24 h to 264 h), while in bulk soil there was almost no change with time (Fig. 4).

The kinetics of metal resupply were obtained from the best-fitting curve of experimental R values against time using the 2D DIFS software.

Figure 5 shows the 2D DIFS model simulation of R for Ni over time: the continuous line shows the best fitting combination of K_D and T_C , and the two dotted lines show the adjustment after modifying this combination by a maximum of 50%. While these values cannot be used as absolute values of K_D and T_C in these soils, they do allow us to make qualitative comparisons of the Ni resupply dynamics between soils before and after modification by the two plant species. The model

Table 3 Ni, Mn, Fe, Co and Al C_{DGT} values ($\mu\text{g L}^{-1}$) determined at the four deployment times. Different letters indicate significant differences between soils ($p < 0.05$)

		Ni	Mn	Fe	Co	Al
		C_{DGT} ($\mu\text{g L}^{-1}$)				
24 h	Bulk	141.6 ± 10.9 c	157.9 ± 43.7 c	22.0 ± 3.8 c	2.3 ± 1.0 c	6.7 ± 1.1 b
	<i>O. serpyllifolia</i>	668.8 ± 17.3 a	3783.0 ± 78.0 a	70.9 ± 12.8 b	65.2 ± 1.6 a	60.6 ± 4.8 a
	<i>H. lanatus</i>	271.0 ± 19.0 b	2339.5 ± 144.3 b	159.5 ± 14.2 a	52.8 ± 4.1 b	42.4 ± 3.5 b
62 h	Bulk	133.1 ± 4.9 c	51.4 ± 4.3 c	10.0 ± 2.8 b	1.5 ± 0.2 b	2.8 ± 0.7 b
	<i>O. serpyllifolia</i>	409.1 ± 22.2 a	2137.0 ± 98.5 a	12.2 ± 3.8 b	41.0 ± 2.5 a	16.7 ± 2.7 a
	<i>H. lanatus</i>	232.0 ± 11.1 b	1581.6 ± 62.2 b	73.2 ± 12.4 a	42.9 ± 2.6 a	19.1 ± 3.4 a
168 h	Bulk	94.7 ± 7.5 b	13.6 ± 1.2 c	4.9 ± 1.0 b	0.7 ± 0.1 b	2.0 ± 0.3 a
	<i>O. serpyllifolia</i>	285.9 ± 4.7 a	1163.4 ± 101.6 a	28.6 ± 10.7 b	27.7 ± 2.0 a	8.8 ± 2.6 a
	<i>H. lanatus</i>	227.0 ± 64.9 a	307.8 ± 43.3 b	121.5 ± 3.9 a	27.2 ± 4.0 a	18.5 ± 6.5 a
264 h	Bulk	82.4 ± 7.1 c	5.0 ± 0.6 b	11.3 ± 3.9 a	0.5 ± 0.1 c	3.4 ± 1.2 a
	<i>O. serpyllifolia</i>	234.9 ± 8.1 a	653.1 ± 113.1 a	8.3 ± 2.2 a	18.3 ± 0.6 a	3.1 ± 0.6 a
	<i>H. lanatus</i>	155.3 ± 6.1 b	55.9 ± 7.2 b	12.4 ± 4.6 a	12.9 ± 1.0 b	3.7 ± 1.1 a

Fig. 4 Measured accumulated Ni mass in the resin gel (mol cm⁻²) and the Ni *R* ratio (C_{DGT} / C_{soln}) with deployment time (24, 62, 168, 264 h). Different letters indicate significant differences between soils at each deployment time ($p < 0.05$)

results show that the solid phase reservoir of labile Ni in the bulk soil was very small with slow resupply of dissolved Ni from the solid phase (K_D of 5 and T_C of 40,000; $E = 1.14$). The modelled *R* values in the *O. serpyllifolia* rhizosphere soil suggest that the solid phase reservoir of labile Ni has increased, and the resupply from this solid phase is faster than in the bulk soil (K_D of 100 and T_C of 10; $E = 4.36$). The largest model-estimated reservoir of labile solid phase-bound Ni was observed in the *H. lanatus* rhizosphere, (K_D 750 and T_C 8; $E = 4.28$).

Chemical imaging of DGT-available Ni along the root

High-resolution imaging of DGT-measured Ni fluxes around *O. serpyllifolia* and *H. lanatus* roots was carried out to show the spatial variability of root activities and potential root-induced Ni solubilisation, metal depletion and/or accumulation in the rhizosphere. Figure 6 shows the DGT-measured Ni fluxes, around a single root of *O. serpyllifolia* and *H. lanatus*. Figure 7 shows horizontal profile plots of Ni fluxes (pg cm⁻² s⁻¹) along root cross sections labelled as O1, O2 and O3 (corresponding to *O. serpyllifolia* roots) and profiles labelled as H1, H2

Fig. 5 Experimental data and 2D DIFS model simulation of *R* for Ni with error margins (T_C and K_D were changed by 30–50% from the best model fit). k_f (sorption rate; s⁻¹) and k_b (desorption rate; s⁻¹)

and H3 (corresponding to *H. lanatus* roots). Nickel depletion zones were observed along the root axes of both species, with the depletion extending up to 3 mm into the soil along the root of the Ni hyperaccumulator (line O2), while the depletion area was only up to 0.8 mm broad along the root of the excluder (line H1).

Discussion

Assessing the dynamics of nickel in the rhizosphere and related physicochemical processes might help to better understand fundamental processes related to nickel mobilization and uptake by hyperaccumulator and excluder plants growing on serpentine soils. Ultimately, a better mechanistic understanding may also contribute to potential technological applications, such as nickel phytomining. In this study, the Ni-hyperaccumulator

Fig. 6 High resolution two-dimensional mapping of Ni along root axes of *O. serpyllifolia* (a) and *H. lanatus* (b). Nickel is shown as DGT-measured Ni fluxes ($\text{pg cm}^{-2} \text{s}^{-1}$). Root cross sections (boxes) are referred to in the next figure (Fig. 7)

O. serpyllifolia was compared with the non-accumulator *H. lanatus*. Shoot Ni concentrations in *O. serpyllifolia* were similar to values previously reported for this plant species (Álvarez-López et al. 2015; Cabello-Conejo 2015) and confirmed its ability to hyperaccumulate this metal. In contrast, shoot Ni concentrations in *H. lanatus* were very low (10 mg kg^{-1}), and in this species Ni tended to accumulate only in the roots (with a mean root Ni concentration of 397 mg kg^{-1}).

Concentrations of extractable and labile Ni (assessed by conventional extraction approaches and DGT) were significantly higher in the rhizosphere compared to bulk soil. Moreover, extractable and labile Ni concentrations were higher in the rhizosphere of the hyperaccumulator than the excluder. Soil Ni fractionation was also

modified in rhizosphere soils compared to bulk soil and shifts in Ni fractionation towards more plant-available fractions were more pronounced in the Ni-hyperaccumulator than in the excluder. Similarly, Wenzel et al. (2003) found soluble (water-extractable) Ni concentrations in the rhizosphere of the Ni-hyperaccumulator *Nocca goesingensis* Hálácsy collected from the Redlschlag serpentine site (Austria) to be 3- and 2-fold higher than in corresponding bulk soil or rhizosphere soil of non-accumulating plant species.

An increase in metal availability and plant uptake is often associated with a root-induced acidification effect (Hinsinger et al. 2005). However, most studies suggest that root-induced pH changes are not involved in the hyperaccumulation mechanism (Wenzel et al. 2004;

Fig. 7 Horizontal profile plots of Ni fluxes ($\text{pg cm}^{-2} \text{s}^{-1}$) along root cross sections. Profiles labelled as O1, O2, O3 and A4 correspond to *O. serpyllifolia* roots and profiles labelled as H1, H2 and H3 to *H. lanatus* roots

Kukier et al. 2004). Likewise, no pH decrease was found in the rhizosphere of *O. serpyllifolia* and even a slight increase was observed, in agreement with Wenzel et al. (2003) who found an increase in soil pH in the rhizosphere of *N. goesingensis* growing in serpentine soils. It is also well known that root exudates can influence metal availability, through other processes than acidification such as chelation, precipitation and redox reactions (Adriano 2001; Hinsinger et al. 2005; Puschenreiter et al. 2003; Tao et al. 2004; Uren and Reisenauer 1988). DOC concentrations were higher in the rhizosphere of the hyperaccumulator than the excluder, and soil extractable and labile Ni concentrations were positively correlated with DOC concentration. Moreover, in the rhizosphere of the hyperaccumulator, the percentage and concentration of Ni bound to organic matter (fraction F4) was also higher than in the bulk soil

or in the rhizosphere of the excluder. Previous studies have also related enhanced metal availability in the rhizosphere of metal hyperaccumulators to higher DOC concentrations. Thus, Wenzel et al. (2003) found that a higher concentration of water-extractable Ni in the rhizosphere of *N. goesingensis* than in non-vegetated soil was correlated with a higher DOC concentration in the former. Using chemical speciation analysis (MINTEQA2), these authors also suggested that the higher soluble Ni concentration in the hyperaccumulator rhizosphere was driven by the formation of Ni-organic complexes. Moreover, the nature of DOC in the rhizosphere likely differs among plant species. Wenzel et al. (2003) suggested that DOC in the rhizosphere of hyperaccumulators may form stronger complexes with Ni than DOC derived from bulk soil or the rhizosphere soil of Ni-excluders (such as the *H. lanatus* in this

study). Likewise, Cattani et al. (2009) suggested that DOC released by the As-hyperaccumulator *Pteris vittata* could have different effects on As bioavailability compared to the DOC released by the As-tolerant *Agrostis capillaris* (increasing its availability in the case of the hyperaccumulator rhizosphere) and they attributed this possibility to the difference in DOC composition of both plant species. Divergent results are found in the literature regarding the capacity of the hyperaccumulators to access to different soil Ni fractions than excluders and the capacity of root-driven mineral dissolution by hyperaccumulators. For example, Massoura et al. (2004) found identical Ni available pools for plants with different strategies in Ni uptake, Chardot-Jacques et al. (2013) found enhanced root-driven dissolution of minerals by the hyperaccumulator *Bornmuellera emarginata* when the concentration of soil solution Ni was low. According to these authors, root-induced dissolution of soil minerals in the present study would be unlikely due to the high initial soluble Ni in soil solution. However, experimental conditions of these studies (external nutrient inputs and short growth period) were different to field conditions (where substantial nutrient limitation is typical, and plants grow for longer periods).

Nutrient deficiency is common in serpentine soils and root exudation of organic compounds likely plays a vital role in the mobilisation and uptake of essential elements, such as Ca, P, K or Fe (Marschner 1995; Marschner et al. 2011; Neumann and Römheld 2007). The shoot ionome of the two studied species clearly showed that the Ni-hyperaccumulator was more effective than the grass species in overcoming the nutrient limiting conditions to which they were both exposed. Shoot macronutrient (Ca, K, P) and micronutrients (Fe) concentrations were significantly higher in the hyperaccumulator. In contrast to the excluder, the hyperaccumulator was able to notably increase exchangeable Ca and K in the rhizosphere soil and significantly improved the strong nutritional disequilibrium. In parallel, C_{DGT} (at 24 h) showed a clear enrichment in elements such as Al, Co, Fe, Mn and Ni in the rhizosphere of this plant compared to bulk soils. Most of these increments were maintained with deployment time. An enhanced weathering of primary minerals (such as orthopyroxene or amphibole) by the Ni-hyperaccumulator could well explain the observed increments in extractable and labile concentrations of all these elements. Similarly, Wenzel et al. (2003) also

found elevated concentrations of Ca, Mg and K in water extracts of the rhizosphere soil of *N. goesingensis* compared to non-vegetated soil. Moreover, authors considered the increase in pH in the rhizosphere of *N. goesingensis* (also observed in the present study) to be consistent with the release of hydroxyl ions during mineral dissolution of Mg/Ni-bearing orthosilicates. Root-driven dissolution of minerals in conditions of limited nutrient availability was found for example, in soils with low available K: depletion of K can lead to the disaggregation of shale particles and the accumulation of amorphous iron and aluminium oxyhydrates indicative of enhanced weathering of soil material at the soil-root interface (Sarkar et al. 1979; Kong and Steffens 1989). A previous study (not published) carried out using X-ray diffraction showed a more intense mineral weathering in the rhizosphere of *O. serpyllifolia* compared to *H. lanatus*. Moreover, chlorite and serpentine were identified as the dominant clay minerals in these soils but the presence of smectite (a weathering product of serpentine) was only identified in the rhizosphere soil of the hyperaccumulator. At the same time, mineralisation of soil organic matter present in the rhizosphere of the hyperaccumulator could also be contributing to nutrient cycling. In consequence, root activity and ligand-induced dissolution of soil minerals would not only lead to the release of essential plant nutrients but also to the Ni associated with these solid phases. The role of organic matter in determining Ni availability to plants has also been demonstrated by other authors for ultramafic areas. Some recent publications (Estrade et al. 2015; Zelano et al. 2020) clearly show that the isotopic signature of Ni in ultramafic topsoils is to a large extent (>50%) deriving from plant-recycled (absorbed) Ni, that was originally taken up from the soil and subsequently released from decaying plant matter. Antić-Mladenović et al. (2011) also suggested that organic matter transformations (together with dissolution and precipitation of Fe/Mn oxides and adsorption on soils), were important processes controlling Ni solubility in ultramafic soils. Fe-oxides in ultramafic soils were shown to be a key fraction controlling soil Ni solubility: this fraction can bear 2- to 3- fold higher Ni than primary silicates, and these can be the source of labile Ni (Massoura et al. 2004; Chardot et al. 2005). A high proportion of Ni bound to Fe oxides was also found in the present study (between 20 to 25%) although no differences were observed among plant species or rhizospheres and bulk soils. Fitz et al. (2003) also found

that increased concentrations of DOC were correlated with enhanced Fe solubility in the rhizosphere of the As-hyperaccumulator *Pteris vittata*. These authors related a DOC-triggered dissolution of iron hydroxide surfaces with an enhanced co-dissolution of As and Fe. Next to than plants, also soil microorganisms play a key role in influencing soil metal availability. For example, Becerra-Castro et al. (2013) found an important mineral weathering activity of two *Arthrobacter* strains isolated from *O. serpyllifolia* rhizosphere. Moreover, authors found that the two strains were able to act on different mineral phases which was correlated with the secretion of different carboxylates when microbes were incubated with ultramafic rock as the only Ni source.

The capacity to pre-concentrate analytes in situ and to determine element fluxes instead of equilibrium concentrations like in conventional extraction methods makes DGT highly suitable for studying high resolution element distribution around single roots (Kreuzeder et al. 2015). Ni distribution patterns around roots of the plants cultivated in rhizotrons showed that both plants species depleted labile Ni due to plant uptake, but the extent of this depletion was higher in the rhizosphere of the hyperaccumulator. Similarly, studies involving IEK approaches, found that several species of Ni hyperaccumulators (including *Odontarrhena* sp.) cultivated for 3 months in a serpentine soil not only depleted soluble Ni but also the most reactive pools and replenishment from less reactive pools was not enough to resupply the initial Ni concentration (Chardot et al. 2005). These results appear contradictory to the results of the bulk DGT measurements on field rhizosphere samples. In the latter, rhizosphere soil of adult plants (older than 2 y) was assessed, whereas in the rhizotron single roots that were only a few days old were investigated. It can be assumed that the effect of root activity on mineral weathering and Ni availability is more pronounced in the field-collected rhizosphere soils. Our results suggest that, at the root tip of very young roots, exploring non-vegetated soil, Ni is effectively and quickly depleted; whereas over time (i.e. several months to years) the effect of root activities of the whole root system lead to an overall net mobilisation of Ni in the rhizosphere as a result of the plant's attempts to improve its nutritional status. *O. serpyllifolia* induced a slightly more pronounced depletion zone due to its hyperaccumulation trait, also indicating that the root activity of these plants can influence a larger soil volume than other plant species such as the *H. lanatus* studied

here. In spite of its metal-excluding nature, *H. lanatus* also induced a Ni depletion zone around the investigated root. This was likely due to the fact that excluder plants also accumulate/adsorb substantial Ni concentrations in their roots and on the root surface.

Understanding the exchange of trace metals between the solid and the solution phase is critical when trying to estimate their mobility and bioavailability in the soil (Harper et al. 2000; McLaughlin et al. 1998). The DIFS model can provide information related to the kinetics of metal replenishment by quantifying the relationship between R (C_{DGT}/C_{soln}), K_D (distribution coefficient between the solid and solution phases) and T_C (response time of the soil to reach equilibrium) (Harper et al. 1998). The accumulated Ni mass in DGT followed the order *O. serpyllifolia* > *H. lanatus* > bulk soil, and this was consistent across the increasing deployment times. The higher accumulated Ni mass from the rhizosphere soil of the hyperaccumulator compared to the excluder is in agreement with its higher labile Ni concentration. Experimental R values at 24 h differed somewhat between the two rhizosphere soils ($R = 0.59$ (*O. serpyllifolia*) vs. 0.74 (*H. lanatus*)), however, the best DIFS model fit for R and deployment time predicted R values to peak at 0.74 and 0.75 for both rhizosphere soils before the 24 h deployment time. This indicates that the resin took up a similar proportion of labile Ni from both soils in the initial hours of DGT deployment. After this peak, the R values progressively decreased with time in both rhizosphere soils, indicating an initially greater Ni flux from the soluble phase. However, the decline in R values was more pronounced in the rhizosphere of the hyperaccumulator than the excluder. The rhizosphere soil of the hyperaccumulator effectively buffered the Ni concentration in the soil solution at the first deployment time (24 h), but with time the Ni resupply was not sufficient to maintain the high initial concentrations of soluble Ni ($1130 \mu\text{g L}^{-1}$) in the soil solution. While in the excluder rhizosphere soil the lower initially soluble Ni concentration ($340 \mu\text{g L}^{-1}$) appeared to be more easily compensated for through replenishment from the solid phase. In contrast, in bulk (initial concentrations of soluble Ni: $1121 \mu\text{g L}^{-1}$) soils there was practically no resupply from the solid phase and the data suggest that there is no net exchange reaction of metal between the sorbed phase and the soil solution (indicated by a very low K_D and high T_C). The similar initial concentration of C_{soln} in the rhizosphere of the hyperaccumulator and the bulk soils also seems an

indicator of the capacity of the hyperaccumulator to sustain a high Ni solubility despite its capacity of Ni uptake. The estimated K_D values for both plant species were significantly higher than those of the bulk soil ($K_D = 5$), confirming their greater solid-phase labile Ni pool. The predicted model results suggest that Ni resupply to the DGT device in the rhizosphere soil of the hyperaccumulator is partially sustained by a labile pool of Ni sorbed to solid phases while Ni resupply to the DGT device in the rhizosphere soil of the excluder was almost completely sustained by the solid phase. The lower K_D values associated with the hyperaccumulator could also indicate that the hyperaccumulator maintains a higher proportion of Ni in the dissolved phase than in the sorbed phase at equilibrium. This could be explained by the release of nickel-chelating compounds from the hyperaccumulator roots (see also Wenzel et al. 2003). It is important to keep in mind that the soil subdomain in the 2D DIFS model extends 40 mm from the DGT probe interface into the soil. Therefore, the accuracy with which the model represents the Ni depletion in the soil water in these experiments may not be optimal, since the soil layer used to determine C_{DGT} and R was only ~8–10 mm thick, owing to the small amount of available rhizosphere soil. However, given that only one soil was used and the amount used was consistent between deployments, the model can be used to make qualitative comparisons between the resupply kinetics of non-vegetated soil and the two plant rhizospheres, while absolute values of K_D and T_C should be considered with caution.

The DIFS model also has some other limitations, such as the assumption of a single pool of labile element associated with the solid phase or the linear (de)sorption relationship for simplicity (Menezes-Blackburn et al. 2019). For example, DIFS assumes that metal in solution is in a fully labile form, and not present as metal-complexes that have slow-release kinetics and/or are large enough to diffuse slowly (Lehto et al. 2008). The DIFS model does not directly consider metal complexation with dissolved organic ligands (such as DOC) and some recent studies have found that DGT measurements might be affected when a significant amount of metal is bound by humic substances in solution (Lehto et al. 2006b; Lehto et al. 2008; Davison et al. 2015). Metal complexes (such as organic complexes) can have diffusion coefficients as low as 20% of the free metal

(Lehto et al. 2006b) and the kinetics of metal release from the complex may be slowed down compared to the free ion. Due to this behaviour, these organic complexes can be perceived as components of K_D by the DIFS model. As mentioned above, the DOC concentration in the rhizosphere soil of both plant species differed and, moreover, the nature of this DOC is likely to differ between plant species. Plant-specific complexing agents may be present in the rhizosphere soils and are likely to influence the DGT measurements and DIFS predictions: grasses are well known for releasing phytosiderophores for Fe solubilisation, and indeed DGT-labile Fe concentrations were higher in the rhizosphere of *H. lanatus* than in *O. serpyllifolia*. In addition to increasing Fe uptake these chelates can also mobilise other trace metals, in particular Zn, Cu and Ni (Awad and Römheld 2000; Zhang et al. 1991; Puschenreiter et al. 2017). Finally, it is worth pointing out that the hyperaccumulator plant clearly modified soil extractable, labile and Ni fractionation to a greater extent than the excluder. The fact that the DIFS model showed that the initial high soluble Ni concentration in the soil solution of this plant could not be sustained by the soil buffering, even reinforces the importance of the hyperaccumulator root activity and its influence on soil Ni availability. Since the plant was not present this effect is lost (for example, the in vivo effect of root exudates on metal availability), and it could be assumed that in a dynamic system (such as the rhizosphere) the labile Ni concentrations will be sustained over time.

Our results support other studies suggesting that infinite-sink approaches such as the DGT technique can give more information on the estimation of plant-available metal fractions and their kinetics of resupply than conventional equilibrium-based soil extractions (Tandy et al. 2011; Zhang et al. 2001). However, it is important to also denote that some limitations on DGT performance can happen according to the element of study: DGT performance and thus predictability of plant element concentrations is superior in cases where metal uptake is controlled by diffusion (e.g. Ni), compared to cases where mass flow is the dominating mechanisms for element supply to plants (such as in the case of Mg) (Degryse et al. 2009).

Conclusions

Both plant species influenced soil physicochemical characteristics in the rhizosphere, however, plant-induced changes in soil extractable, labile and Ni fractions were more pronounced in the case of the hyperaccumulator. The DGT technique was a more sensitive tool for investigating the plant-induced changes than the conventional extractions. Our results suggest that the enhanced Ni extractability and lability in the rhizosphere of *O. serpyllifolia* was not due to active nickel mobilisation by the hyperaccumulator but rather a response to the plant's efforts to improve nutrition through mineral weathering and/or mineralisation of organic matter. Indeed, our data indicate that increasing DOC is leading to enhanced potentially plant-available Ni concentrations.

DIFS modelling showed that the rhizosphere soil of *O. serpyllifolia* effectively buffered Ni concentration in the soil solution at the first deployment time (24 h), but with time Ni resupply was not sufficient to maintain the high initial concentrations of soluble Ni in the soil solution. However, in *H. lanatus* rhizosphere soil the lower initial soluble Ni concentration was more easily compensated for through replenishment from the solid phase. In addition, the much higher Ni uptake of the hyperaccumulator could lead to a more strongly depleted pool of labile Ni, although it is important to highlight that the absolute size of this pool was larger compared to the excluder.

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Declarations

Conflicts of interest/competing interests Not applicable.

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