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REGULAR ARTICLE

Root allocation in metal-rich patch by *Thlaspi caerulescens* from normal and metalliferous soil—new insights into the rhizobox approach

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Abstract We compared root responses to spatial heterogeneity of Zn and Ni in *Thlaspi caerulescens* J. and C. Presl from normal (NM plants) and metalliferous soil (M plants). We investigated whether the strong metal accumulation capacity of NM plants (compared to M plants) was related to a greater capacity of roots to grow towards metal-enriched soil compartments. Two similar experiments were conducted in summer (slow growth) and spring (high growth), respectively. Our study is the first to show that NM plants of *T. caerulescens* have the ability to allocate more roots in the Zn-enriched compartment of soil. However, the positive response to Zn by roots of NM plants does not explain their higher Zn accumulation capacity as M plants express a similar level of root allocation in Zn-enriched compartment of soil. In M plants, root response to the Zn-rich compartment appears to be more susceptible to variations in growth conditions. Preferential root allocation in Ni-enriched compartment was consistently found in M plants only, suggesting that Ni supply is critical in their native metalliferous soil. Our study also illustrates bias in the interpretation of root allocation studies using two dimensional boxes, as interferences between root response to metal and root chirality have been highlighted.

Keywords Heavy metal · Nickel · Plasticity · Rhizobox · Root chirality · Zinc

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Introduction

Root morphological plasticity, which allows plants to preferentially proliferate roots in nutrient-rich patches of soil, is recognized as the most common morphological response to acquire patchily distributed soil resources. This process is called root foraging (Hutchings and de Kroon 1994; Hodge 2004). Several studies showed that various plant species can proliferate roots in nutrient-rich patches of soil (e.g. Drew 1975; Drew and Saker 1978; Larigauderie and Richards 1994; Johnson and Biondini 2001). This behaviour seems to be adaptive as it is often associated with enhanced plant performance (Birch and Hutchings 1994; Wijesinghe and Hutchings 1997, 1999; Einsmann et al. 1999; Wijesinghe et al. 2001). Some studies examined morphological responses of

roots to heterogeneous metal supply in heavy metal hyperaccumulators (Schwartz et al. 1999; Whiting et al. 2000; Haines 2002; Saison et al. 2004). These plants are able to accumulate very high amounts of metals in shoots (e.g. >1% Zn, >0.1% Ni or >0.01% Cd of shoot dry weight) (Baker and Brooks 1989). Acquiring metals could be important for hyperaccumulator species as their growth may be stimulated by increased metal supply (Tolrà et al. 1996; Shen et al. 1997).

Preferential allocation of root mass in metal-rich patch (referred to as metal root foraging) has been described in the model metal hyperaccumulator *Thlaspi caerulescens* (Brassicaceae) and has been proposed as one of the mechanisms involved in its high metal uptake (Schwartz et al. 1999; Whiting et al. 2000; Haines 2002; Saison et al. 2004). This species is known for its constitutive ability to tolerate and accumulate Zn, Cd and Ni up to very high concentrations in leaves. When grown in soil where Zn and Cd are heterogeneously distributed, *T. caerulescens* shows preferential root allocation in the Zn- and Cd-enriched patches (Schwartz et al. 1999; Whiting et al. 2000; Haines 2002). Growing *T. caerulescens* in soils differing only in the spatial distribution of Zn (heterogeneous vs. homogeneous soil Zn concentration), Haines (2002) observed that zincophilic root foraging in the heterogeneous treatment was associated with a better growth and a lower leaf Zn concentration. In the same study, by comparing plants from a highly Zn-contaminated site (Prayon) and a slightly Zn-contaminated site (Bradford Dale), Haines (2002) observed zincophilic root foraging only for the Prayon population, the strongest Zn accumulator. Whiting et al. (2000) showed Cd root foraging in a Cd hyperaccumulating population (Clough Wood), but not in a non Cd accumulating population (Prayon). In addition to root foraging, other mechanisms involved in the uptake of metals have been partly elucidated. When *T. caerulescens* is compared with the non-hyperaccumulator congener *Thlaspi arvense*, higher expression of Zn transporter genes in roots (Lasat et al. 1996, 2000; Pence et al. 2000; Assunção et al. 2001), enhanced xylem loading of Zn and enhanced uptake of Zn into leaf cells were found (Lasat et al. 1998; Lasat and Kochian 2000).

Up to now, metal root foraging in *T. caerulescens* has only been studied in metallicolous (M) populations (Schwartz et al. 1999; Whiting et al. 2000; Haines 2002; Saison et al. 2004) but never with non-metallicolous (NM) populations. Root foraging for Ni, which can also be accumulated by *T. caerulescens*, has never been investigated. The NM populations, which grow on non-contaminated soils with very low metal concentrations (in mg kg^{-1}; Zn<20; Cd<1; Ni<1; Molitor et al. 2005), are known for their strong capacity to accumulate Zn, Cd and Ni (Meerts and Van Isacker 1997; Escarré et al. 2000; Assunção et al. 2003; Molitor et al. 2005; Dechamps et al. 2007). *In natura*, NM plants have very high plant/soil concentration factors (CF) for Zn, Cd and Ni (CF_{Zn}~1,000, CF_{Cd}>30, CF_{Ni}>180) (Molitor et al. 2005) compared with those of M plants (CF_{Zn}<1, CF_{Cd}<1, CF_{Ni}<1). The high plant/soil CF of NM plants could be partly attributable to an increased root proliferation in metal-enriched patches of soil. A localized root proliferation in metal-enriched patches could be adaptive for NM plants in allowing them to efficiently acquire the metal required for healthy growth (Tolrà et al. 1996; Shen et al. 1997).

Two-dimensional boxes called rhizoboxes can be used to study root growth (e.g. Schwartz et al. 1999; Whiting et al. 2000; Goodson et al. 2003). To clearly visualize the root system rhizoboxes are generally slightly inclined to favour root growth close to the box surface. However, due to the design of the rhizobox, growth artefacts consisting of root slanting to one side of the box may occur (Migliaccio and Piconese 2001). Such slanting results from the interaction of root circumnutation (intrinsic circular movement, amplified by the box inclination) with the negative root thigmotropism (growing away from obstacle) triggered by contact with box surface. Up to now, root slanting was only demonstrated in *Arabidopsis thaliana* grown in tilted Petri dishes (Simmons et al. 1995; Migliaccio and Piconese 2001; Piconese et al. 2003).

In this study, using a rhizobox system, we compared root growth of M and NM plants of *T. caerulescens* in soils varying for metal distribution (homogeneous vs. heterogeneous metal distributions). Two metals, Zn and Ni, at two concentrations were considered in homogeneous and heterogeneous soil conditions. We addressed the following questions:

– Could the high concentration factor of NM plants be related with an increased root allocation in

metal-rich soil compartments? Would this root foraging be higher in NM than in M plants?
- Does root allocation in metal-rich compartments differ according to metal nature, Zn or Ni, and to metal concentration?
- Does selective root placement in metal-rich compartments increase metal uptake and growth? As Zn uptake was shown to influence the absorption of certain nutrients (Ca, Mg, etc.), how does heterogeneous metal supply influence nutrient uptake?

Two experiments are reported here. The second experiment was designed to answer questions raised by the first one. These questions concern the interaction between metal root foraging and root chirality.

Materials and methods

Studied populations

Two populations have been used in this study. The first is a metallicolous population originating from a calamine metalliferous site (enriched with Zn, Pb, Cd), located at Prayon (province of Liège, Belgium). This site has been contaminated for about 150 years by dust from a Pb–Zn–Cd smelter. The second is a non-metallicolous population growing on steep road banks situated at Winseler in the Oesling area (Grand-Duchy of Luxembourg). Seeds were collected on 20 plants randomly selected within each site. See Dechamps et al. (2008) for further details on those populations.

Soil preparation

The experimental substrate consisted of arable soil (sieved at 2 mm) in Experiment 1, and of a mixture of arable soil and sand (2:3, 1:3, *v*/*v*) in Experiment 2. The mineral composition of the arable soil was measured before metal contamination (in mg kg^{-1}, extraction ammonium acetate–EDTA 1 N pH 4.65: Ca, 2,147; Mg, 13; K, 170; Fe, 302; Mn 27; Zn, 9; Cd, <1; Ni, 1). This experimental substrate was used as control soil (CT). To obtain metal treatments, metallic salts (ZnO and $Ni(OH)_2$) were added and thoroughly mixed with the experimental substrate.

Experimental design

Plants were grown in flat boxes (rhizoboxes, as in Whiting et al. 2000) allowing visualization of roots in a two-dimensional system after several weeks. Rhizoboxes were constructed from square Petri dishes (12×12×2 cm), as conceived by Marschner and Römheld (1983, cited in Whiting et al. 2000). A slot (10 mm), situated on the top, allows the stem to pass through. Six smaller slots (4 mm), located on Petri dish base and covered by a cloth wick, allow homogeneous watering. Rhizoboxes were virtually divided in two equal left and right compartments (Fig. 1). In homogeneous treatments, both left and right compartments were filled with the same substrate (metal-contaminated or control). In the heterogeneous treatments, the left and the right compartments of one rhizobox were filled with substrates with of contrasting metal concentrations. A piece of cardboard was used as a temporary barrier to separate the two box halves during filling. In homogeneous treatments, the two box halves were also filled separately to ensure similar soil packing. Despite the removal of the partition between the two box halves, Whiting et al. (2000) showed that metal patches of heterogeneous treatments remain discrete.

Seeds were first sown in seed trays filled with compost in a glasshouse. At two cotyledons stage, one seedling was transplanted into each rhizobox, with the root positioned on the central line. Plants were grown either in control conditions or in various metal treatments in which Zn or Ni were added, either heterogeneously or homogeneously. The lid of the box was placed over the soil and fixed with tape. Rhizoboxes were placed on a sloping board holding them at an angle of 50° from the horizontal plane, with the lid positioned underside to ensure root growth close to the lid. This system was maintained in a glasshouse without additional lighting for a 3-month period.

Experiment 1

In heterogeneous treatments, the control soil (CT) was in the left compartment of the rhizobox, and the metal-contaminated soil was in the right one (as in Whiting et al. 2000). Zn and Ni were tested at two different concentrations each (Zn=500 and 1,000 mg kg^{-1}; Ni=125 and 250 mg kg^{-1}). The heterogeneous

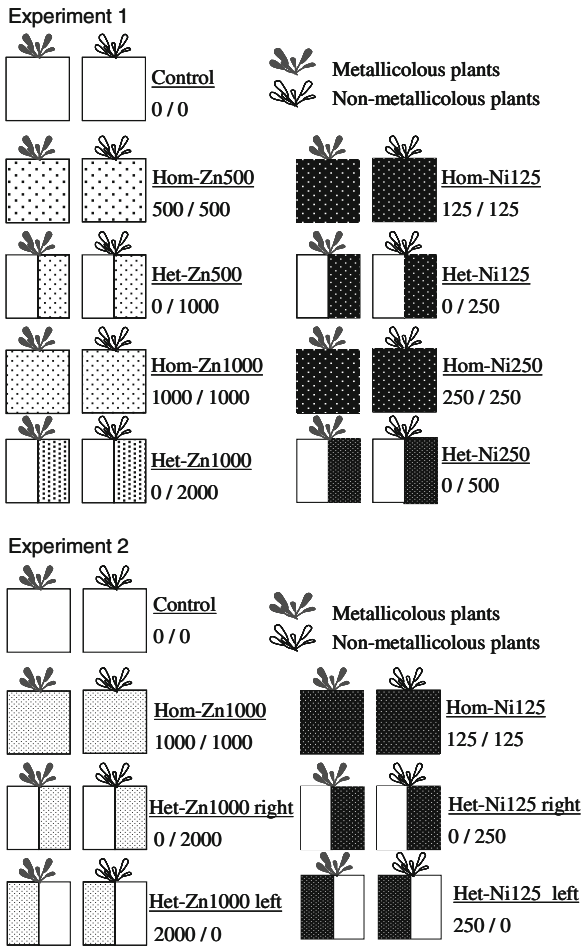

Fig. 1 Experimental design of two rhizobox experiments examining root allocation in *Thlaspi caerulescens* according to concentration and spatial distribution of metals in soil. Metallicolous and non-metallicolous plants were grown in uncontaminated control soil (*CT*) or in soils variously contaminated with metals (*Zn* or *Ni*). In homogeneous treatments (*Hom*), soil was uniformly contaminated with metals. In heterogeneous treatments (*Het*), one rhizobox half was filled with uncontaminated, and the other with metal-contaminated soil

treatments (Het-Zn and Het-Ni) were as follows: Zn0/Zn1000, Zn0/Zn2000, Ni0/Ni250, Ni0/Ni500 (Fig. 1). The homogeneous treatments (Hom-Zn and Hom-Ni) were: Zn500/Zn500, Zn1000/Zn1000, Ni125/Ni125, Ni250/Ni250 (Fig. 1). An uncontaminated control treatment (CT) was added. Each pair of treatments (e.g. HetZn500: Zn0/Zn1000 vs. HomZn500: Zn500/Zn500) differed by the spatial distribution of metal only but not in total metal supply. Six replicate rhizoboxes were prepared for each of the nine treatments (Fig. 1). What is called the right side of the rhizobox here is based on experimenter's view of the lid. This first experiment was conducted from July to September 2004.

Experiment 2

In heterogeneous treatments, the metal-enriched compartment was alternately placed in the right and the left compartment of rhizoboxes. Zn and Ni were considered in this experiment at one concentration each. The heterogeneous treatments (Het-Zn and Het-Ni) included Zn0/Zn2000 (Het-Zn right), Zn2000/Zn0 (Het-Zn left), Ni0/Ni250 (Het-Ni right), Ni250/Ni0 (Het-Ni left), corresponding to the following homogeneous treatments Zn1000/Zn1000 (Hom-Zn) and Ni125/Ni125 (Hom-Ni) (Fig. 1). An uncontaminated control treatment (CT) was added. For each treatment (CT, Hom-Zn, Het-Zn, Hom-Ni, Het-Ni), twelve replicates rhizoboxes were used. Within both heterogeneous treatments (Het-Zn and Het-Ni), six replicate rhizoboxes had their metal compartment in the left, and six in the right. This second experiment was conducted from February to April 2005.

Root allocation analysis

After 3 months, a digital photograph of the root system developed on rhizobox surface was taken. Root length in each left and right compartments of the box was determined by image analysis (Image J software, 2007, version 1.38). The great density of roots would have made root length measure after washing impossible. Soil and roots were divided down the central line using a scalpel and the two halves were separated. For each half, roots were washed, dried (60°C—48 h) and weighed to the nearest 0.1 mg. Root mass and root length allocated to the metal-enriched compartment is expressed as a percentage of the total root mass or length. Similar calculations were performed for roots in the right compartment of rhizoboxes containing homogeneous soils (CT or metal-contaminated). Values that are not significantly different from 50% indicate no effect of Zn distribution on root growth.

Mineral analyses of shoots

Shoots were harvested, rinsed in deionised water, dried (60°C—48 h), and weighed to the nearest 0.1 mg. They were mineralised in a mixture of nitric and perchloric

acid with a Tecator digestor. Zn, Ni, Ca, K, Mg, Fe concentrations were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES; Vista MPX, Varian Inc., California, USA).

Statistical analyses

The percentage of roots in the metal-enriched compartment, the total dry weight and the shoot elemental concentrations were analysed by three-way ANOVAs for Experiment 1 and by two-way ANOVAs for Experiment 2 (STATISTICA 7, Statsoft, 2005). In Experiment 1, the fixed factors tested were (1) population (M vs. NM), (2) pattern of metal distribution in soil (Hom- vs. Het-) and (3) concentration (low vs. high). In Experiment 2, the fixed factors tested were (1) population (M vs. NM) and (2) treatment (Hom- vs. Het-right vs. Het-left). Means were compared with Fisher's post hoc tests. To fulfil the normality assumption of ANOVAs, logarithmic transformations were applied. The uncontaminated control (CT) was not included in the ANOVAs described above, but it was compared to all metal treatments using Fisher's post hoc tests. The percentages of root mass and length developed in the right compartment of homogeneous treatments (metal-contaminated or control) and in the metal-enriched compartment of heterogeneous treatment were compared to the expected value of 50% with a *t*-test for single means (STATISTICA 7, Statsoft, 2005).

Results

Experiment 1

Allocation of root mass and root length in Het-Zn and Hom-Zn treatments

In both heterogeneous treatments (Het-Zn500 and Het-Zn1000), NM plants had greater root mass in the right, Zn-enriched compartment (~63%; significantly different from 50%, *t*-test: $t=12.4$, $P<0.001$; Fig. 2a), than in the left uncontaminated compartment. Soil Zn concentration did not influence the intensity of root proliferation in the Zn-enriched compartment (Table 1). In contrast, M plants did not preferentially allocate roots in the right Zn-enriched compartment (*t*-test: $P>0.05$; Fig. 2a). These results account for the significant effect of pattern (Hom-Zn vs. Het-Zn) and the marginally significant effect of population × pattern in the ANOVA (Table 1). In the homogeneous soil treatments (Hom-Zn and CT), both NM and M plants developed similar root mass in left and right compartments (no deviation from the expected mean of 50%; *t*-tests: $P>0.05$).

As for root mass, NM plants had higher root length in the right Zn-enriched compartment of Het-Zn treatments than in the right compartment of Hom-Zn treatments (Fig. 2a, Table 1: significant effects of pattern and pattern × population). However, it is worth noting that root length allocated to the right compart-

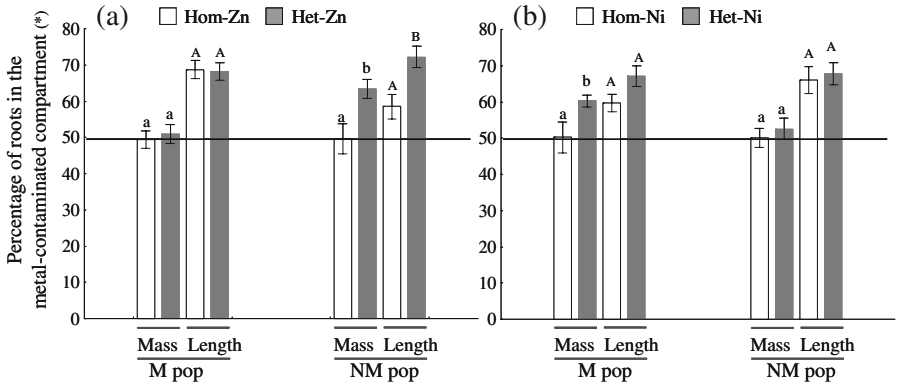

Fig. 2 Root allocation (mass and length) of metallicolous (*M*) and non-metallicolous (*NM*) plants of *Thlaspi caerulescens*, according to metal distribution in soil (homogeneous or heterogeneous metal concentration in the rhizobox) in Experiment 1. Root foraging was investigated for Zn (**a**) and for Ni (**b**). High and low concentrations of Zn and Ni are pooled as no effect of concentration on root allocation was detected. Data are means±SE. *Asterisk* for homogeneous treatments, *y*=percentage of roots in right compartment of rhizobox. *Bars topped with similar small* (for mass) or *capital* (for length) *letters* are not significantly different ($P>0.05$, Fisher's post hoc test)

Table 1 ANOVAs of the percentage of root (mass and length) allocated to the metal (Zn or Ni) enriched compartment, total mass and leaf metal concentration (Zn or Ni)

	Percentage of root mass in the metal-enriched compartment				Percentage of root length in metal-enriched compartment				Total mass				Leaf metal concentration			
	df	MS	*F*	*P*	*df*	MS	*F*	*P*	*df*	MS	*F*	*P*	*df*	MS	*F*	*P*
Experiment 1																
Zn																
Population	1	0.021	2.56	0.118	1	0.005	1.17	0.287	1	0.001	0.002	0.893	1	1.310	98.67	<0.001
Concentration	1	0.003	0.36	0.552	1	0.003	0.71	0.407	1	0.040	1.46	0.234	1	0.021	1.54	0.224
Pattern	1	0.048	5.95	0.019	1	0.022	5.16	0.030	1	0.410	14.76	<0.001	1	0.079	5.95	0.021
Population × Conc	1	0.001	0.13	0.723	1	0.009	2.12	0.154	1	0.016	0.58	0.45	1	0.001	0.05	0.82
Population × Pattern	1	0.031	3.79	0.059	1	0.023	5.41	0.026	1	0.023	0.84	0.364	1	0.029	2.22	0.146
Pattern × Conc	1	0.011	1.35	0.253	1	0.003	0.61	0.441	1	0.032	1.69	0.286	1	0.002	0.12	0.733
Pop × Pattern × Conc	1	0.001	0.08	0.778	1	0.0004	0.10	0.758	1	0.0001	0.002	0.961	1	0.036	2.70	0.111
Residual	38	0.008			33	0.004			38	0.028			32	0.013		
Ni																
Population	1	0.006	0.58	0.449	1	0.004	1.09	0.305	1	0.00043	1.01	0.323	1	18.45	124.5	<0.001
Concentration	1	0.001	0.07	0.797	1	0.000	0.01	0.925	1	0.00002	0.04	0.851	1	1.31	8.83	0.005
Pattern	1	0.100	10.35	0.003	1	0.012	2.87	0.100	1	0.00002	0.05	0.824	1	0.70	4.73	0.037
Population × Conc	1	0.004	0.38	0.544	1	0.005	1.34	0.255	1	0.00031	0.73	0.400	1	0.13	0.90	0.349
Population × Pattern	1	0.020	2.08	0.158	1	0.003	0.75	0.394	1	0.00017	0.38	0.540	1	0.81	5.48	0.025
Pattern × Conc	1	0.001	0.06	0.802	1	0.001	0.27	0.607	1	0.00002	0.05	0.826	1	0.091	0.61	0.439
Pop × Pattern × Conc	1	0.006	0.61	0.441	1	0.009	2.21	0.148	1	0.00064	1.47	0.233	1	0.12	0.81	0.375
Residual	36	0.010			31	0.004			36	0.00043			34	0.15		
Experiment 2																
Zn																
Population	1	0.008	0.76	0.390	1	0.007	0.9	0.349	1	0.094	3.31	0.076	1	1.33	110.7	<0.001
Treatment	2	0.017	1.61	0.212	2	0.075	9.58	<0.001	2	0.124	4.37	0.019	2	0.73	60.3	<0.001
Population × Treat	2	0.001	0.08	0.924	2	0.005	0.62	0.544	2	0.146	5.14	0.010	2	0.19	15.8	<0.001
Residual	40	0.011			39	0.008			40	0.028			41	0.012		
Ni																
Population	1	0.005	1.52	0.224	1	0.007	1.10	0.301	1	0.025	0.79	0.378	1	17.63	235.7	<0.001
Treatment	2	0.014	4.51	0.020	2	0.02	2.66	0.083	2	0.046	1.46	0.244	2	0.19	2.60	0.086
Population × Treat	2	0.003	1.01	0.375	2	0.002	0.36	0.700	2	0.005	0.15	0.864	2	0.079	1.06	0.358
Residual	40	0.003			37	0.006			40	0.032			40	0.075		

Sources of variation in Experiment 1: population (Pop: metallicolous vs. non-metallicolous), concentration (Conc: 500 vs. 1,000 mg Zn kg^{-1} or 125 vs. 250 mg Ni kg^{-1}), and pattern (homogeneous vs. heterogeneous).

Sources of variation in Experiment 2: populations (Pop) and Treatment (Treat: homogeneous vs. heterogeneous right vs. heterogeneous left).

All factors were considered as fixed.

ment was superior to 50% in all treatments (CT, Hom-Zn and Het-Zn) for both M plants (*t*-tests; CT: $t=6.27$, $P<0.05$; Hom-Zn: $t=7.24$, $P<0.001$; Het-Zn: $t=7.5$, $P<0.001$; Fig. 2a) and NM plants (CT: $t=4.42$, $P<0.05$; Hom-Zn: $t=2.49$, $P<0.05$; Het-Zn: $t=7.68$, $P<0.001$; Fig. 2a).

Allocation of root mass and root length in Het-Ni and Hom-Ni treatments

In both Het-Ni treatments (Het-Ni125 and Het-Ni250), M plants developed more root mass in the right, Ni-enriched compartment (~60%; significantly different from 50%: $t=6.1$, $P<0.001$), independently of soil Ni concentration (Fig. 2b; Table 1: significant pattern effect: Hom-Ni vs. Het-Ni). In contrast, NM population did not show preferential root mass allocation in the right Ni-enriched compartment of Het-Ni treatments ($P>0.05$; Fig. 2b). In the homogeneous treatments (Hom-Ni and CT), both populations had equal root masses in the left and right compartments (no deviation from the expected mean of 50%, $P>0.05$).

As for Zn and CT treatments, plants had greater root length in the right compartment of boxes in all Ni treatments. This was observed for both M

Table 2 Leaf elemental concentrations of metallicolous (M) and non-metallicolous (NM) plants of *Thlaspi caerulescens* in uncontaminated control soil (CT) and in metal treatments (Zn or Ni)

Experiment 1

	M population					NM population				
	CT	HOM-Zn500	HET-Zn500	HOM-Zn1000	HET-Zn1000	CT	HOM-Zn500	HET-Zn500	HOM-Zn1000	HET-Zn1000
Zn treatments										
Mg	2,285^{a}	3,123^{b}	3,358^{bc}	4,866^{c}	3,760^{bc}	5,059^{AB}	4,809^{A}	5,214^{AB}	6,629^{B}	5,365^{AB}
	113	*95*	*210*	*891*	*469*	*960*	*398*	*433*	*365*	*714*
Ca	17,177^{a}	18,206^{a}	18,268^{a}	20,967^{a}	20,728^{a}	22,211^{B}	15,917^{A}	20,790^{B}	21,350^{B}	19,284^{AB}
	1631	*1454*	*971*	*842*	*3067*	*3054*	*1560*	*662*	*1814*	*2392*
K	20,286^{a}	21,324^{a}	28,653^{b}	27,174^{b}	23,786^{ab}	23,546^{B}	12,998^{A}	15,555^{AB}	18,145^{AB}	15,893^{AB}
	2293	*179*	*1822*	*1361*	*1013*	*3922*	*1915*	*781*	*3184*	*239*
Fe	195^{b}	174^{a}	174^{a}	192^{ab}	165^{a}	187^{B}	114^{A}	143^{B}	134^{AB}	174^{B}
	28	*13*	*3*	*28*	*32*	*24*	*15*	*9*	*8*	*16*

Experiment 2

	M population				NM population			
	CT	HOM-Zn	HET-Zn right	HET-Zn left	CT	HOM-Zn	HET-Zn right	HET-Zn left
Zn treatments								
Mg	1,892^{a}	2,191^{a}	2,465^{a}	1,762^{a}	2,484^{A}	6,555^{B}	2,532^{A}	2,591^{A}
	256	*212*	*318*	*55*	*148*	*691*	*398*	*292*
Ca	16,843^{a}	14,720^{a}	15,319^{a}	12,430^{a}	20,421^{AB}	27,151^{B}	15,908^{A}	16,045^{A}
	1802	*1679*	*1346*	*508*	*1311*	*2783*	*2757*	*2382*
K	36,889^{a}	38,696^{a}	42,620^{a}	28,335^{a}	35,195^{B}	32,553^{AB}	25,049^{A}	28,711^{AB}
	4906	*4851*	*4517*	*1474*	*2064*	*3593*	*3927*	*2204*
Fe	64^{ab}	53^{a}	69^{b}	65^{ab}	96^{B}	59^{A}	74^{AB}	69^{AB}
	5	*6*	*6*	*7*	*10*	*8*	*11*	*10*
Ni treatments								
Mg	1,892^{a}	2,223^{a}	2,116^{a}	1,824^{a}	2,484^{AB}	2,699^{B}	2,489^{AB}	1,994^{A}
	256	*370*	*516*	*179*	*148*	*229*	*305*	*124*
Ca	16,843^{a}	15,712^{a}	13,166^{a}	14,103^{a}	21,733^{B}	16,541^{A}	18,340^{AB}	16,002^{A}
	1802	*1533*	*2234*	*2379*	*1776*	*1254*	*2835*	*1039*
K	36,889^{a}	31,554^{a}	29,708^{a}	31,059^{a}	35,195^{B}	27,832^{AB}	31,289^{AB}	24,446^{A}
	4906	*2544*	*4957*	*2366*	*2064*	*2425*	*4134*	*6208*
Fe	64^{a}	59^{a}	61^{a}	76^{a}	96^{A}	109^{A}	85^{A}	107^{A}
	5	*4*	*11*	*11*	*10*	*8*	*5*	*20*
Ni	5^{a}	144^{b}	115^{b}	124^{b}	178^{A}	3,522^{C}	1,810^{B}	1,837^{B}
	1	*10*	*26*	*23*	*11*	*263*	*209*	*185*
Zn	1,645^{a}	1,768^{a}	1,400^{a}	1,874^{a}	2,695^{B}	1,786^{A}	2,061^{A}	1,843^{A}
	160	*141*	*172*	*205*	*131*	*120*	*203*	*159*

In Experiment 1, only Zn treatments was showed: homogeneous (Hom) and heterogenous (Het) Zn distribution at two concentrations (500 and 1000 mg Zn kg^{-1}).

In Experiment 2, both Zn and Ni treatments were showed: homogeneous (Hom) and heterogeneous (Het) Zn and Ni distributions at one concentration (1000 mg Zn kg^{-1}, 125 mg Ni kg^{-1}). The metal-enriched compartment of heterogeneous treatment was alternately located on the right (Het-Zn right and Het-Ni right) and on the left (Het-Zn left and Het-Ni left) compartment of rhizobox.

Data are means ±SE. Values sharing one identical superscript letter (small for M, capital for NM) are not significantly different ($P>0.05$, Fisher's post-hoc test).

plants (*t*-tests; Hom-Ni: $t=4.04$, $P<0.01$; Het-Ni: $t=5.85$, $P<0.001$; Fig. 2b) and NM plants (*t*-tests; Hom-Ni: $t=4.3$, $P<0.01$; Het-Ni: $t=5.97$, $P<0.001$; Fig. 2b). As for root mass, M plants tended ($P=0.10$) to allocate higher root length in the right Ni-enriched compartment of Het-Ni treatment than in the right compartment of Hom-Ni treatment (Fig. 2b; Table 1).

Growth in Zn treatments

At corresponding Zn concentrations, plants exposed to Het-Zn treatments had a higher total mass than those exposed to Hom-Zn treatments (Fig. 3a; Table 1: significant pattern effect). This effect was significant at both concentrations (500 and 1,000 mg Zn kg^{-1}) for M plants and only at the lowest

concentration for NM plants (Fig. 3a, $P<0.08$). Neither M nor NM populations showed differences in mass between CT and both Hom-Zn treatments (Fig. 3a). In each of the five treatments, M and NM populations had similar masses (Fig. 3a). M plants invested proportionally less roots in the heterogeneous treatment than in the homogeneous treatment, at the lowest Zn concentration only (Fig. 3a). NM plants had similar root/shoot ratios across all treatments, except in Hom-Zn1000 treatment, where the root/shoot ratio steeply decreased (Fig. 3a).

Leaf Zn and nutrient concentrations in Zn treatments

Across all treatments, NM plants accumulated significantly more Zn in shoots compared to M plants (Fig. 3b, Table 1: significant population effect). At the lowest Zn concentration in soil (500 mg Zn kg^{-1}), M plants had a higher leaf Zn concentration in Hom-Zn treatment than in Het-Zn treatment (Fig. 3b, $P<0.08$). The same trend was observed for NM plants at the highest Zn concentration (Fig. 3b, $P<0.08$). These results match the significant pattern effect in the ANOVA (Table 1).

Variations of leaf Mg concentrations across Zn treatments were similar to those of Zn concentrations ([Mg] in NM plants>[Mg] in M plants; [Mg] tended to be higher in Hom-Zn1000 treatment than in Het-Zn1000 treatment for both populations; Table 2). Moreover, leaf Mg concentration of both populations increased with Zn concentration in soil in homogeneous treatments (Table 2). Compared to CT treat-

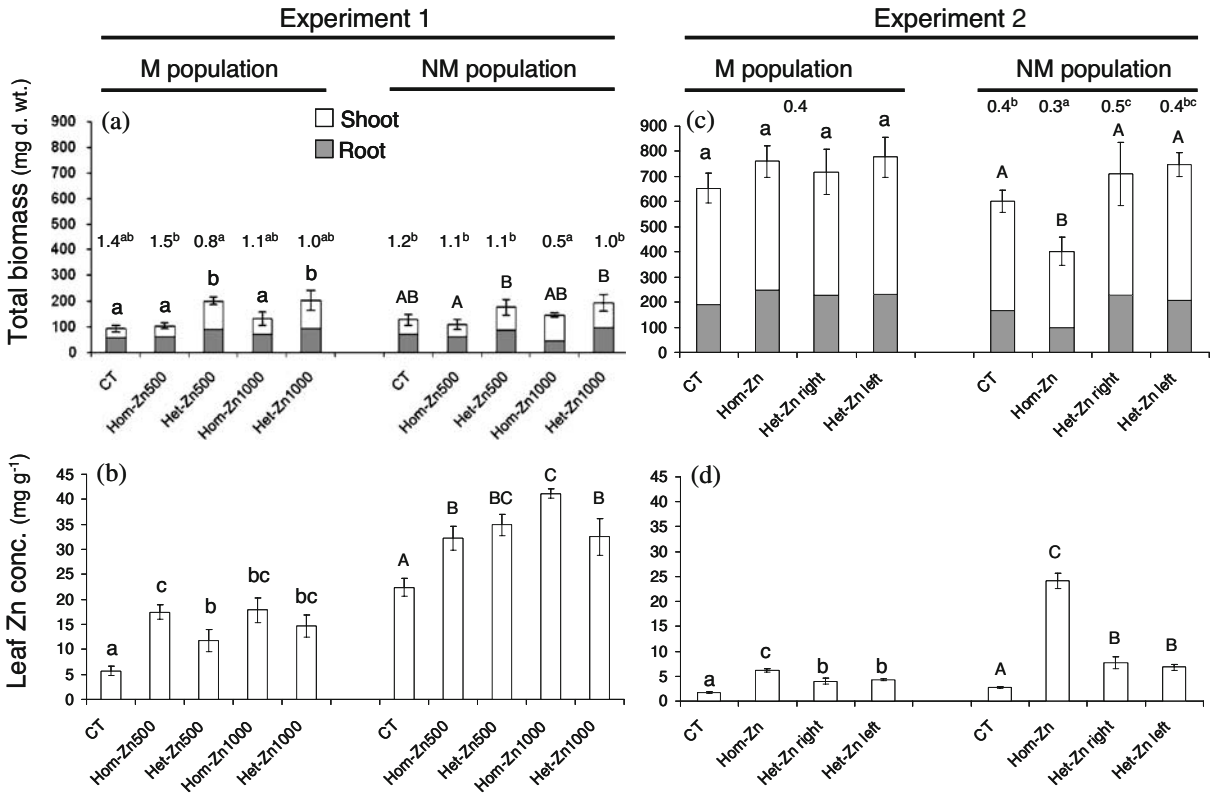

Fig. 3 Total mass (**a**, **c**) and leaf Zn concentration (**b**, **d**) of metallicolous (*M*) and non-metallicolous (*NM*) plants of *Thlaspi caerulescens* in uncontaminated control (*CT*) soil and various Zn treatments of Experiments 1 (**a**, **b**) and 2 (**c**, **d**). The root/shoot ratios are shown above each bars of total mass. Zn treatments of Experiment 1: homogeneous (*Hom*) and heterogeneous (*Het*) Zn distribution at two concentrations (500 and 1,000 mg Zn kg^{-1}). Zn treatments of Experiment 2: homogeneous (*Hom*) and heterogeneous (*Het*) Zn distribution at one concentration (1,000 mg kg^{-1}). In Experiment 2, the Zn-enriched compartment of heterogeneous treatment was alternately located in the right (*Het-Zn right*) and in the left (*Het-Zn left*) compartment of rhizobox. Data are means±SE. *Bars topped with different small* (for *M*) or *capital* (for *NM*) *letters* are significantly different ($P<0.08$, Fisher's post hoc test)

ment, M plants accumulated significantly more Mg in Zn treatments (Table 2). Significant positive correlations between Mg and Zn leaf concentrations were also found for both populations across CT and Zn treatments (M plants: r=+0.57, P<0.01, n=21; NM plants: r=+0.73, P<0.001, n=23). Ca, K and Fe concentrations in leaves of NM plants decreased in Hom-Zn500 compared to all other treatments (Table 2). Higher leaf K concentrations in Het-Zn500 treatment than in Hom-Zn500 treatment was observed in M population.

Growth in Ni treatments

Population origin, pattern of Ni distribution and Ni concentration in soil did not influence the total plant mass (Table 1). However, plants growing in CT treatment had a mass significantly lower than those growing in Ni treatments (115±14 mg vs. 149±7 mg dry weight, Fisher's post hoc test, P=0.04).

Leaf Ni and nutrient concentrations in Ni treatments

Across all Ni treatments (homogeneous and heterogeneous), NM plants accumulated much more Ni (~2,000 to 6,500 mg Ni kg^{-1}) than M plants (~90 to 160), accounting for the significant population effect (Table 1). Even in the CT treatment, NM plants accumulated 60 times more Ni than M plants (400 vs. 7 mg Ni kg^{-1}). For both Ni concentrations in soil (125 and 250 mg Ni kg^{-1}), NM plants had about twice as much Ni in their shoots in Hom-Ni treatments than in Het-Ni treatments (means±SE, in mg Ni kg^{-1}; Hom-Ni125=3,729±595 vs. Het-Ni125=2,325±546; Hom-Ni250=6,512±144 vs. Het-Ni250=3,093±431). For both Ni concentrations in soil, M plants showed similar Ni accumulation in homogeneous and heterogeneous treatments (means±SE, in mg Ni kg^{-1}; Hom-Ni125=86±7 vs. Het-Ni125=93±6; Hom-Ni250=155±9 vs. Het-Ni250=156±5). These results explain the significant effects of pattern and pattern×population (Table 1). Concentration of Ni in soil (125 and 250 mg Ni kg^{-1}) significantly influenced the Ni leaf concentration of both M plants (respectively ~90 and 150 mg Ni kg^{-1}) and NM plants (respectively ~3,100 and 4,600 mg Ni kg^{-1}) (Table 1). Leaf nutrient concentrations were not influenced by the presence of Ni and the pattern of Ni distribution in soil (homogeneous vs. heterogeneous) (data not shown).

Experiment 2

Experiment 2 was designed to specifically investigate the systematic higher root length production in the right compartment of boxes, as well as to check the lack of Zn root foraging in the M population that had already been observed in previous studies (Schwartz et al. 1999; Whiting et al. 2000; Haines 2002).

Allocation of root mass and root length in Het-Zn and Hom-Zn treatments

Overall, in Het-Zn treatments (Het-Zn right and Het-Zn left), M and NM plants did not allocate more root mass in the Zn-enriched compartment than in the non-contaminated compartment of boxes (not significantly different from 50%, t-test: P>0.05). However, both M and NM plants tended to allocate lower root mass in the Zn-enriched compartment when it was placed in the left compartment (M: t-test: P=0.09; NM: t-test: P=0.14; Fig. 4a). In Hom-Zn treatment, all plants developed similar root mass in the right and left compartments of boxes (not significantly different from 50%, t-test: P>0.05).

Both populations developed more root length in the Zn-enriched compartment of Het-Zn treatments, but only when Zn was placed in the right compartment of boxes (~68% for M and NM, Fig. 4a; t-tests; M population: Het-Zn right: t=4.9, P=0.004 vs. Het-Zn left: t=0.8, P>0.05; NM population: Het-Zn right: t=2.7, P=0.04 vs. Het-Zn left: t=1.1, P>0.05). These results explain the significant treatment effect (Table 1). In contrast to Experiment 1, in Hom-Zn and CT treatments, no difference of root length allocation between the left and right compartments of boxes was detected for both populations (comparison with the expected value of 50% by t-tests: P>0.05; Fig. 4a).

Allocation of root mass and root length in Het-Ni and Hom-Ni treatments

In both Het-Ni treatments (Het-Ni right and Het-Ni left), M plants developed more than 50% of root mass (t-tests; Het-Ni right: t=3.3, P=0.02; Het-Ni left: t=2.2, P=0.07; Fig. 4b) and root length (Het-Ni right: t=3.5, P=0.024 vs. Het-Ni left: t=2.1, P=0.086; Fig. 4b) in the Ni-enriched compartment of boxes. It is worth noting that this effect was stronger when Ni

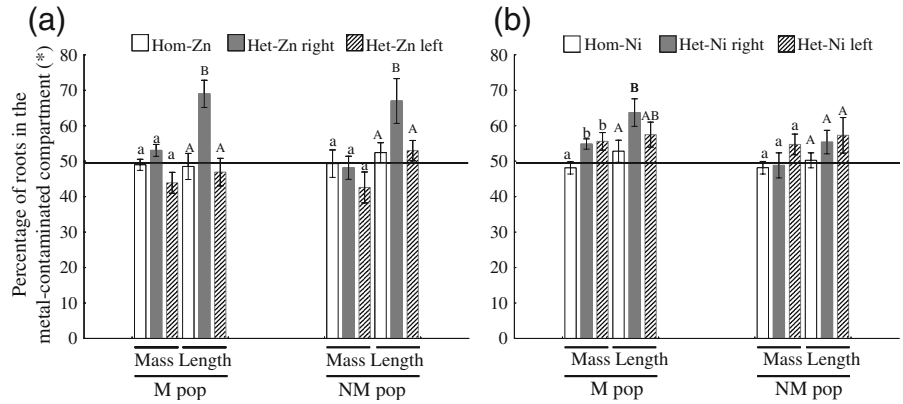

Fig. 4 Root allocation (mass and length) of metallicolous (*M*) and non-metallicolous (*NM*) plants of *Thlaspi caerulescens*, according to metal distribution in soil (homogeneous or heterogeneous metal concentration in the rhizobox) in Experiment 2. Root foraging was investigated for Zn (**a**) and for Ni (**b**). In heterogeneous treatments, the metal-enriched compartment in the rhizobox was alternately located on the right and on the left. Data are means±SE. *Asterisk* for homogeneous treatments, *y*=percentage of roots in right compartment of rhizobox. Data are means±SE. *Bars topped with similar small* (for *M*) or *capital* (for *NM*) *letters* are not significantly different ($P>0.05$, Fisher's post hoc test)

was placed on the right (Fig. 4b). For the NM population, preferential allocation of roots in the Ni-enriched compartment was not detected (slightly greater root mass and length in the left Ni-enriched compartment, not significant). In Hom-Ni treatment and CT, no difference of root mass and length allocation between the left and right compartments of boxes was detected (comparison with expected value of 50% by *t*-test: $P>0.05$). These results are reflected in the significant and the marginally significant treatment effects (Table 1).

Growth in Zn treatments

In Experiment 2, plants produced three to four times more total mass compared to those growing in Experiment 1 (Fig. 3a,c). M plants did not show mass variation across treatments (CT, Hom-Zn1000, Het-Zn1000 right, Het-Zn1000 left) (Fig. 3c). In contrast, and as in Experiment 1, mass of NM plants significantly decreased in the Hom-Zn treatment (Fig. 3c), explaining the significant treatment and treatment×population effects (Table 1). The position of Zn in Het-Zn treatments (right vs. left) had no effect on mass of both populations (Fig. 3c). The root:shoot ratio was on average two times lower in Experiment 2 compared to Experiment 1 (0.4 vs. 1; Fig. 3a,c). As in Experiment 1, NM plants had a significantly lower root:shoot ratio in the Hom-Zn treatment than in CT and both Het-Zn treatments (Fig. 3c). For M plants, no variation of root:shoot ratio was observed (Fig. 3c).

Leaf Zn and nutrient concentrations in Zn treatments

As in Experiment 1, NM plants did accumulate more Zn than M plants in all treatments (Fig. 3d; Table 1: significant population effect). As in Experiment 1, a higher leaf Zn accumulation was observed in the Hom-Zn treatment compared with both Het-Zn treatments for both populations (Fig. 3d; Table 1: significant treatment effect). The position of the Zn compartment (right vs. left) in Het-Zn treatments had no effect on Zn accumulation for both populations (Fig. 4c).

In NM plants, Mg and Ca concentrations were higher in the Hom-Zn treatment compared with both Het-Zn treatments. As in Experiment 1, a positive and significant correlation between Zn and Mg was found across CT and Zn treatments for both populations (M: $r=+0.43$, $P<0.01$, $n=35$; NM: $r=+0.83$, $P<0.001$, $n=34$). An inhibition of Fe uptake was observed in Hom-Zn treatment compared to CT treatment for NM plants.

Growth in Ni treatments

As in Experiment 1, plant origin and pattern of Ni distribution had no influence on the mass of both populations (Table 1). In contrast to Experiment 1, the

mass of plants growing in CT treatment was not significantly different from the mass of plants growing in all other treatments ($P>0.05$). It is worth noting that masses produced in Experiment 2 were four times higher than those developed in Experiment 1 (data not shown).

Leaf Ni and nutrient concentrations in Ni treatments

As in Experiment 1, NM plants accumulated much more Ni than M plants (in mg Ni kg^{-1}; ~2,500 for NM vs. ~120 for M), accounting for the significant population effect (Table 1). In the control treatment, NM plants accumulated 100 times more Ni than M plants (~500 vs. ~5). As in Experiment 1, NM plants had significantly more Ni in their shoots in Hom-Ni treatment than in both Het-Ni treatments (in mg Ni kg^{-1}; Hom-Ni=3,522±263; Het-Ni right=1,810±209; Het-Ni left=1,837±185; Table 1: significant treatment effect). For M plants, no difference in Ni accumulation between the three Ni treatments was found (as in Exp. 1; Table 2).

The presence of Ni in soil and the pattern of Ni distribution had no influence on leaf nutrient composition of M plants. The presence of Ni in soil inhibited Zn and Ca accumulation (comparison with control) in NM plants but not in M plants (Table 2).

Discussion

Before discussing results in details, it is worth emphasizing the experimental differences between the two experiments. Plants were grown in a glasshouse subject to seasonal variations (no control of photoperiod and temperature). Low spring temperatures in Experiment 2 obviously provided better growth conditions for *T. caerulescens* as shoot mass produced in 3 months was six times higher than that produced in 3 months in summer (Exp. 1) (in mg dry weight: ~470 vs. ~80). Similarly, root mass was three times higher in Experiment 2 than in Experiment 1 (~200 vs. ~70). These differences in root growth indisputably influenced the assessment of metal root foraging. That metal root foraging was only detected through differences of root length, and not of root mass in Experiment 2 is thus not surprising as rhizoboxes were full of roots. Although zincophilic root foraging based on mass measure could probably have been detected at earlier stage, at the end of the experiment, roots equally occupied the two rhizobox compartments. This exact phenomenon was investigated by Hutchings and John (2004) in several species growing in heterogeneous nutrient treatments. For all species, root foraging in nutrient-rich patch was no more detectable above a certain root mass. In contrast to root mass, root elongation in Zn patch (architectural response) would remain visible longer, at least until root death. In both experiments the percentage of root length in the metal-enriched compartment was positively correlated to the percentage of root mass (data not shown).

In both experiments, we have demonstrated for the first time that NM plants known for their strong Zn accumulation, do proliferate roots in Zn-enriched patches of soil. The detection of this response in Experiments 1 and 2 shows that root foraging is more constantly expressed in NM than in M plants. NM plants growing in soils with low Zn concentrations, Zn root foraging in normal soils would always be essential. Interestingly, root foraging for Zn in NM plants was detected in a concentration range (500–1,000 mg Zn kg^{-1}) much higher than *in natura* (~20 mg Zn kg^{-1}), and these concentrations are known to decrease NM plant fertility (Dechamps et al. 2007). This suggests a lack of regulation for this character in NM plants. If root foraging for Zn in NM plants probably contributes to improve their Zn uptake, it does however not account for their higher Zn accumulation capacity as M plants can express a similar level of Zn root foraging. Root foraging rather appears as a secondary mechanism optionally used for Zn uptake (see below).

In contrast to NM plants, M plants expressed root foraging for Zn in Experiment 2 only. Due to faster growth in Experiment 2, M plants probably required more Zn, which they could not have acquired without Zn root foraging. This result therefore suggests that the root foraging for Zn in M plants is more influenced by experimental conditions than that of NM plants. In several plant species, root foraging has been shown to depend on the scale and position of nutrient patch (Wijesinghe and Hutchings 1997, 1999; Wijesinghe et al. 2001), on the depletion of nutrient patch (Fransen et al. 1998) and on the developmental stage of plants (Hutchings and John 2004).

In both experiments, Ni-rich patches in soil induced root proliferation in M plants only, indicating

that root foraging in *T. caerulescens* is influenced by metal nature. Root foraging for Ni is here demonstrated for the first time in *T. caerulescens*. The nickelophilic behaviour of M plants could have evolved in response to the low availability of Ni in metalliferous soils, due to high competition with other metals accumulated by *T. caerulescens* (Zn, Cd). Indeed, Ni is an essential nutrient for plants and Ni concentration in the soil of Prayon is only slightly higher than that in normal soils in Luxembourg (5 vs. 1 mg kg^{-1}, unpublished data). As for Zn, Ni root foraging does not explain the difference in Ni accumulation between the two populations (Ni root foraging was only expressed in the lowest Ni accumulator, M population).

For both populations, root foraging was not influenced by the different concentrations of Zn and Ni (Exp. 1). This is in line with previous studies on Zn root foraging in the M population of Prayon (Whiting et al. 2000; Haines 2002). Nevertheless, lower metal concentrations (similar to natural soils of NM plants) should be considered in future studies to better assess the plasticity of metal root foraging.

In the present study, plants grown in soils with heterogeneous Zn and Ni supply had similar or lower leaf metal concentrations than those grown in soils with the same metal content but supplied homogeneously. For Zn, these results confirm those of Whiting et al. (2000) and Haines (2002). If root proliferation in the metal-enriched compartment of heterogeneous treatments is a metal acquisition mechanism, plants showing metal root foraging should uptake more Zn or Ni compared with plants allocating roots equally between the two compartments of heterogeneous treatments. To assess the advantage of metal root foraging on metal uptake, the actual total shoot metal content must be compared to the total metal content expected if roots had been equally distributed in both compartments of rhizoboxes (i.e. no root foraging). In the present study, such calculations (similar as those of Whiting et al. 2000) can be performed for NM plants showing Zn foraging, and for M plants showing Ni foraging (Exp. 1 only). Assuming no root foraging in NM plants in Het-Zn500 (i.e. 0/1,000), the expected leaf Zn content should be 2.9 mg Zn (calculated as 50% of the mean leaf content of Zn in CT (i.e. 0/0) plus 50% of that in Hom-Zn1000 (i.e. 1,000/1,000). The actual Zn content measured in NM plants that did express root foraging was 3.4±0.7. This value is higher but not significantly different from 2.9 (*t*-test: $P=0.89$). For M plants in Het-Ni125 treatment (Exp. 1), the actual Ni content is higher and almost significantly different from that expected if no root foraging had been observed (in μg; 5.1±0.7 vs. 3.2, *t*-test: $P=0.06$). These calculations show that enhanced root allocation in metal-enriched compartment does not necessarily result in significantly higher metal uptake. Nevertheless, these calculations assume no regulation of metal uptake by internal metal concentration and must therefore be considered with caution. Moreover, the advantage of metal root foraging should be tested for lower metal concentrations.

Plant growth was influenced by the distribution of Zn in soil only (not by Ni), but in different ways according to Experiments. In Experiment 1, both populations grew better in heterogeneous than in homogeneous treatments as observed by Haines (2002). Nevertheless, in contrast to Haines (2002), this growth stimulation was not systematically associated with a Zn root foraging response. The increased growth in heterogeneous treatments could result from an enhanced nutrient availability in the non-contaminated compartment of the rhizobox. Nevertheless, no consistent impact of soil Zn distribution on leaf nutrient concentrations was detected in Experiment 1 (except for M plants: higher leaf K in Het-Zn500 compared with Hom-Zn500, and for NM plants: higher leaf Ca and Fe in Het-Zn500 compared with Hom-Zn500). More investigations, as the analyses of other essential nutrients (N, P) are needed to better understand the growth stimulation observed in Zn heterogeneous conditions. In Experiment 2, the growth inhibition of NM plants in the Zn homogeneous treatment was likely due to internal Zn intoxication (two-fold higher leaf Zn concentration in Hom-Zn1000 than in Het-Zn1000). Analyses of leaf nutrient concentrations mainly revealed across both experiments a great synergy between Zn and Mg uptakes in both populations (as observed in *T. caerulescens* by Dechamps et al. 2005), which probably reflects the use of common transporters.

New insights into the rhizobox approach: root growth asymmetry and influence of Zn patch location on Zn root foraging

In Experiment 1, plants of both origins had a higher root length in the right compartment of all treatments

(Ni and Zn, homogeneous and heterogeneous), which interfered with root foraging response. To our knowledge, root growth asymmetry has never been reported in any previous study of root foraging. Chirality of roots has mainly been investigated by physiologists interested in root spiralizations and tropism (Migliaccio and Piconese 2001), and very few ecological studies have taken into account these root characteristics. In *A. thaliana*, root slanting to one side of the rhizobox would result from the interaction of root circumnutation with the negative root thigmotropism created by contact with box surface (Migliaccio and Piconese 2001). The chirality of root length observed in Experiment 1 could therefore be due to a similar phenomenon. In Experiment 2, we tried to separate what may be called root artefact (i.e. right root slanting) from what we wanted to quantify (i.e. root foraging) by placing the metal-enriched compartment alternately in the right and the left compartments of rhizoboxes. Overall, chirality of root length was no more detected (e.g. in CT treatment), probably because boxes were full of roots due to the better growth in Experiment 2. Interestingly, however, Zn root foraging was observed only when the Zn-enriched compartment was placed in the right compartment, but not when it was in the left. A similar trend was observed for the expression of Ni root foraging in the left Ni-enriched compartment, but to a lesser extent. We might hypothesize that in two-dimensional rhizoboxes, root foraging and root slanting cancel each other when they occur in opposite direction, but reinforce each other when they occur in the same direction. This observation indisputably questions the use of the rhizobox design to study root foraging.

Conclusion

Our study demonstrates for the first time that NM plants of *T. caerulescens* exhibit root foraging for Zn. If zincophilic root foraging in NM plants probably contributes to improve their Zn uptake, it does however not explain their higher Zn accumulation as M plants can express a similar degree of Zn root foraging. Root foraging for Zn is more influenced by experimental conditions in M plants than in NM plants. Ni root foraging ability was found in M plants only, suggesting that Ni supply is critical in the native metalliferous soils. The present study also raises doubts about the use of two dimensional rhizoboxes to study root foraging. Indeed, results from such experiments are liable to systematic bias due to root chirality.

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