Plant Soil (2009) 318:205–215
DOI 10.1007/s11104-008-9830-y

REGULAR ARTICLE

Effect of nitrogen form on the rhizosphere dynamics and uptake of cadmium and zinc by the hyperaccumulator *Thlaspi caerulescens*

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Received: 7 June 2008 / Accepted: 4 November 2008 / Published online: 25 November 2008

Abstract The effect of N form (NO_3^- versus NH_4^+) on growth and uptake of Cd and Zn by the hyperaccumulator *Thlaspi caerulescens* (Ganges ecotype) was investigated in hydroponic and rhizobox experiments. In the hydroponic experiments, NO_3^- or NH_4^+ was supplied to plants with the pH of the nutrient solution being unbuffered or buffered at around 6.0. A moderately contaminated soil was used in the rhizobox experiment with or without additions of NO_3^-, NH_4^+ or NH_4^+ + DCD (dicyanodiamide, a nitrification inhibitor). A higher biomass was obtained when N was supplied as NO_3^- in both experiments, indicating that *T. caerulescens* prefers NO_3^- over NH_4^+. In the hydroponic experiments, supplying NO_3^- resulted in a doubling of Cd concentration in the shoots compared with the NH_4^+ treatment, regardless whether solution pH was buffered or not. The form of N also had a noticeable effect on root Zn concentrations. In the rhizosphere box experiment, rhizosphere pH was markedly influenced by the N treatment. The acidification in the NH_4^+ and NH_4^+ + DCD treatments increased the concentrations of extractable Cd and Zn, both of which showed a considerable depletion in the rhizosphere. However, total uptake of Cd and Zn were highest in the NO_3^- treatment, despite the fact that concentrations of extractable Cd and Zn in the rhizosphere were the lowest in this treatment. The results showed that supplying N as NO_3^- promoted growth and phytoextraction of Cd and Zn by *T. caerulescens* compared with NH_4^+.

Keywords Ammonium · Nitrate · Hyperaccumulation · Rhizosphere · Phytoremediation · *Thlaspi caerulescens*

Responsible Editor: Juan Barcelo.

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Introduction

Thlaspi caerulescens J. & C. Presl is a well-known Zn hyperaccumulator, being able to accumulate more than 3% Zn in the shoot dry matter (Brooks 1998). Some populations of this species are also able to hyperaccumulate Cd or Ni (Assunção et al. 2008; Assunção et al. 2003). The ecotype from south France near Ganges is particularly efficient in Cd accumulation, with shoot Cd concentration reaching 0.3% in the field and 1% in hydroponic experiments (Lombi et al. 2000; Robinson et al. 1998; Roosens et al. 2003). Phytoremediation of soil moderately contaminated with Cd appears to be feasible with the Ganges

ecotype of *T. caerulescens* (Chaney et al. 2007; Maxted et al. 2007; McGrath et al. 2006; Zhao et al. 2003).

The mechanisms responsible for Zn/Cd hyperaccumulation in *T. caerulescens* are not fully understood yet, but are likely to involve enhanced root uptake, efficient root to shoot translocation and cellular detoxification (Chaney et al. 2007; McGrath and Zhao 2003). The rate of Zn influx into root cells of *T. caerulescens* is markedly higher than that of the non-hyperaccumulator *Thlaspi arvense* (Lasat et al. 1996). Uptake and root to shoot translocation of Cd vary greatly among different populations of *T. caerulescens*, with the Ganges ecotype showing a much higher influx rate than other ecotypes (Lombi et al. 2001b; Xing et al. 2008; Zhao et al. 2002). A number of genes putatively involved in metal transport and detoxification are constitutively highly expressed in *T. caerulescens* compared with non-hyperaccumulator species (Hammond et al. 2006; Pence et al. 2000; van de Mortel et al. 2006).

The dynamics of metals in the rhizosphere influence their bioavailability to plants. The rhizosphere of *T. caerulescens* has been investigated in a number of studies. There is some evidence that the roots of *T. caerulescens* tend to proliferate in Zn/Cd-rich patches of soil, whereas non-hyperaccumulators tend to avoid metal-rich patches (Schwartz et al. 1999; Whiting et al. 2000). On the other hand, there is no evidence for a significant mobilisation of metals in soil by the root exudates of *T. caerulescens* (Zhao et al. 2001). Soil pH has a strong influence on metal bioavailability. It has been shown that decreasing soil pH increased Zn and Cd uptake by *T. caerulescens*, but only if the decrease in pH did not causes mobilisation of Al to which *T. caerulescens* appears to be rather sensitive (Brown et al. 1994; Wang et al. 2006; Yanai et al. 2006). The form of N supplied to plants affects rhizosphere pH greatly (Marschner 1995). It has been reported that supplying NH_4^+ to plants led to rhizosphere acidification and enhanced Zn and Cd uptake by the non-hyperaccumulators sunflower and tobacco (Loosemore et al. 2004; Zaccheo et al. 2006). Manipulation of rhizosphere pH through the use of NH_4^+ and a nitrification inhibitor has been suggested as an effective way to enhance phytoextraction of Cd and Zn from contaminated soil by sunflower (Zaccheo et al. 2006). However, it is not presently known whether the N form can influence Cd and Zn accumulation by *T. caerulescens* and, if so, whether the effect is due to the N form directly or to changes in pH. Answers to these questions would be useful for improving the phytoremediation efficiency of *T. caerulescens*.

The objective of the present study was to investigate the effect of supplying NO_3^- or NH_4^+ on growth and the uptake of Cd and Zn by *T. caerulescens*. Experiments were carried out in both hydroponic culture with the pH of the nutrient solution being buffered or unbuffered, and in a rhizobox experiment with a moderately contaminated soil.

Materials and methods

Hydroponic experiments

Seeds of *Thlaspi caerulescens* (the Ganges ecotype) were sterilised in 10% H_2O_2 for 10 min and then germinated in a tray of vermiculite. Ten days after germination, seedlings were transferred to an aerated hydroponic solution containing 1 mM NH_4NO_3, 0.5 mM $MgSO_4$, 0.25 mM K_2HPO_4, 1 mM K_2SO_4, 1 mM $CaCl_2$, 10 μM H_3BO_3, 1.8 μM $MnSO_4$, 0.2 μM $NaMoO_4$, 0.31 μM $CuSO_4$, 5 μM $ZnSO_4$ and 50 μM Fe-EDDHA (pH 6.0), and grown for 8 days. Two hydroponic experiments were carried out to test the effect of NH_4^+ versus NO_3^- on plant growth and the uptake of Cd and Zn, one with solution pH unbuffered and the other buffered with 5 mM MES (2-morpholinoethanesulphonic acid) to maintain the solution pH at around 6.0. The nutrient compositions of the different treatments are shown in Table 1. Four seedlings were grown in each of 1 l pot, and each treatment was replicated in four pots. The nutrient solutions were aerated continuously and renewed every other day. Solution pH was recorded every day at 9:00 AM before nutrient solution renewal or pH adjustment. In the -MES experiment, solution pH was adjusted to the initial value of 6.0 with either 1 M HCl or NaOH every day. The -MES and + MES experiments were conducted separately, with experimental durations of 24 and 30 days, respectively. $CdSO_4$ (10 μM) was added to the nutrient solutions during the last 2 and 3 weeks of the -MES and + MES experiments, respectively. Plants were grown inside a growth room with 14 h/10 h day/night, 350 μmol m^{-2} s^{-1} light intensity, and 25°C/18°C day/night temperature. At the

Table 1 The composition of nutrient solutions in different treatments in the hydroponic experiments[a]

Nutrient composition	NH_4^+ treatment	NO_3^- treatment
$(NH_4)_2SO_4$ (mM)	1.0	0
KNO_3 (mM)	0	2.0
KH_2PO_4 (mM)	0.25	0.25
K_2SO_4 (mM)	1.0	0
$MgSO_4$ (mM)	0.5	0.5
$CaCl_2$ (mM)	1.0	1.0
H_3BO_3 (μM)	10	10
$MnSO_4$ (μM)	1.8	1.8
$CuSO_4$ (μM)	0.31	0.31
$ZnSO_4$ (μM)	10	10
$CdSO_4$ (μM)[b]	10	10
Na_2MoO_4 (μM)	0.2	0.2
Fe-EDDHA (μM)	50	50

[a] 5 mM MES was added to the nutrient solution in the pH buffered experiment.

[b] Cd was added only during the last 2 and 3 weeks of the pH unbuffered and pH buffered experiments, respectively.

end of the experiments, plants were rinsed with deionised water, separated into roots and shoots, and dried at 65°C for 48 h before dry weights were recorded. Samples were ground to <0.5 mm for analysis. Plant materials (0.25 g) were digested with 4 ml HNO_3 and 1 ml $HClO_4$, and the concentrations of Cd and Zn were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES, Perkin Elmer DV3300).

Speciation of Cd and Zn in the nutrient solutions was calculated using Geochem-PC (Parker et al. 1995).

Rhizobox experiment

Seedlings of *T. caerulescens* (Ganges) were pre-cultured in a nutrient solution for 10 days as described above. Two seedlings were transferred to a 12×10×11 cm (L×W×H) rectangular rhizobox, which was divided into two equal halves with two layers of nylon mesh (40 μm) installed in the middle. Roots were placed inside the two nylon layers, which prevented roots from growing outside the mesh. On each side of the nylon mesh was placed 500 g air-dried soil (<2 mm). The soil was moderately contaminated with Cd and Zn due to the irrigation with sewage effluent water in the past. Selected soil properties are shown in Table 2. There were four treatments: Control (without N addition), NH_4^+, NH_4^+ + DCD (dicyanodiamide, a nitrification inhibitor) or NO_3^-. Each treatment was replicated in three boxes. Nitrogen was added at a rate of 150 mg kg^{-1} and mixed thoroughly with the soil. DCD was added at a rate of 10% of the total N dose. A previous study has shown that DCD was effective at inhibiting nitrification in the first 30 days after addition (Ju et al. 2004). Other basal nutrients included 100 mg K kg^{-1} and 80 mg P kg^{-1} added as KH_2PO_4. Deionised water was added daily to maintain soil moisture content at 20–25% (w/w). The experiment was conducted in a glasshouse with natural sunlight supplemented with sodium vapour lamps to maintain a minimum light intensity of 350 μmol m^{-2} s^{-1} for 14 h per day. Temperature was maintained at 18–25°C. Plants were harvested 90 days after transfer to the rhizoboxes. Shoots and roots were separated, rinsed with deionised water, and dried at 65°C for 48 h before dry weights were determined. The soils in the two rhizobox halves were frozen at −20°C for 12 h, and then sectioned into 0–2, 2–4, 4–6, 6–8 and >8 mm distance from the root surface (nylon mesh). The same sections from the two rhizobox halves were combined. A portion of the soil was sieved to <5 mm and frozen at −20°C until analysis, and the remaining portion was air dried and sieved to <1 mm.

Plant samples were analysed for Cd and Zn concentrations using ICP-AES. Fresh soil was used for the measurement of pH, extractable Cd and Zn concentrations. pH was determined in a suspension of 1:2.5 (v/w) of soil to water. Soil (2 g) was extracted with 50 ml 1 M NH_4NO_3 (DIN 1995) and the concentrations of Cd and Zn in the extracts were determined by ICP-AES.

Table 2 Selected properties of the soil used in the rhizobox experiment

Soil properties	
Soil texture	Medium loam
pH (in water)	6.5
Organic matter (%)	2.88
Total N (%)	0.14
NH_4^+ -N (mg kg^{-1})	5.2
NO_3^-N (mg kg^{-1})	67.9
Total Zn (mg kg^{-1})	176
Total Cd (mg kg^{-1})	2.75
NH_4NO_3-extractable Zn (mg kg^{-1})	2.43
NH_4NO_3-extractable Cd (mg kg^{-1})	0.19

The significance of treatment effects was evaluated by analysis of variance (ANOVA). Treatment means were compared using least significant difference (LSD) at $p<0.05$.

Results

In both hydroponic and rhizobox experiments, two ecotypes of *T. caerulescens* (Ganges and Prayon) were grown. The results were similar, except that Ganges accumulated much more Cd than Prayon, which was consistent with previous reports (Lombi et al. 2000; Lombi et al. 2001b; Zhao et al. 2002). Therefore, only the results of the Ganges ecotype are presented here.

Hydroponic experiments

Without the addition of the pH buffer MES, solution pH showed substantial daily changes from the initial value (6.0) (Fig. 1a). As expected, the NH_4^+ treatment led to acidification in the nutrient solution, by up to 1.8 pH units. In contrast, the NO_3^- treatment led to increased pH (mostly within 0.5 unit). MES (5 mM) was effective in buffering pH in the nutrient solutions, with pH being maintained at ±0.25 units of the initial value (Fig. 1b).

In the -MES experiment, the NO_3^- treatment produced significantly higher shoot and root biomass than the NH_4^+ treatment (Fig. 2a), as well as a significantly higher root to shoot ratio (data not shown). In the + MES experiment, although plant growth was still better in the NO_3^- treatment than in the NH_4^+ treatment, the difference was not significant (Fig. 2b).

In both -MES and + MES experiments, the concentration of Cd in roots was higher than that in shoots (Fig. 2c,d). In the absence of the MES buffer, both shoot and root Cd concentrations were markedly higher (2–2.2 fold, $p<0.001$) in the NO_3^- treatment than in the NH_4^+ treatment (Fig. 2c). When MES was used to buffer solution pH, the difference in root Cd concentration between the two N treatments became insignificant, but the shoot Cd concentration was 2.3 fold higher in the NO_3^- treatment than in the NH_4^+ treatment ($p<0.001$, Fig. 2d). Even though the -MES and + MES experiments were not strictly comparable because of different lengths of plant growth and Cd exposure, it is interesting to note that Cd concentrations in shoots were comparable in the same N treatment of the two experiments, whereas Cd concentrations in roots were comparable only in the NH_4^+ treatment.

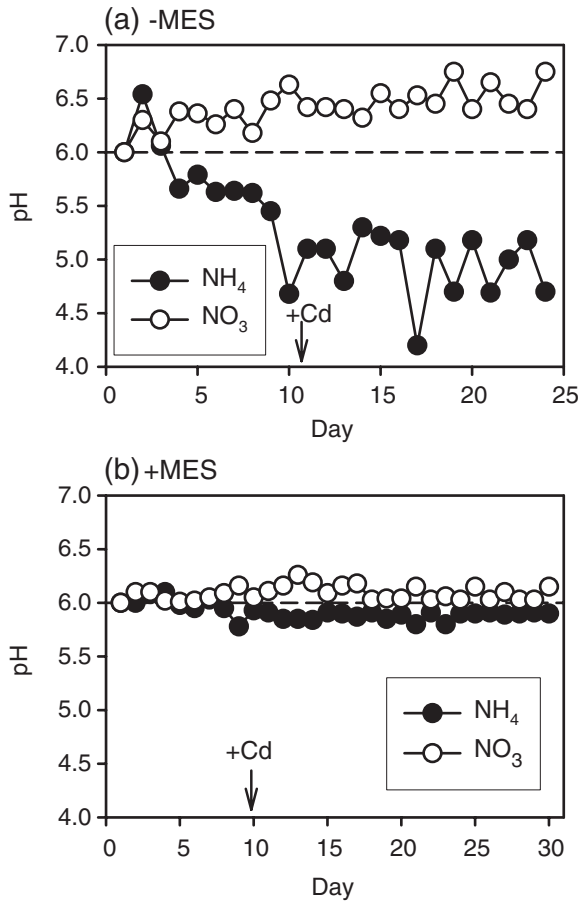

Fig. 1 Nutrient solution pH in the pH unbuffered (**a**) and pH buffered (**b**) hydroponic experiments

In contrast to Cd, Zn concentrations in shoots were always higher than those in roots (Fig. 2e,f). In both -MES and + MES experiments, there were no significant differences in shoot Zn concentration between the NH_4^+ and NO_3^- treatments, whereas root Zn concentration was significantly ($p<0.001$) higher in the NO_3^- treatment than in the NH_4^+ treatment. Zinc concentrations in roots were comparable in the same N treatment of the two experiments, whereas Zn concentrations in shoots were higher in the + MES than in the -MES experiment.

Calculations of metal speciation in the nutrient solutions showed that Cd was more likely to precipitate with phosphate than Zn within the pH

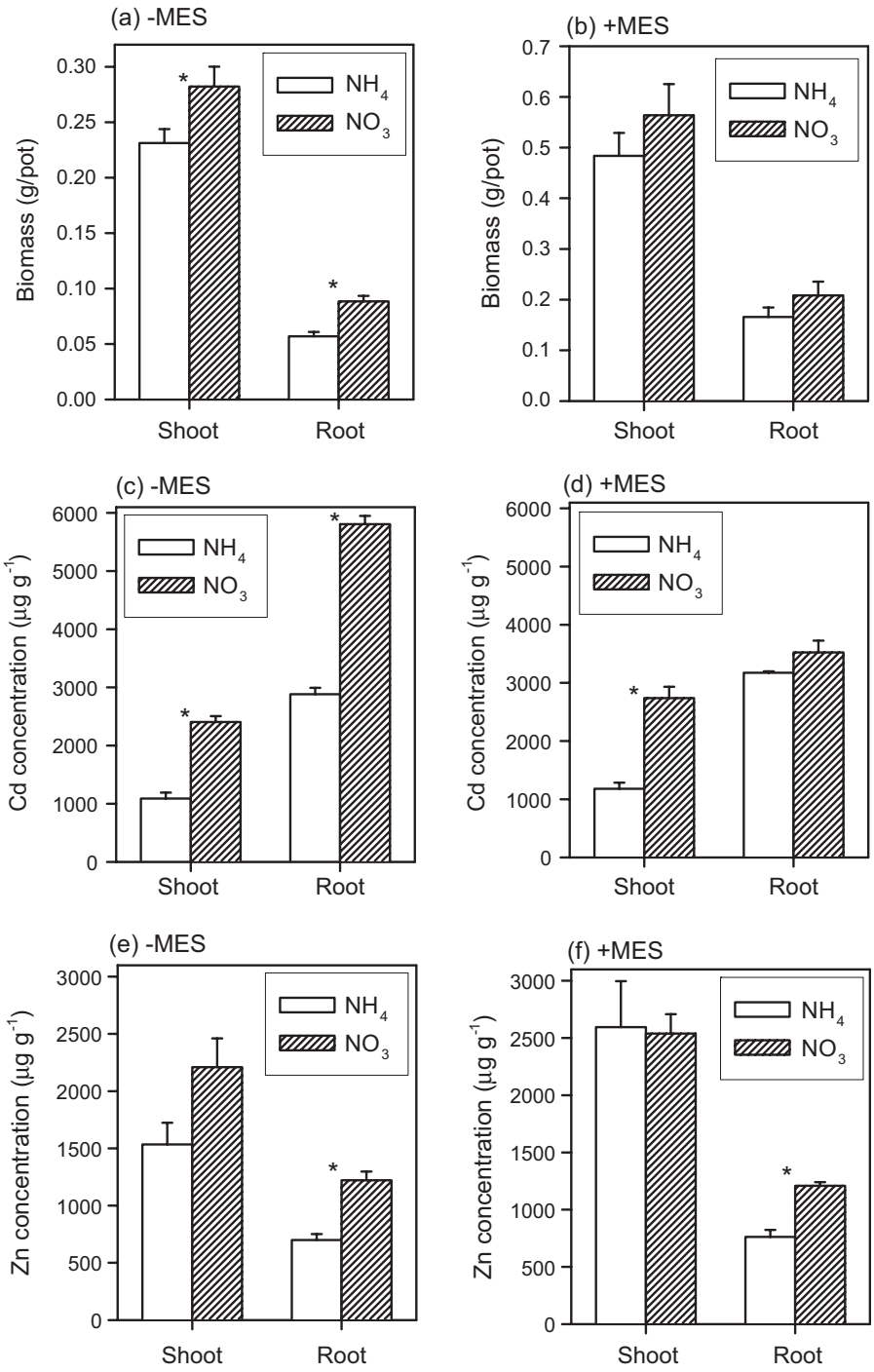

Fig. 2 Effects of N forms on the biomass of shoots and roots of *Thlaspi caerulescens* (**a**, **b**), concentrations of Cd (**c**, **d**) and Zn (**e**, **f**) in shoots and roots in the pH unbuffered (a, c, e) and pH buffered (b, d, f) hydroponic experiments. Error bars are SE (n=4). * indicates a significant difference between the NH_4^+ and NO_3^- treatment ($p<0.05$)

range observed in the experiments. Precipitation of cadmium phosphate was predicted to occur at pH≥6.1 under our experimental conditions; at pH 6.6 (the highest solution pH observed in the unbuffered NO_3^- treatment) 76.4% of the Cd in the solution would precipitate with phosphate. In contrast, precipitation of zinc phosphate was not predicted to occur in all treatment solutions with pH ≤ 6.6.

Rhizobox experiment

At the end of the experiment, soil pH differed between different N treatments, and also varied spatially from the root surface to the bulk soil (Fig. 3a). Soil pH was the lowest in the NH_4^+ treatment, most probably as a result of nitrification. In both Control and NH_4^+ + DCD treatments, soil pH showed a small decrease (~0.3 units) from the bulk soil toward the root surface, although pH was about 0.3 units higher in the Control than in the NH_4^+ + DCD treatment. In contrast, both NH_4^+ and NO_3^- treatments resulted in an increasing pH gradient (~0.5 unit) toward the root surface. Soil pH was approximately 0.6 units higher in the NO_3^- treatment than in the NH_4^+ treatment along the rhizosphere gradient.

The concentrations of ammonium nitrate-extractable Cd and Zn were affected by different N treatments. The extractable concentrations were the highest in the NH_4^+ treatment and the lowest in the NO_3^- treatment (Fig. 3b,c); this difference can be attributed to the difference in the pH of the rhizosphere soil. Both metals showed a similar pattern of depletion from the bulk soil toward the root surface; on average the depletion from the >8 mm section to the 0–2 mm section was 49% and 30% for Cd and Zn, respectively.

Shoot biomass was significantly ($p<0.001$) affected by the treatments; the addition of NH_4^+ or NO_3^- increased shoot biomass by 72 and 141%, respectively, over the control (Fig. 4a). The NH_4^+ + DCD treatment increased shoot biomass by 20% compared with the control, but this difference was not significant. The effects of treatments on root biomass were similar to those on shoot biomass, although the overall treatment effects were not significant ($p=0.065$).

Shoot Cd concentration varied from 67 to 555 mg kg^{-1} dry weight (Fig. 4b). The bioconcentration factor for Cd (i.e. the ratio of shoot Cd to soil Cd concentrations) ranged from 25 to 202. Shoot Cd concentration was significantly ($p<0.001$) affected by the additions of different N forms, and followed the order of Control > NO_3^- > NH_4^+ > NH_4^+ + DCD (Fig. 4b). The total amount of Cd accumulated in the shoots accounted for 1–7.8% of the total Cd in the soil and followed the order of NO_3^- > Control > NH_4^+ > NH_4^+ + DCD (data not shown). The total amount of Cd accumulated in the shoots in the NO_3^- treatment was 2.6 and 8 fold higher than that in the

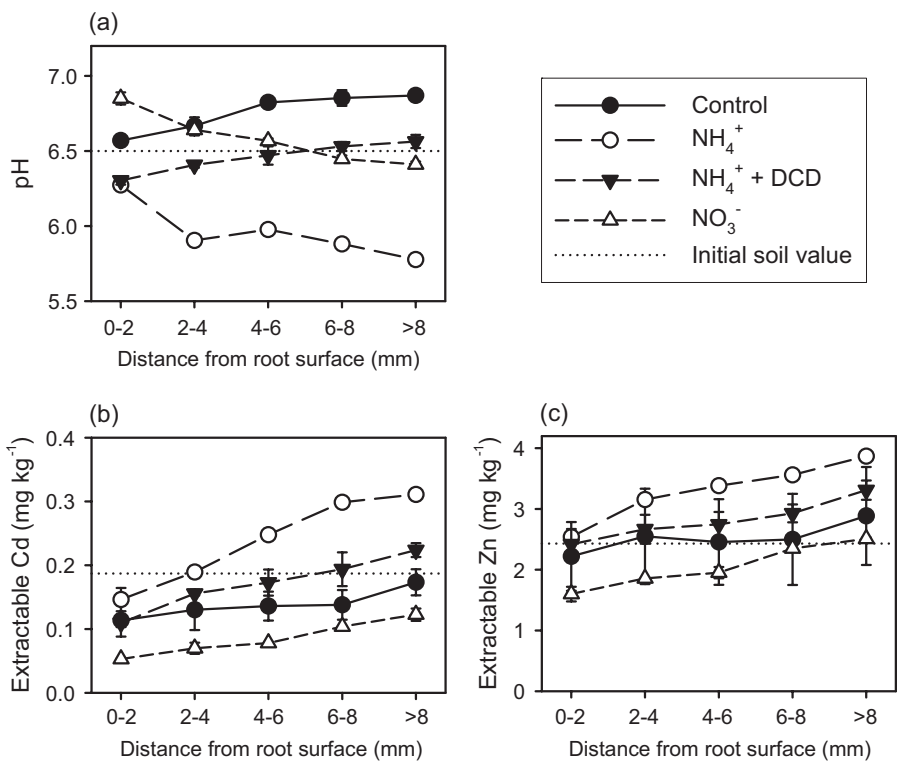

Fig. 3 Effects of N forms on the rhizosphere soil pH (**a**), NH_4NO_3-extractable Cd (**b**) and Zn (**c**) in the rhizobox experiment after growth of *Thlaspi caerulescens* for 90 days. Error bars are ±SE ($n=3$)

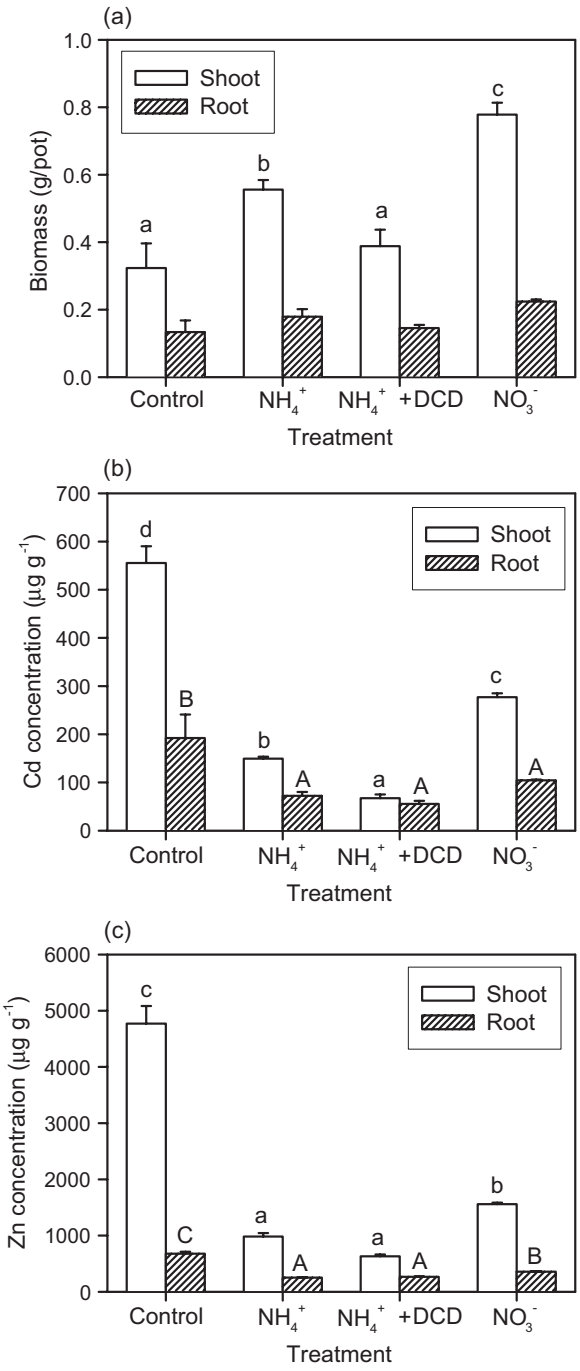

Fig. 4 Effects of N forms on the biomass of shoots and roots of *Thlaspi caerulescens* (**a**), and the concentrations of Cd (**b**) and Zn (**c**) in shoots and roots in the rhizobox experiment. Different letters indicate significant difference at $p<0.05$. There were no significant differences between treatments in root biomass. Error bars are ±SE ($n=3$)

NH_4^+ and NH_4^+ + DCD treatments, respectively. A dilution effect due to increased biomass probably accounted for the decreased Cd concentration in the shoots of the NO_3^- treatment compared with the control. In contrast, the NH_4^+ and NH_4^+ + DCD treatments decreased shoot Cd concentration by mechanisms other than or in addition to the dilution effect. Root Cd concentrations were smaller than those in the shoots. The treatment effects on root Cd were only significant between the Control and the three + N treatments (Fig. 4b).

Shoot Zn concentration ranged from 630 to 4770 mg kg^{-1} dry weight (Fig. 4c), and were influenced by the treatments in a similar way as shoot Cd concentration. The addition of N, particularly NH_4^+ and NH_4^+ + DCD, significantly decreased shoot Zn concentration. The bioconcentration factor for Zn ranged from 3.6 to 27, and the Zn uptake by shoots accounted for 0.1–0.9% of the total Zn in the soil. The concentrations of Zn in roots were markedly smaller than those in shoots, but showed a similar trend among treatments as shoot Zn.

Discussion

Consistent with previous reports (Lombi et al. 2000, 2001a; Roosens et al. 2003; Zhao et al. 2002), the Ganges ecotype of *T. caerulescens* showed an exceptional ability to accumulate Cd in the shoots in both the hydroponic and the rhizobox experiments. Large values (>25) of the bioconcentration factor for Cd were obtained in the rhizobox experiment, even though the roots were confined to the central compartment inside two layers of nylon mesh without a direct contact with soil. A bioconcentration factor of >10 is considered to be necessary for an efficient phytoremediation of moderately contaminated soils within a reasonable time frame (McGrath and Zhao 2003; Zhao et al. 2003). Wang et al. (2006) reported that 36–40% of soil Cd was extracted by *T. caerulescens* shoots in a single planting in a pot experiment. Our results were smaller (1–7.8%), which can be attributed to the lack of a direct root-soil contact and a relatively short growth period. In contrast, *T. caerulescens* shoots removed <1% of the total soil Zn in the rhizobox experiment. This low rate of phytoextraction was because of the relatively high concentration of Zn in the soil (relative to Cd), and

consequently the bioconcentration factor for Zn was substantially lower than that for Cd.

Growth of *T. caerulescens* was better when N was supplied as NO_3^- than as NH_4^+ in both hydroponic and rhizobox experiments (Figs. 2 and 4). This difference could be due to a pH effect or an effect of N form, or both. As expected, N supply as NH_4^+ resulted in acidification in the nutrient solution in the unbuffered hydroponic experiment and in the soil of the rhizobox experiment, although the mechanisms of acidification were likely to be different. In the hydroponic experiment, acidification of the nutrient solution was a result of excess uptake of cations over anions (Marschner 1995). In the rhizobox experiment, a decrease in the bulk soil pH in the NH_4^+ treatment was caused by nitrification in soil, whereas rhizosphere acidification in the NH_4^+ + DCD treatment was a result of excess uptake of cations. The trend of increasing pH in the rhizosphere toward the root surface in the NO_3^- and NH_4^+ treatments suggests that more anions than cations were taken up by roots, whereas opposite was true for the Control and NH_4^+ + DCD treatments. In soil experiments, acidification of some soils to a pH of <5 has been found to inhibit the growth of *T. caerulescens* mainly because of the mobilisation of toxic Al and Mn (Maxted et al. 2007; Wang et al. 2006; Yanai et al. 2006). In our hydroponic experiments, the difference in plant growth between the NO_3^- and NH_4^+ treatments appeared to persist in both the pH unbuffered and buffered experiments, though more pronounced in the former than in the latter. In the rhizobox experiment, shoot growth showed significant differences among the three + N treatments in the order of NO_3^- > NH_4^+ > NH_4^+ + DCD, even though the rhizosphere pHs were higher in the NH_4^+ + DCD treatment than in the NH_4^+ treatment. Also, soil pH was higher than 5.6 in all treatments of the rhizobox experiment; thus Al or Mn toxicity was unlikely. These results suggest that the main reason for the growth difference between different N treatments was the form of N supplied rather than pH, and that *T. caerulescens* preferred NO_3^- to NH_4^+ as the N source.

In the hydroponic experiments, supplying N as NO_3^- resulted in a doubling of Cd concentration in the shoots compared with the NH_4^+ treatment, regardless whether solution pH was buffered or not (Fig. 2). In contrast, the effect of N form on the Cd concentration in roots was dependent on solution pH; the NO_3^- treatment significantly increased the concentration of Cd in roots compared with the NH_4^+ treatment only when solution pH was unbuffered. The form of N supplied to plants may influence Cd accumulation in plants through several possible mechanisms. First, precipitation of cadmium phosphate was predicted to occur at pH ≥ 6.1 under our experimental conditions, and the cadmium phosphate precipitate was likely to deposit on to the root surfaces (Küpper et al. 2000). This precipitation would largely explain why root Cd concentration in the unbuffered NO_3^- treatment (solution pH varying from 6.0 to 6.7) was more than double of that in the unbuffered NH_4^+ treatment (solution pH≤6.0), but not in the pH buffered experiment (solution pH around 6.0 in both N treatments). Zaccheo et al. (2006) also observed a much larger accumulation of Cd in sunflower roots in the NO_3^- treatment than in the NH_4^+ treatment in a pH unbuffered hydroponic experiment. Although they did not attribute this difference to the possibility of cadmium phosphate precipitation onto the root surfaces, this was likely to occur. Second, a higher pH in the nutrition solution in the NO_3^- treatment would increase the binding of metals to the root cell walls (Plette et al. 1999), which may in turn increase metal uptake into the root symplast (Marschner 1995). Third, uptake of NH_4^+ leads to a rapid depolarization of the membrane potential (Wang et al. 1994), which may decrease influx of metal ions to the root symplast (Marschner 1995). Fourth, assimilation of NO_3^- in roots enhances synthesis of organic anions, which may increase uptake and xylem translocation of cations (Kirkby and Knight 1977). Results from the hydroponic experiments suggest that the effect of the N treatments on root Cd concentration can be attributed mainly to the difference in nutrient solution pH, but the effect on shoot Cd concentration was largely unrelated to solution pH, and possibly attributable to the third and fourth explanations given above. The exact mechanism for the NO_3^- enhanced Cd accumulation in *T. caerulescens* remains to be investigated in the future.

In the hydroponic experiments N form had a significant effect on the concentration of Zn in the roots only, and this effect was independent of solution pH (Fig. 2). As precipitation of zinc phosphate was not predicted to occur in any of the treatment solutions, the increased Zn concentration in the roots of NO_3^- treated plants may be a result of increased

cell wall binding and/or increase uptake into the root cells. However, it appears that translocation of Zn to shoots, unlike that of Cd, was not increased by NO_3^-.

The positive effect of NO_3^- on Cd and Zn uptake and, particularly, Cd accumulation in the shoots was also apparent in the rhizobox experiment with a moderately contaminated soil (Fig. 4), even though the bioavailability of Cd and Zn, as indicated by the amounts of ammonium nitrate-extractable metals in the rhizosphere soil, was considerably higher in the NH_4^+ and NH_4^+ + DCD treatments than in the NO_3^- treatment as a result of the pH difference (Fig. 3). These results indicate that, compared with NH_4^+, NO_3^- enhanced Cd and Zn uptake and/or Cd translocation from roots to shoots in *T. caerulescens*, and that this effect was substantially greater than the NH_4^+ -induced increase in the bioavailability of Cd and Zn in the rhizosphere soil. This result is opposite to that reported for the non-hyperaccumulator sunflower by Zaccheo et al. (2006), who found that supplying sunflower plants with NH_4^+ in combination with a nitrification inhibitor increased metal (Cd and Zn) uptake from contaminated soils. Our results show that rhizosphere acidification as a result of supplying NH_4^+ to plants would not enhance phytoextraction of Cd and Zn by *T. caerulescens*, because NH_4^+ suppresses growth and metal uptake in this plant species. In a pot study with the Ni hyperaccumulator *Thlaspi goesingense*, Puschenreiter et al. (2001) found little effect of the addition of ammonium sulphate and DCD on plant metal uptake, possibly because DCD was degraded and there was no acidification in the rhizosphere.

In the rhizobox experiment, the concentrations of ammonium nitrate-extractable Cd and Zn decreased considerably toward the root surface, indicating that a depletion zone developed in the rhizosphere for both metals as a result of fast uptake by *T. caerulescens* (Fig. 3). Depletion of both Cd and Zn in the rhizosphere occurred in all treatments, which differed in the rhizosphere pH and the levels of extractable metals. Similarly, Puschenreiter et al. (2005) found that ammonium nitrate-extractable Ni was depleted in the rhizosphere of *T. goesingense*. These results support the prediction by Whiting et al. (2003) using a solute transport model, who showed that a depletion of Zn in the rhizosphere of *T. caerulescens* is likely to occur in moderately contaminated soils and that the rate of Zn diffusion is crucial for maintaining sufficient Zn at the root surface. In contrast, mass flow delivers only a small percentage of Zn to the root surface of *T. caerulescens* (McGrath et al. 2001; Whiting et al. 2003). Methods that can enhance metal solubility and hence the rate of diffusion to the root surface may enhance the phytoremediation potential, but only if these methods do not suppress plant growth and interfere with the metal absorption processes in the roots. Field experiments showed that additions of the chelators EDTA and NTA did not enhance metal uptake by *T. caerulescens* (McGrath et al. 2006). Furthermore, use of chelators may result in unacceptable leaching of metals to subsoil or groundwater (Wenzel et al. 2003).

Acknowledgements We thank the Changjiang Scholars Programme and the Innovative Research Team in the University Scheme (IRT0511) and the "863 Project" (2007AA061001) for financial support. Rothamsted Research receives grant-aided support from the UK Biotechnology and Biological Sciences Research Council.

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