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Ammonium-based fertilizers enhance Cd accumulation in *Carpobrotus rossii* grown in two soils differing in pH

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Abstract
Nitrogen fertilization has been shown to improve Cd uptake by plants but there is little information on the effects of N forms. This study examined the effects of N form on Cd bioavailability and phytoextraction in two soils differing in pH. Plants of halophytic species *Carpobrotus rossii* were grown in an acidic Sodosol [pH ($CaCl_2$) 4.9] and a neutral Vertosol (pH 7.2) spiked with 20 mg kg^{-1} Cd as $CdCl_2$. Three N forms, KNO_3, $(NH_4)_2SO_4$ and $(NH_2)_2CO$ at a rate of 24 mg N kg^{-1} were applied at weekly intervals, together with nitrification inhibitor dicyanodiamide (DCD). Cadmium availability was measured, and Cd speciation in the rhizosphere analysed using synchrotron-based X-ray absorption spectroscopy. The uptake, translocation and accumulation of Cd in plants were also assessed. The reduced N forms (NH_4^+ and urea), compared to NO_3^--N, decreased rhizosphere pH by 0.25 units in Sodosol and 0.72 units in Vertosol, but decreased Cd-phosphate (by 23%) only in the Vertosol. Moreover, the reduced N forms increased the extractable Cd concentration in the rhizosphere of the Vertosol by 92% and of the Sodosol by 14%. They increased root Cd concentration by 70% and Cd uptake per unit root length by 40% in the Vertosol, and increased the translocation of Cd from the roots to the shoots by 76% in the Sodosol. The results suggest that the supply of NH_4^+-based N favors Cd phytoextraction in *C. rossii*.

Key words: Cd translocation, Cd speciation, halophytes, N form, rhizosphere pH, XANES

1. Introduction

Cadmium (Cd) is a serious toxin in the food chain polluting cultivated soils from a variety of sources, including application of phosphate fertilizers, sewage sludge, and recycled wastes such as manure and compost (Nicholson et al., 1994). Cadmium poses a risk through being taken up by plants and accumulating to levels that are harmful in animal and human diets. Effective ways to remediate Cd-contaminated soils are needed to reduce the risk of Cd poisoning. Phytoextraction is considered to be one promising technique that utilizes the capacity of accumulator and hyperaccumulator plants to extract heavy metals from the soil, because it is cost-effective and environmentally friendly (McGrath et al., 2006; Mahar et al., 2016). However, a current problem with phytoextraction is the long time needed for remediation.

The bioavailability of Cd in soils is affected by soil properties including pH, texture, organic matter, and nutrient levels. It has been well documented that pH is the most important single soil property that affects available Cd and uptake by plants (Eriksson, 1989; Adams et al., 2004; Wang et al., 2006; Yanai et al., 2006). In general, Cd uptake by plants increases with decreasing pH, with an inverse relationship between soil pH and bioavailability or plant uptake of Cd (Tudoreanu and Phillips, 2004; Yanai et al., 2006), because decreasing soil pH increases releases of Cd from sesquioxides and variable-charged clays and increases the solubility of solid phases of Cd such as $CdCO_3$ and $Cd_3(PO_4)_2$ (McBride, 1994; Adriano, 2001). Therefore, increasing Cd bioavailability

through rhizosphere acidification is expected to be an effective way to enhance the efficiency of phytoextraction and shorten the remediation time

Nitrogen (N) fertilization is an agronomic strategy to improve phytoextraction not only by improving the growth of N-deficient plants but also by influencing the rhizosphere pH. The common N forms taken up by plant are nitrate (NO_3^-), ammonium (NH_4^+) and urea. Urea, however, is usually hydrolysed to NH_4^+ prior to uptake by plants. When nitrification is inhibited by nitrification inhibitors, the uptake and assimilation of NH_4^+ results in greater net extrusion of H^+ and consequent decrease in rhizosphere pH that potentially increases Cd availability in soils (Wu et al., 1989; Marschner, 2011). The opposite occurs in NO_3^- uptake, which increases rhizosphere pH. However, many studies investigating Cd accumulation by plants supplied with different N forms have shown conflicting results. It has been shown that supply of NH_4^+ increased Cd accumulation in sunflower (*Helianthus annuus*), potato (*Solanum tuberosum*), and hyperaccumulator *Rorippa globosa* and *Sedum alfredii* when grown in soils (Zaccheo et al., 2006; Zhu et al., 2010; Larsson Jönsson and Asp, 2011; Wei et al., 2015). In contrast, the opposite observation has been reported in rice, potato, and hyperaccumulator *Thlaspi caerulescens*, where the supply of NO_3^- not NH_4^+ enhanced Cd accumulation in plants (Maier et al., 2002; Alpha et al., 2009; Jalloh et al., 2009; Xie et al., 2009). In another study, the application of different N fertilizers had little influence on Cd accumulation in hyperaccumulator *S. plumbizincicola* (Arnamwong et al., 2015).

The inconsistent findings in the effect of N form on Cd phytoextraction from different studies may be partly due to the different properties of the soils used, especially soil pH. First, the response of plant growth to N form depends on soil pH (Marschner, 2011). Acidification induced by NH_4^+ uptake enhances Cd mobilization in neutral and alkaline soils, but may cause adverse effects on plant growth in acid soils. For example, the Cd hyperaccumulator *T. caerulescens* did not grow well at low soil pH, with nearly 10 times lower biomass in the soil pH of 4.4 than at pH 5.0 (Yanai et al., 2006). Second, the extent of N-induced changes in soil pH depends on the initial soil pH. For example, compared to sole NO_3^-, combined supply of NO_3^- and NH_4^+ to rape and tomato plants did not affect rhizosphere pH in calcareous soils, but decreased it in acid soils (Chaignon et al., 2002). In addition, soil pH buffer capacity is another factor determining the extent of changes in rhizosphere pH, with smaller pH changes in more buffered soils (Schubert et al., 1990; Hinsinger et al., 2003). However, little information is available regarding the response of Cd bioavailability and phytoextraction to N forms in different soils in the same study.

Carpobrotus rossii is an Australian native halophytic succulent plant species with potential for Cd phytoextraction (Zhang et al., 2014). Our previous studies have shown that the supply of NH_4^+ as the sole N source had similar effects to the effect of NO_3^- on the growth of *C. rossii*, but increased Cd accumulation in shoots of plants grown in an acid soil or hydroponic culture (Liu et al., 2015; Cheng et al., 2016). But it remains unknown whether the N form has a consistent effect on plant growth and Cd accumulation when *C. rossii* is grown in soils differing in pH. Understanding this would help to formulate fertilizer programs to improve phytoremediation efficiency in Cd-contaminated soils.

The objectives of this study were 1) to examine the effect of N form on soil pH, Cd speciation and extractable Cd concentration in the rhizosphere of *C. rossii* grown in an acidic Sodosol and a neutral Vertosol, and 2) to correlate these changes with the efficiency of Cd phytoextraction by *C. rossii* plants. It was hypothesized that NH_4^+ and urea, but not NO_3^-, would increase Cd accumulation in plants due to increased Cd bioavailability through rhizosphere acidification. Furthermore, the effects of N on Cd phytoextraction would be greater in the Vertosol than the Sodosol because of its higher pH and lower pH buffer capacity.

2. Materials and methods

2.1. Soil collection and Cd-contaminated soil

The surface layer (0-15 cm) of two Victorian soils were collected for this study, a Sodosol (Isbell, 1996) or Solonetz (4) Group, 201 from the La Trobe University farm (37°72′S, 145°4′E) and a Vertosol (Isbell, 1996) or Vertisol (Group, 2014) from the Plant Breeding Centre farm of

Department of Economic Development, Jobs, Transport and Resources (36°44'S, 142°06'E). The properties of Sodosol and Vertosol had pH 4.91 and 7.2, pH buffer capacity 22.0 and 16.8 $cmol_c$ pH^{-1} kg^{-1}, total N 2.9 and 0.9 g kg^{-1}, and clay content 24.8% and 52.4%, respectively (Table S1). After sieving (< 2 mm), 20 mg kg^{-1} Cd ($CdCl_2 \cdot 2.5H_2O$) in distilled water was added to 1.5 kg air-dried soil in each plastic bag. Basal nutrient salts (mg kg^{-1}) were added as a solution to each bag: 150 KH_2PO_4, 99.4 KCl, 151 $CaCl_2 \cdot 2H_2O$, 15.9 $MgCl_2 \cdot 6H_2O$, 17.6 $MnCl_2 \cdot 4H_2O$, 1.41 $CuCl_2 \cdot 2H_2O$, 10.3 $ZnSO_4 \cdot 7H_2O$, 0.67 H_3BO_3 and 0.17 $Na_2MoO_4 \cdot 2H_2O$. After thorough mixing, the soils were watered to 80% of field capacity and incubated for 3 weeks at a constant temperature of 35°C with daily re-mixing to ensure uniform distribution of Cd and nutrients.

Cuttings of *C. rossii* (family Aizoaceae) were propagated in plastic nursery cells (5×5×8 cm) filled with sand to ensure well-developed root systems. Two uniform seedlings were transferred to a rhizobag, a cylindrical solute-permeable nylon 400 mesh bag (20 cm high and 5 cm diameter) with 0.4 kg soil surrounded by 1.1 kg bulk soil of the same treatment. The rhizobag, being impervious to roots, separated the rhizosphere soil from the bulk soil. Two porous soil moisture samplers (Rhizon MOM, Rhizosphere Research Products, Wageningen, the Netherlands) were installed in each pot, one in the rhizobag and one in the bulk soil to collect soil solutions.

2.2. Treatments and experimental design

The experiment consisted of six treatments with four replicates arranged in a completely randomized design. The six treatments were three N forms [KNO_3, $(NH_4)_2SO_4$ and $(NH_2)_2CO$)] × two soil types (Sodosol and Vertosol). Nitrogen was applied at 24.0 mg N kg^{-1} as solution weekly from 27 d after transplanting until 2 weeks before harvesting (i.e. a total of six times). Nitrification inhibitor dicyandiamide (DCD) was applied at a rate of 7.2 mg kg^{-1} to all the pots to minimize NH_4^+ oxidation, and hence maintain plant NH_4^+ uptake from the NH_4^+-fertilized soils. Additionally, 149 mg K_2SO_4 kg^{-1} was added to the KNO_3 and $(NH_2)_2CO$ treatments to ensure a constant SO_4^{2-} concentration. The pots were arranged randomly in a controlled-environment room with 14-h photoperiod, a light intensity of 400 μmol m^{-2} s^{-1}, 20 °C during the day and 18 °C during the night. Deionized water was added daily by weighing to maintain 80% of field capacity. Soil solutions were collected from the soil moisture samplers on days 28 39, 53, 60, 67 and 71, 2 d prior to each N application.

2.3. Plant and soil analysis

On day 75, the experiment was harvested. Shoots were cut at their base, washed with deionized water and then oven-dried at 80°C until completely dry. After washing to remove soil particles, roots were immersed in 20 mM Na_2-EDTA for 15 min to remove surface-adsorbed Cd and then rinsed with deionized water. The roots were examined using a root scanner at 600 dpi (Epson Perfection 4990 Scanner, model J131B, Epson Inc.) to determine morphological parameters (length, average diameter and surface area) before being oven-dried for chemical analysis. The dried plant samples were weighed and ground in a stainless steel mill (ZM200 Retsch Technology GmbH), and then digested using HNO_3 in a microwave digester (Multiwave 3000, Anton Paar). The concentrations of elements in digests were analyzed using inductively coupled plasma optical emission spectrometry (ICP-OES) (Perkin Elmer Optima 8000, MA, USA).

The soil adhering to roots was collected as rhizosphere soil by shaking roots gently, while soil sampled from 2 cm outside of the rhizobag was considered as bulk soil. All soil samples were air-dried at room temperature and sieved through a 2-mm stainless mesh. Plant-available Cd was extracted by shaking at 25°C for 16 h with 0.01 M $CaCl_2$ solution (soil: $CaCl_2$ ratio, 1:10), centrifuging (10 min at 1000 *g*), filtering to 80 mm and determined using ICP-OES. Soil pH was measured in the supernatant (Thermo Orion 720, USA) after shaking for 1 h with 0.01 M $CaCl_2$ (soil: $CaCl_2$ ratio, 1:5), and then centrifuged (5 min at 700 *g*).

2.4. Cadmium speciation by X-ray absorption spectroscopy (XAS)

Cadmium K-edge X-ray absorption near edge structure (XANES) spectra of the rhizosphere soils supplied with NH_4^+ and NO_3^- were determined at the XAS beamline of the Australian Synchrotron, Melbourne (Cheng et al., 2016). The energy of each spectrum was calibrated by simultaneous measurement in transmission mode of a metallic Cd foil reference (K-edge at 27,711 eV). The spectra were collected in the fluorescence mode with a 100-element solid-state Ge detector. Six standard compounds, CdS, CdO, $CdCO_3$, $Cd_3(PO_4)_2$, $Cd(NO_3)_2$, and $Cd(NO_3)_2$ mixed with citrate, were analyzed. The spectra (average of two scans) were energy-normalized using Athena (version 0.9.22) (Ravel and Newville, 2005). Principal component analysis (PCA) of the normalized sample spectra was employed to assess the likely number of species contained in the samples, and target transformation (TT) was used to identify relevant standards for linear combination fitting (LCF) of the sample spectra. PCA and TT were undertaken using SixPack (Webb, 2005). The LCF was performed using Athena to identify the relative proportions of standard spectra within the sample spectra, and the fitting energy range was –30 to +100 eV relative to the Cd K-edge.

2.5. Statistical analysis

The data were statistically analyzed using a two-way analysis of variance. Significant ($P \leq 0.05$) differences between means were identified using Duncan's multiple range test using GenStat v. 11 (VSN international).

3. Results

3.1. Soil pH

The Sodosol had solution pH ranging from 4.15 to 4.88 in the rhizosphere and from 4.45 to 5.22 in the bulk soil during plant growth after N application (Fig.1). The solution pH in the rhizosphere decreased over time in all N treatments, but the decrease was greater when N was supplied as NH_4^+ and urea than as NO_3^-. Reduced N (NH_4^+ and urea), especially of NH_4^+, decreased soil solution pH of the bulk soil, but there was little change with NO_3^- application. The volume of solution collected from the Vertosol was insufficient to permit analysis.

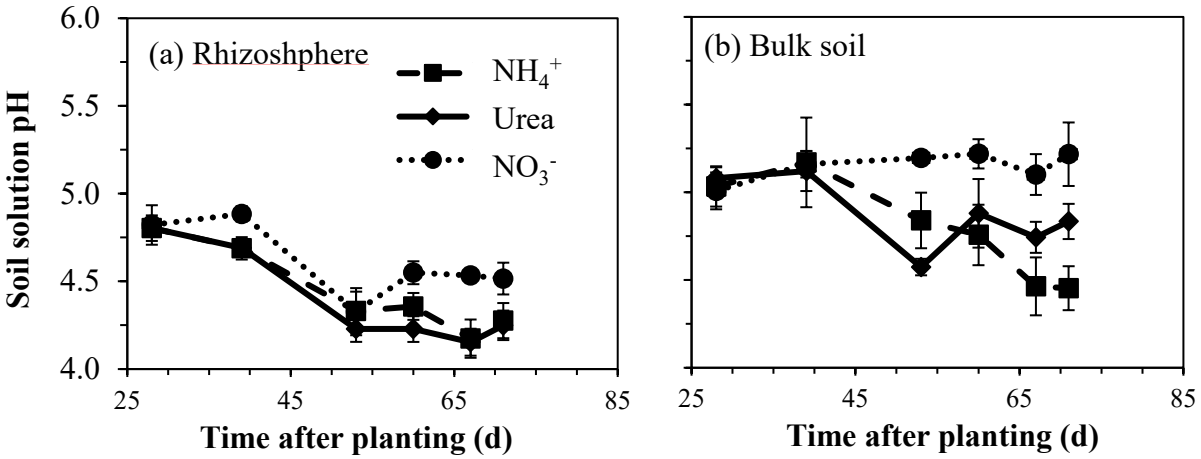

Fig. 1. Effect of N form on soil solution pH of the rhizosphere (a) and bulk soil (b) of the Sodosol during the growth of *Carpobrotus rossii*. Bars represent ± standard error (n = 4).

At the end of the experiment, rhizosphere pH (0.01 M $CaCl_2$) was 0.37-0.90 units lower than bulk soil pH for the Vertosol, and 0.21-0.58 units lower for the Sodosol (Fig. 2a). Specifically, the rhizosphere pH was 0.21 and 0.29 units higher in the NO_3^- treatment than in NH_4^+ and urea treatments, respectively, in the Sodosol, but was 0.87 and 0.58 units, respectively, higher in the Vertosol. In comparison, the pH of bulk soils supplied with NH_4^+ was 0.3 units lower than those supplied with NO_3^- and urea.

3.2. *Extractable Cd concentration*

The concentration of 0.01 M $CaCl_2$-extractable Cd in soils varied from 0.2 to 7 mg kg^{-1} depending on soil type and N supply (Fig. 2b). Overall, the Sodosol had 6-fold higher extractable Cd in the rhizosphere and 14-fold higher in the bulk soil than the Vertosol. Compared to the NO_3^- treatment, the reduced N treatments increased the concentration of extractable Cd in the rhizosphere by 71-113% for the Vertosol and by 14% for the Sodosol. Similar trends were observed in the bulk soil, extractable Cd being highest when NH_4^+ was supplied; there was a greater effect of N form in the Vertosol (136%) than in the Sodosol (24%).

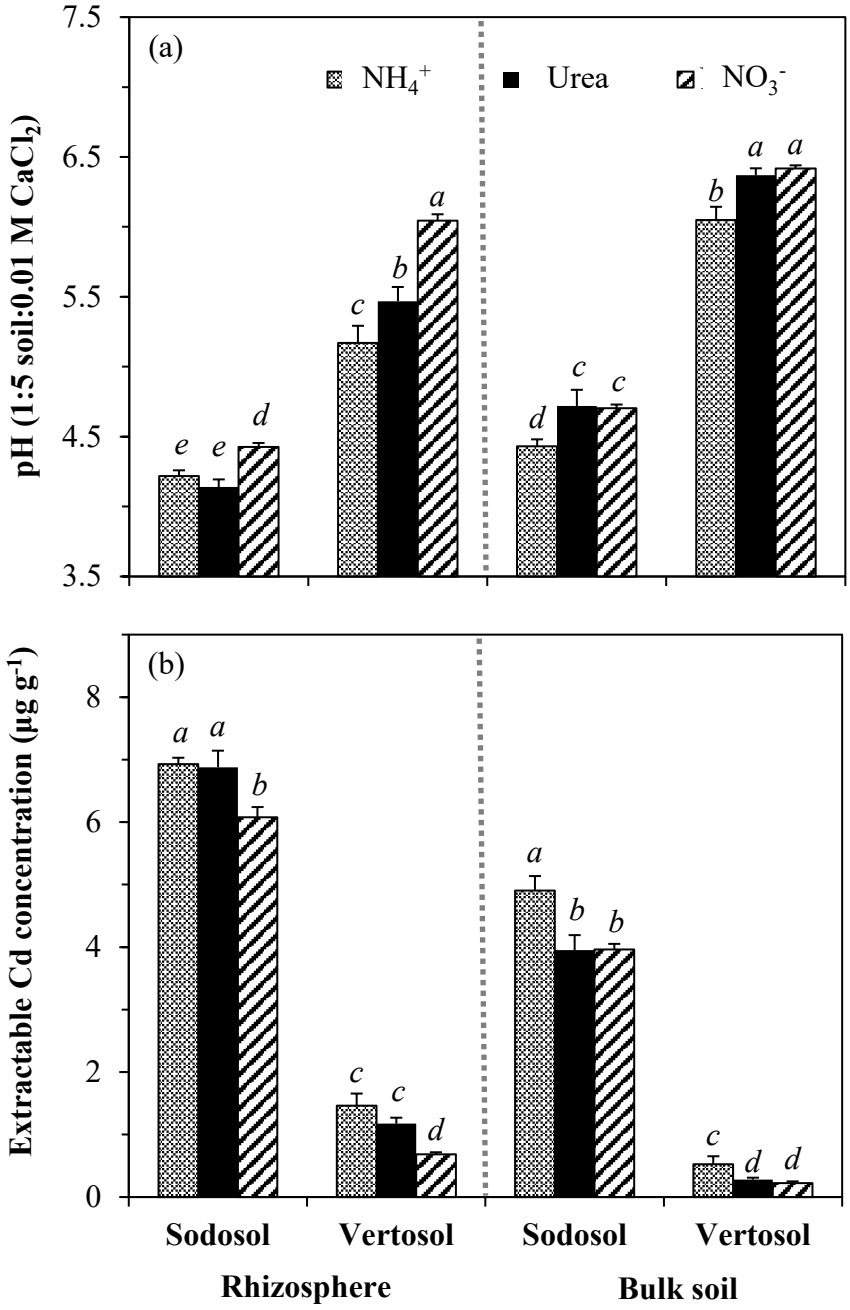

Fig. 2. Effects of N form on soil pH (a) and 0.01 M $CaCl_2$-extractable Cd concentration (b) in the Sodosol and Vertosol after *Carpobrotus rossii* were grown for 75 days. Bars represent the standard error (n = 4). For each panel, means with the same letter above the bars indicate non-significant differences between the treatments for rhizosphere soil or bulk soil (Duncan's test, $P<0.05$).

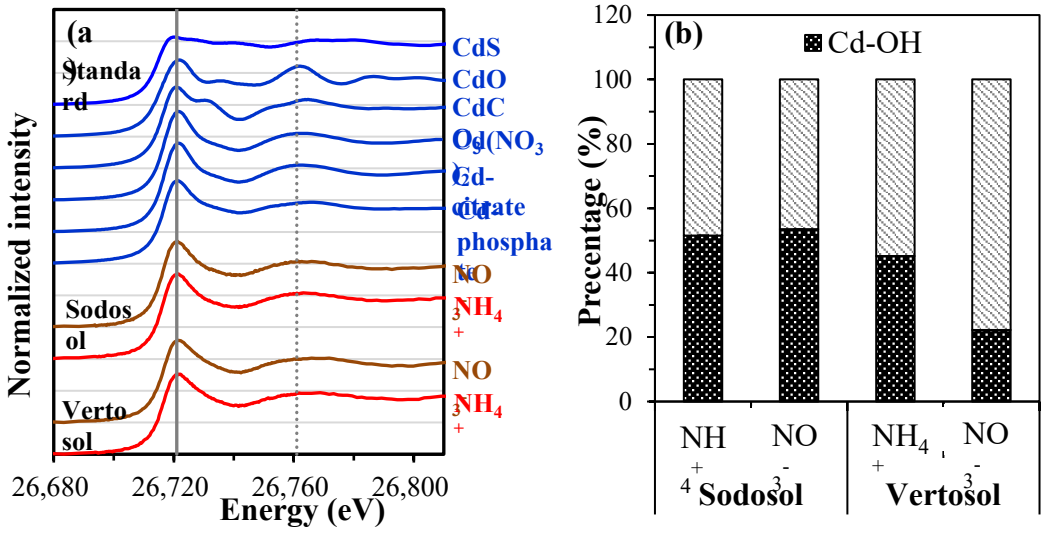

Fig. 3. Normalized Cd K-edge XANES spectra (a) and the proportion of Cd speciation (b) in the rhizosphere of the Sodosol and Vertosol after *Carpobrotus rossii* had been grown for 75 days. The horizontal grey lines represent a value of 1 for each of the normalized spectra, while the vertical grey lines represent white-line peak at 26,721.5 eV (solid line) and the spectral feature at 26,761 eV (dotted line) for Cd-OH standard.

3.3. Cd speciation in soils

Given that the spectra of $Cd(NO_3)_2$ and Cd-citrate were similar and indistinguishable from each other, data are grouped together and referred to as 'Cd-OH'. The XANES spectra of all rhizosphere samples could be fitted with a combination of Cd-phosphate and Cd-OH compounds, which had different magnitudes of the white-line peak and spectral features from 26761 to 26767 eV (Fig. 3a). Indeed, LCF of the XANES data revealed that approximately 50% of Cd in the rhizosphere of the Sodosol was free Cd^{2+} or complexed with carboxyl groups (Cd-OH complexes), and the remaining 50% was Cd-phosphate (Fig. 3b). The spectra of rhizosphere in the Vertosol were visually more similar to that of Cd-phosphate, and this was confirmed by LCF prediction that 61-73% Cd was in Cd-phosphate in the rhizosphere of Vertosol. Furthermore, the N form did not change Cd speciation in the rhizosphere of the Sodosol, but the supply of NH_4^+ increased the Cd-OH forms by 23% in the rhizosphere of the Vertosol compared to the NO_3^- treatment.

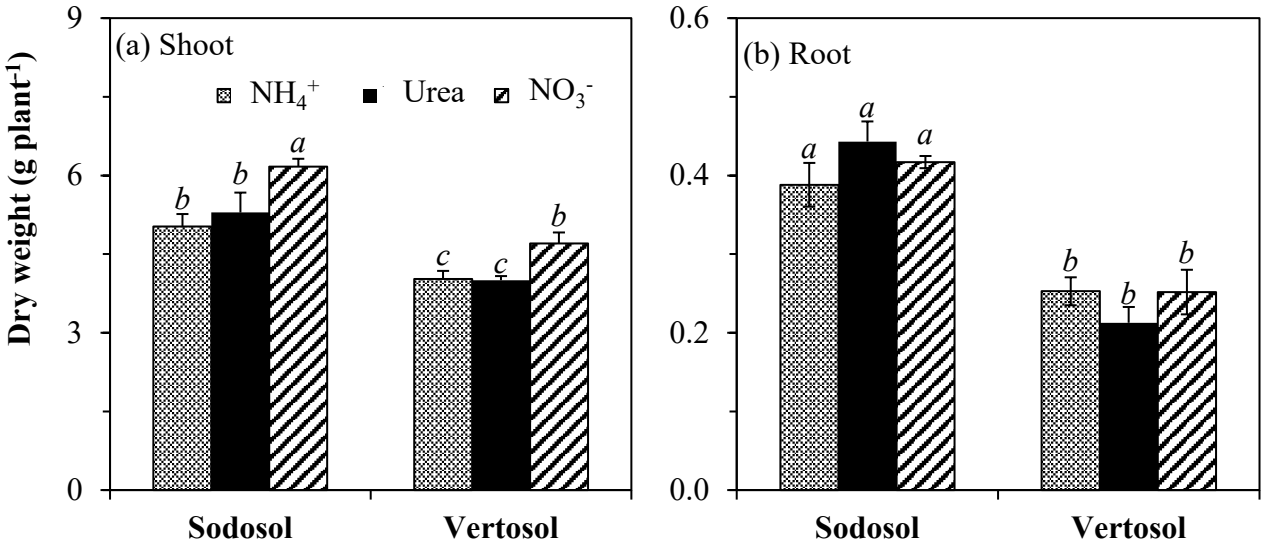

Fig. 4. Effects of N form on the dry weight of shoots (a) and roots (b) of *Carpobrotus rossii* grown for 75 days in two Cd-contaminated soils. Error bars represent ± standard errors (n = 4). Means with the same letter within a panel did not differ significantly (Duncan's test, $P<0.05$).

3.4. Plant growth

All plants appeared healthy throughout the experimental period but from 30 d after transplanting, those grown on the Sodosol were visibly larger than those on the Vertosol, this being reflected in the 30 % higher shoot dry weight of plants on the Sodosol (Fig. 4a). The better growth on the Sodosol was evident in the harvested root dry weight, which was 70% higher than that on the Vertosol (Fig. 4b). Despite the little visual difference between the N treatments, there was 17% and 20% higher shoot dry weight with NO_3^- than with either NH_4^+ or urea in the Sodosol and Vertosol, respectively. There was, however, no difference in root dry weight among the three N forms.

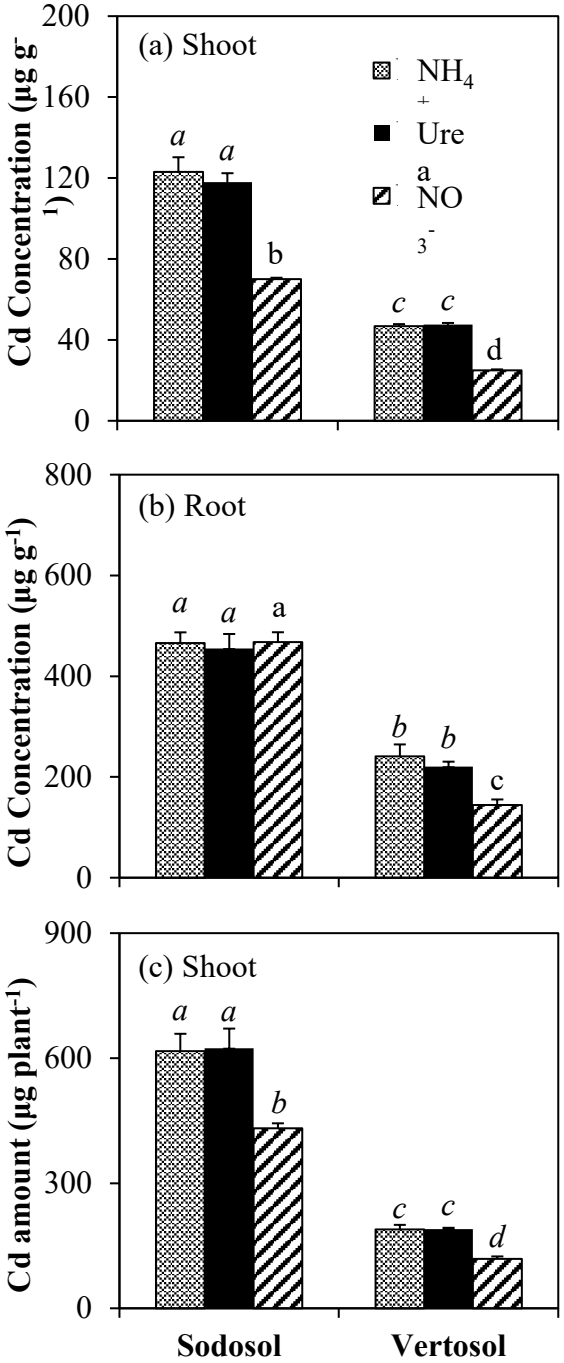

Fig. 5. Effects of N form on Cd concentration in the shoots (a) and roots (b), and on shoot Cd accumulation (c) of *Carpobrotus rossii* grown for 75 d in two Cd-contaminated soils. Error bars represent ± standard errors (n = 4). Means with the same letter within a panel did not differ significantly (Duncan's test, $P<0.05$).

As with the root biomass, the root length and surface area were 2.5-fold greater in plants grown in the Sodosol than in the Vertosol (Table S2). However, the roots were thicker in the Vertosol than in the Sodosol. Furthermore, the N form only influenced root length and root surface area of plants grown in Sodosol, with greater root length and surface area when supplied with urea than supplied with NH_4^+ and NO_3^-.

3.5. Plant Cd concentration and accumulation

The Cd concentrations in plants were significantly higher when grown in the Sodosol than in the Vertosol (Fig. 5), ranging from 25-123 μg g^{-1} in the shoots and from 145-468 μg g^{-1} in the roots. For a given N treatment, plants had 2.5-fold higher Cd concentration in both shoots and roots when grown in the Sodosol than in the Vertosol. Accordingly, plants accumulated three times more Cd in the shoots when grown in the Sodosol than in the Vertosol.

Shoot Cd concentrations were significantly higher when plants were supplied with reduced N than when supplied with NO_3^- (Fig. 5a). For example, when grown in the Sosodol, shoot Cd concentration was 76% higher (123 μg g^{-1} DW) in the plants supplied with NH_4^+ than those with NO_3^-. A similar trend was observed in the Vertosol, where the shoot Cd concentrations was 87% higher in the plants supplied with reduced N (48 μg g^{-1}) than with NO_3^-. In addition, N form did not affect root Cd concentrations (ca. 460 μg g^{-1}) in the Sodosol while root Cd concentration was 67% higher in reduced N-fed plants (ca. 230 μg g^{-1}) than NO_3^--fed plants when grown in the Vertosol (Figure 5b). As with shoot Cd concentration, plants supplied with reduced N accumulated 620 and 190 μg Cd in the shoot of per plant in the Sodosol and Vertosol, respectively, while the plants supplied with NO_3^- accumulated approximately 65% of these, being 430 μg per plant in the Sodosol and 120 μg per plant in the Vertosol.

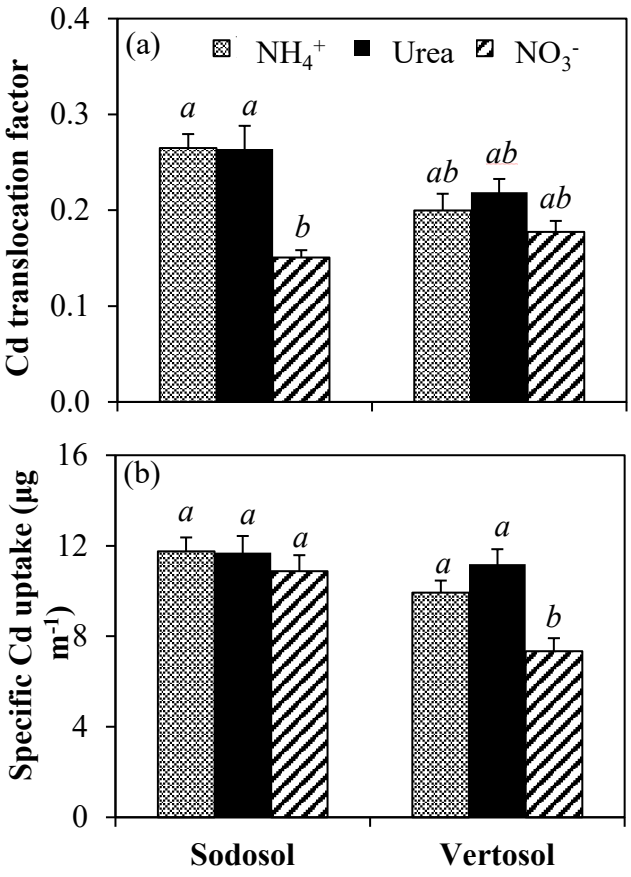

Fig. 6. Effects of N form on shoot/root cadmium concentration ratio (translocation factor) (a) and specific Cd uptake (Cd uptake per root length) (b) of *Carpobrotus rossii* grown for 75 d in two Cd-contaminated soils. Error bars represent the standard errors (n = 4). Means with a common letter within a panel did not differ significantly (Duncan's test, $P<0.05$).

Although the tissue Cd concentrations had significant difference between soil types, for a given N form, the Cd translocation factors (shoot-to-root Cd concentration ratios) were similar in plants grown in both soils, except for the NH_4^+ treatment where the Cd translocation factor was 35% higher in plants grown in the Sodosol than in the Vertosol (Fig. 6a). Among the N treatments, the Cd translocation factor was 76% higher in reduced N-fed plants than NO_3^--fed plants in the Sodosol. In contrast, N form had no statistically significant effect on Cd translocation when plants grown in the Vertosol, although the reduced N tended to increase the translocation factor.

Similarly, there was a significant N form × soil type interaction on the specific Cd uptake (Fig. 6b). The Cd uptake per root length was not altered by N form when plant grown in the Sodosol, but it was 43% higher when plants were supplied with reduced N in the Vertosol.

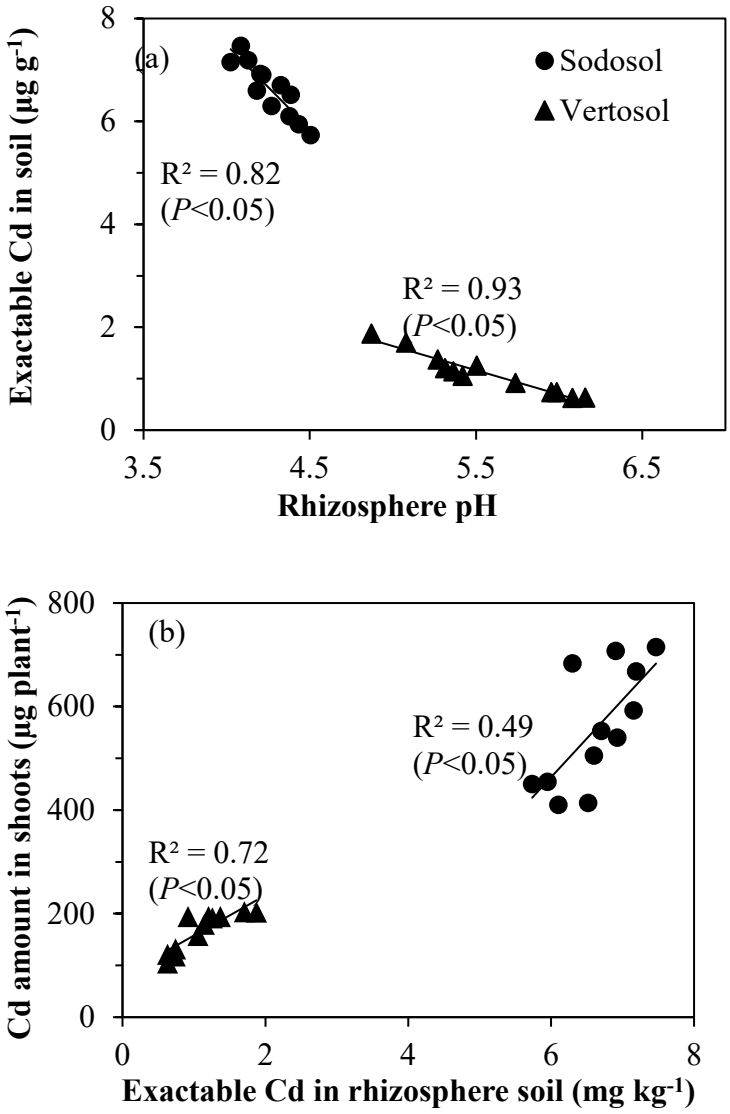

Fig. 7. Relationships between the concentration of 0.01 M $CaCl_2$-exactable Cd and rhizosphere pH (a), and between the Cd content in shoots and $CaCl_2$-exactable Cd concentration in rhizosphere soil (b) after *Carpobrotus rossii* had been grown in two soils for 75 d.

3.6. Correlations between measurements

Irrespective of N treatments, there was significantly negative correlation between the extractable Cd concentration and pH in both soils (Fig. 7a). However, with soil pH decreasing, the increase of extractable Cd concentration in the Sodosol was greater in the rhizoshphere than the bulk soil, with no difference in the Vertosol. Furthermore, the shoot Cd amounts positively correlated with the concentrations of extractable Cd in the rhizosphere soils, such correlation being closer in the Vertosol (R^2=0.72) than in the Sodosol (R^2=0.49) (Fig. 7b).

4. Discussion

4.1. The efficiency of Cd phytoextraction by C. rossii *differs in soil types*

This study showed that shoot Cd accumulation in *C. rossii* was generally three times higher when grown in the Sodosol than in the Vertosol, which is consistent with previous finding that the efficiency of Cd phytoextraction was higher in acidic than in alkaline or calcareous soils (Li et al., 2014). This variation was partly attributed to the better growth of *C. rossii* in the Sodosol with higher root and shoot dry weight (Table 2 and Fig. 4). A bigger root system would be particularly important, contributing to a higher acquisition of Cd from the soil and by a stronger rhizosphere activation of Cd availability (Mench and Martin, 1991; Naidu and Harter, 1998; Marschner, 2011). Furthermore, the 30% higher shoot dry weight when grown in the Sodosol would increase the sink for Cd absorbed by roots. The suppressed growth of *C. rossii* in the Vertosol might due to the reduced water availability or oxygen supply resulting from the high content of clay (>50%), since this species is mainly grown in coastal areas, particularly on sand dunes (Geraghty et al., 2011; Zhang et al., 2014). In addition, the high Cd accumulation in plants grown in the Sodosol had also resulted from the higher Cd bioavailability in the soil, evidenced by the lower average proportion of Cd phosphate (Figs. 2 and 3). The Cd phosphate has been considered as a stable species with low availability in the long term (Hamon et al., 1998; Huguet et al., 2015).

The present study confirmed that *C. rossii* has the potential of Cd phytoextraction in Cd-polluted soils, as shown in previous studies (Zhang et al., 2014; Liu et al., 2015). After 75 d growth, *C. rossii* removed 3.7% of total Cd from the Sodosol and 1.1% from the Vertosol. This efficiency was lower than that of *T. caerulescens* 1-7.8% when grown in soil with 2.75 mg kg^{-1} Cd for 90 d (Xie et al., 2009). However, due to its big biomass, *C. rossii* had average daily Cd uptake per plant of 7.4 and 2.2 μg d^{-1} in the Sodosol and Vertosol, respectively, which is within the range of *T. caerulescens* (2-12 μg d^{-1}) and *S. plumbizincicola* (0-10 μg d^{-1}), although shoot Cd concentration was lower (Xie et al., 2009; Li et al., 2014). Considering the confined roots by the rhizobag in the present study, the Cd removal efficiency of *C. rossii* may be higher in naturally Cd-contaminated soils, especially in low pH soils.

4.2. Effects of N form on plant Cd accumulation

This study supported the first hypothesis that reduced N forms (NH_4^+ and urea), compared to NO_3^-, increased Cd accumulation in plants in both the Sodosol and the Vertosol. This was evident in Cd concentration and total amount of Cd in shoots despite the plants growing better when supplied with NO_3^- (Fig. 4). Furthermore, the results supported our second hypothesis that the increased Cd accumulation with reduced N form was modulated by soil type, with a greater impact when plants grown in the Vertosol than in the Sodosol. Generally, two processes determine Cd accumulation in plants: root uptake activity and efficiency of translocation (Clemens, 2006). In the present study, the reduced N form increased Cd uptake by root in the Vertosol due to the greater soil acidification (Marschner and Römheld, 1983) and consequent increased extractable Cd (Wu et al., 1989; Zaccheo et al., 2006), but increased Cd translocation from root to shoot in the Sodosol, indicating the key process for the increased Cd accumulation in NH_4^+- and urea-fed plants differed in soils.

4.3. Effects of N form on Cd uptake

Interestingly, the supply of reduced N increased the root Cd uptake only in the Vertosol but not in the Sodosol, although the reduced N lowered the rhizosphere pH in both soil types. It was evident that the reduced N enhanced not only the root Cd concentrations but also the Cd uptake per unit root length when plant grown in the Vertosol (Figs. 5b and 6b). The increased root capacity to require Cd in the Vertosol was mainly due to the increased available Cd in soils rather than the changes of root length or surface area, which differs from the previous hydroponic experiment (Cheng et al., 2016). The apparent discrepancy about root Cd uptake between the soil types could be mainly explained by the greater effect of N form on the Cd bioavailability in the Vertosol compared to the Sodosol. This is supported by the increased Cd-OH proportion and doubled extractable Cd in the rhizosphere of the Vertosol with little changes in the Sodosol (Figs. 2 and 3). This is not unexpected because the Vertosol had a higher initial pH and lower pH buffer capacity, and hence was sensitive to acidification under reduced N supply, contributing to the increased available Cd in soils (Schubert et al., 1990; Hinsinger et al., 2003). Although the N form did not affect the root Cd uptake when plants grown in

the Sodosol, both the root Cd concentration and Cd uptake per root length were higher than those in plants grown in the Vertosol, indicating the available Cd was not the main limiting factor affecting Cd uptake by the roots of *C. rossii* (Haghiri, 1973; Cataldo et al., 1983; Lux et al., 2011).

4.4. Effects of N form on Cd translocation

The reduced N forms increased the Cd translocation factor in the Sodosol (Fig. 6) as had been found with *C. rossii* and *Solanum nigrum* grown in nutrient solution (Cheng et al., 2016). However, the reason for this increased Cd translocation by the supply of NH_4^+ was not clear. It has been shown that the increased Cd translocation was not due to the changes of Cd speciation in plant tissues (Cheng et al., 2016). The higher content of proteins in the roots of NH_4^+- and urea-fed plants than NO_3^--fed plants resulting from N assimilation might elevate the expression of *AtHMA4* which mediates the Cd xylem loading (Verret et al., 2004; Verbruggen et al., 2009; Xu et al., 2012) but this still needs further study. Meanwhile, the little change of Cd translocation in plants grown in the Vertosol was consistent with previous studies that Cd uptake and accumulation in NH_4^+-fed plants were higher than that in NO_3^--fed plants, without influencing the Cd distribution when plants grown in the high-pH soils (Florijn et al., 1992; Larsson Jönsson and Asp, 2011). The little impact of N form on Cd translocation in the Vertosol might be because the greater impact of N forms on Cd uptake by roots in the Vertosol, where the available Cd was the primary limitation for Cd uptake.

Our findings of higher shoot Cd accumulation in reduced N-fed plants are consistent with those when *C. rossii* was grown in acidic soils (Liu et al., 2015) and when potato (Larsson Jönsson and Asp, 2011; Larsson Jönsson and Asp, 2013) and sunflower (Zaccheo et al., 2006) were grown in high-pH soils. In contrast, other studies have shown that NO_3^- resulted in higher Cd concentrations in plants than NH_4^+ (Maier et al., 2002; Xie et al., 2009; Arnamwong et al., 2015). Discrepancies between the studies may be due to the counter-ion applied with the N fertilizer. The NO_3^- was added as KNO_3 in this present study but as $Ca(NO_3)_2$ in the study of Maier et al. (2002), with Ca^{2+} potentially more effective in replacing surface-bound Cd and thereby increasing Cd^{2+} in soil solution. Alternatively, plant species differ in their response to N form with NH_4^+ suppressing metal uptake and growth of *T. caerulescens* despite Cd bioavailability in the rhizosphere being higher in the NH_4^+ than in the NO_3^- treatment (Xie et al., 2009).

5. Conclusions

This research investigated how N form influenced the efficiency of Cd phytoextraction by *C. rossii* on two soil types that differed in pH and pH buffer capacity. There is strong evidence that NH_4^+ and urea had a greater effect than NO_3^- on increasing shoot Cd accumulation in both soils. The main mechanism for this enhanced shoot Cd accumulation by reduced N differed between the two soils. In the Sodosol, the reduced N increased shoot Cd accumulation via enhanced Cd translocation from root to shoot compared with NO_3^-, with less dependence on soil pH and Cd bioavailability. In comparison, the reduced N doubled the Cd bioavailability in the Vertosol and consequently enhanced root Cd uptake, contributing to the increased Cd accumulation in shoots. The results imply that the application of reduced N fertilizers is an effective method to increase Cd phytoextraction by *C. rossii*. Further work is required to confirm these findings in the field conditions.

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Table S1. Selected properties of the soils used in this study

Property	Sodosol	Vertosol
Clay content (%)	24.8	52.4
Silt content (%)	59.8	35.3
Sand content (%)	15.4	12.2
Water content at field capacity (g kg^{-1})	232	379
pH (1:5 0.01 M $CaCl_2$)	4.91	7.2
pH buffer capacity ($cmol_c$ pH^{-1} kg^{-1})	22.0	16.8
Electrical conductivity (1:5 H_2O) (µS cm^{-1})	131	134
Organic C (g kg^{-1})	39.0	10.0
Total N (g kg^{-1})	2.9	0.9
NH_4^+-N (mg kg^{-1})	7.35	4.86
NO_3^--N (mg kg^{-1})	8.76	3.32

Table S2. The effects of N form on root morphological characters of *Carpobrotus rossii* grown for 75 d in two Cd-contaminated soils. Data are means ± standard errors (n = 4); means followed by a common letter did not differ significantly (Duncan's test, $P<0.05$).

Soil type	Treatment	Root length (m $plant^{-1}$)	Root diameter (mm $plant^{-1}$)	Root surface area (cm^2 $plant^{-1}$)
Sodosol	$(NH_4)_2SO_4$	62.4±3.5 bc	0.34±0.01 a	665±46 b
	$(NH_2)_2CO$	71.1±3.4 c	0.36±0.01 ab	979±55 c
	KNO_3	58.5±4.1 b	0.35±0.01 ab	649±48 b
Vertosol	$(NH_4)_2SO_4$	25.2±0.7 a	0.38±0.01 bc	303±13 a
	$(NH_2)_2CO$	19.5±2.4 a	0.41±0.02 c	246±21 a
	KNO_3	21.6±2.2 a	0.40±0.01 c	267±23 a
Significance level				
N form		>0.05	>0.05	>0.05
Soil type		<0.001	<0.001	<0.001
N form × Soil type		>0.05	>0.05	>0.05