

# Some effects of nitrogen nutrition on caesium uptake and translocation by species in the Poaceae, Asteraceae and Caryophyllidae

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## Abstract

There is current interest in managing and manipulating <sup>137</sup>Cs transfer from soil to plants. We hypothesized that N source might affect Cs uptake by plants and report experiments that confirm this. Uptake experiments using hydroponics with a variety of species in the Poaceae, Asteraceae and Caryophyllid clade grown in a variety of N regimes with excess N and then acutely exposed to Cs showed that N nutrition could affect Cs uptake rates, total amounts of Cs taken up and root:shoot ratios of Cs. In general, the Caryophyllids tested produced significantly less shoot and root biomass but had higher Cs uptake rates when grown on NH<sub>4</sub><sup>+</sup> rather than NO<sub>3</sub><sup>-</sup>, whilst species from the Poaceae and Asteraceae almost always produced similar shoot and root biomass and had similar Cs uptake on NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup> or glycine as N sources. This is the first time that plants grown on an organic-N source have been demonstrated to take up Cs. Physiological experiments using N-starvation and the N-metabolism inhibitor methionine sulfoxamine (MSX) demonstrated that Cs transport into the root was inversely related to NH<sub>4</sub><sup>+</sup> transport, i.e. NH<sub>4</sub><sup>+</sup>-grown plants had higher Cs uptake rates if there is no NH<sub>4</sub><sup>+</sup> present during uptake but lower Cs uptake rates if NH<sub>4</sub><sup>+</sup> is present. It is suggested that taking account of N ecophysiology might help refine predictions of soil-to-plant transfer of <sup>137</sup>Cs and, in some instances, be useful for managing or manipulating it. It is noted that there is much recent research into N nutrition in plants that might be useful in achieving this.

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## 1. Introduction

Research into <sup>137</sup>Cs transfer from soils to plants has recently begun to expand from making predictions of plant <sup>137</sup>Cs concentrations to managing and manipulating them. For example, in most soils contaminated with <sup>137</sup>Cs it is now clear that it will persist in the rooting zone for many years (Smith et al., 2000), necessitating amelioration strategies based on managing <sup>137</sup>Cs transfer from soils to plants (White et al., 2003). Further, because there are currently no economically viable methods to clean up all <sup>137</sup>Cs-contaminated soils, and enhanced plant uptake of other contaminants from soils is showing potential as a decontamination method (e.g. Huang et al., 1998; Ma et al., 2001), ‘phytoextraction’ has

been investigated for soils contaminated with <sup>137</sup>Cs (Lasat et al., 1997; Dushenkov et al., 1999; Fuhrmann et al., 2003; Willey et al., 2001). These trials have shown that manipulations to enhance soil-to-plant transfer are needed if phytoextraction is to be useful for <sup>137</sup>Cs.

Minotti et al. (1965) and Jackson et al. (1968) reported that NH<sub>4</sub><sup>+</sup>-grown wheat seedlings had significantly higher Cs uptake than NO<sub>3</sub><sup>-</sup>-grown ones, and the experiments of Shaw and Bell (1991) showed that NH<sub>4</sub><sup>+</sup> and Cs could compete during uptake by wheat. Given that NH<sub>4</sub><sup>+</sup> concentrations in soils can vary by several orders of magnitude (Glass et al., 2002), such results indicate that soil NH<sub>4</sub><sup>+</sup> concentrations might affect Cs transfer from soil to plants. Evans and Dekker (1969) provided the first field test of this phenomenon and showed that additions of NH<sub>4</sub><sup>+</sup> to soil significantly increased <sup>137</sup>Cs uptake by oat plants. Similar results have recently been reported with <sup>137</sup>Cs uptake by rye grass (Paasikillio

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and Sormunen-Cristian, 2002). Significant  $\text{NH}_4^+$ -induced increases in Cs uptake have, therefore, all been reported in plants in the family Poaceae (Wheat, Oats and Rye). Experiments at Brookhaven National Laboratory (Lasat et al., 1997; Lasat et al., 1998; Fuhrmann et al., 2002; Fuhrmann et al., 2003) and near Chernobyl (Dushenkov et al., 1999) have shown that  $\text{NH}_4^+$  additions can increase  $^{137}\text{Cs}$  desorption from soils by an order of magnitude in the laboratory but that additions of  $\text{NH}_4^+$  to soil in the field did not increase  $^{137}\text{Cs}$  transfer to plants, almost all of which were in the families Chenopodiaceae or Amaranthaceae on the Caryophyllid clade.

Molecular biological investigations have transformed the understanding of plant N-nutrition (Glass et al., 2002). It is now thought that up to seven transport proteins in the AMT1 family are responsible for  $\text{NH}_4^+$  uptake and transport in plants (Glass et al., 2002), although with the exception of AMT1.1, their Cs transport capacity is unknown. Their expression is dependent on N source, plant N status, plant species and plant organ. AMT1.1 has some Cs transport activity (Ninneman et al., 1994), and given that  $\text{NH}_4^+$  and  $\text{Cs}^+$  have a very similar hydrated size, which causes them to interact during adsorption to soil, interactions between  $\text{NH}_4^+$  and Cs transport seem possible. The uptake of  $\text{NO}_3^-$  by plants is primarily regulated by NRT2 proteins (Glass et al., 2002), which seem unlikely to be able to transport Cs, but the presence of  $\text{NO}_3^-$  up-regulates expression of  $\text{NO}_3^-$  transporters and down-regulates expression of  $\text{NH}_4^+$  transporters, probably via feedback based on cytosolic glutamine concentrations (Crawford and Glass, 1998). Interestingly, it has also become clear in the last decade that many plants can use organic forms of soil nitrogen, such as glycine, in significant quantities (Lipson and Nasholm, 2001), including both plant species that grow in  $^{137}\text{Cs}$  contaminated agricultural (Nasholm et al., 2000) and natural ecosystems (Persson and Nasholm, 2001). Uptake of organic N also affects  $\text{NH}_4^+$  uptake via feedback through cytosolic glutamine concentrations (Glass et al., 2002). The genes and proteins that control numerous aspects of N uptake and metabolism in the model plant *Arabidopsis thaliana* are rapidly being identified (Crawford and Forde, 2002). Overall, such studies are emphasising the central role of N uptake and metabolism in whole-plant physiology and ion uptake (Crawford et al., 2000). Given that such research is opening up the possibility of genetically engineering plant N uptake, with consequent effects on ion uptake, it seems an appropriate moment to reinvestigate the effects of N nutrition on Cs uptake by plants.

It is now known that plants in the Poaceae, such as the wheat used by Minotti et al. (1965), Jackson et al. (1968), and Shaw and Bell (1991) have, on average, much lower Cs uptake than those on the Caryophyllid clade in such families as the Chenopodiaceae, Amaranthaceae and Polygonaceae (Broadley and Willey, 1997; Broadley et al., 1999a,b; Tang et al., 2003). Species in the Asteraceae have also been noted to have high uptake of Cs (Tang and Willey, 2003; Willey et al., 2005). Such inter-taxa differences suggest that efforts

to minimise soil-to-plant transfer of Cs might best focus on species in the Poaceae, whilst efforts to maximise transfer might focus on members of the Caryophyllid clade and the Asteraceae. Significantly, it also appears that there might be some phylogenetic constraints on the sources of nitrogen that plants can utilise, with the Poaceae often reported as preferring a mixed  $\text{NO}_3^-/\text{NH}_4^+$  source, although a wide phylogenetic perspective on plant N preferences has not yet been reported (Glass et al., 2002). Here, using hydroponic systems in which soil interactions have been eliminated, we report five separate experiments that used plants in the Poaceae, Asteraceae and Caryophyllidae to investigate different aspects of plant N-nutrition affects on Cs uptake. One objective was to clarify the apparently contradictory effect of  $\text{NH}_4^+$  nutrition on Cs uptake in plants in the Poaceae and Caryophyllidae but our overall aim was to investigate the effects of the N-nutrition of plants on Cs uptake.

## 2. Methods

In experiments in Sections 2.1–2.3 plants were grown hydroponically in large volumes of different Cs-free N-sources that provided excess readily available nutrients and then given an acute exposure to Cs by immersing their roots in small volumes of solutions containing just radiolabeled Cs and physiological saline ( $\text{CaSO}_4$ ). This eliminated the effects of soil factors and nutrient deficiencies on Cs uptake, prevented interactions (either long-term or short-term) between Cs uptake and nutrient uptake, and gave approximately instant insights into the effects on Cs uptake by minimising the effects of homeostatic mechanisms and other feedback effects during radiolabeling. Protocols of this type were chosen in an attempt to dissect physiological effects of N-source rather than to simulate field conditions. In experiments in Sections 2.1–2.3 there was no significant depletion of radio-caesium during radiolabeling and, because a stable Cs carrier was used, no detectable radio-caesium adsorbed to flasks or tubs.

### 2.1. Nitrogen source and $^{137}\text{Cs}$ activities in plant shoots

The objective of this experiment was to test the effects of the full range of N-sources that plants are now known to utilise on Cs uptake to shoots using plant species known to have contrasting Cs uptake behaviours. Seeds of *Hordeum vulgare*, *Amaranthus cruentus*, *Chenopodium album*, *Carthamus tinctorius* and *Zinnia elegans* were surface sterilised by soaking in 1% NaOCl for 10 min then 5%  $\text{H}_2\text{O}_2$  for 1 min, rinsed in deionised water, and germinated in heat-treated, inert vermiculite in 36 gauze-bottomed tubs of each species for hydroponic culture. Nine replicates of each species were cultured in four Hoagland's solutions modified to contain four different N sources: zero N,  $\text{Ca}(\text{NO}_3)_2$ , glycine and  $\text{NH}_4\text{Cl}$ . The three N solutions each contained 0.715 mM N. Each growth solution had a volume of 40 L, had pH adjusted

to 5.5, contained 10 ppm ampicillin, was continually aerated, changed every two days and located in a greenhouse with 16 h/8 h day/night at ca. 22 °C/18 °C with supplementary lighting to ca. 350  $\mu\text{mol m}^{-1} \text{s}^{-1}$ . Each solution was located in a 40 L compartment of a master tank. At each solution change the compartmental distribution of growth solutions was rotated, and solution samples taken for analysis of  $\text{NO}_3^-$  using NitraVer 5 (Hach Co., USA), analysis of  $\text{NH}_4^+$  using Nessler's solution (Fisher Scientific, UK), and for counts of nitrifying bacteria. After 21 days all plants were transferred to a nuclear laboratory for radiolabeling where each tub of plants was seated for 1 h into the neck of a 275 mL conical flask containing 0.25 mM  $\text{CaSO}_4$  to bathe the roots in physiological saline and acclimatise the plants to radiolabeling conditions, as has been found useful in previous experiments of this type (Broadley et al., 1999a,b). Tubs were then transferred to a 275 mL conical flask containing 10  $\mu\text{M}$   $\text{CsCl}$  radiolabeled with 185 kBq  $^{137}\text{Cs L}^{-1}$  plus 0.25 mM  $\text{CaSO}_4$ . A randomised block design was used to position the flasks in an arena provided with ca. 350  $\mu\text{mol m}^{-1} \text{s}^{-1}$  supplementary lighting. Plants were harvested after 24 h (a period that handheld monitors indicated had produced easily detectable  $^{137}\text{Cs}$  activities in plants), dried at 80 °C for 48 h and shoots analysed for  $^{137}\text{Cs}$   $\gamma$ -emissions on an LKB Wallac Compugamma 1282 (NaI(Tl) detector with 81% efficiency) using appropriate calibration and blanks. Shoot weights, shoot  $^{137}\text{Cs}$  activity concentrations and total Cs uptake to shoot were analysed statistically using two-way ANOVA on SigmaStat 3 for Windows with N regime and species as factors, and the Holm–Sidak procedure for all pairwise comparisons.

### 2.2. N source and root:shoot $^{137}\text{Cs}$ activities in plants

Sixteen tubs each of *Lolium perenne* L. var S23 and *Beta vulgaris* L. var *flavescens* were grown in the greenhouse conditions and in the hydroponic system used for experiment in Section 2.1 above. Eight replicate tubs of each species were grown in a 40 L compartment containing either nitrate or ammonium-based nutrient solutions using the improved hydroponic solutions of Padgett and Leonard (1993) plus the nitrification inhibitor dicyandiamide (DCD) at 20 mg  $\text{L}^{-1}$  (Prasad and Power, 1995). Both solutions contained 10 mM N. The solutions in each tank were aerated and changed weekly, and at each change  $\text{NO}_3^-$  and  $\text{NH}_4^+$  concentrations were measured as for experiment in Section 2.1. After 28 days all plants were radiolabeled as in experiment in Section 2.1 but for 45 min (an exposure time that it was predicted would produce  $^{137}\text{Cs}$  activities that would be easily detectable in the  $\gamma$ -counter), after which time they were transferred to solutions with 5 mM  $\text{CsCl}$  at 4 °C for 30 min. After roots had been immersed in it this cold stable Cs solution contained detectable  $^{137}\text{Cs}$  desorbed from plant roots. The roots and shoots of all plants were then harvested, weighed fresh, and then dried, weighed and counted for  $^{137}\text{Cs}$   $\gamma$ -emissions as in experiment in Section 2.1. The weights and  $^{137}\text{Cs}$  activity concentrations of both roots and shoots, and  $^{137}\text{Cs}$  uptake

rates  $\text{g}^{-1}$  root were statistically analysed using two-way ANOVA as above with N regime and species as factors.

### 2.3. N source, Cs uptake and Cs distribution in Caryophyllids

Nine tubs of eight species (*C. album*, *Chenopodium bonus-henricus*, *A. cruentus*, *Amaranthus giganteum*, *Polygonum fagopyrum*, *Rheum rhaponticum*, *Chenopodium mexicanum*, and *Rheum tataricum*) were grown in the conditions outlined for experiment in Section 2.2 in either the nitrate or ammonium solutions plus the nitrification inhibitor DCD at 20 mg  $\text{L}^{-1}$  (Prasad and Power, 1995). After 42 days, all plants were radiolabeled as in experiment in Section 2.2. The roots, stems and leaves of all plants were harvested, weighed fresh, and counted fresh for  $^{137}\text{Cs}$   $\gamma$ -emissions as in experiments in Sections 2.1 and 2.2 above. Root, stem and leaf weights and  $^{137}\text{Cs}$  activity concentrations were statistically analysed using two-way ANOVA as above with N regime and species as factors.

### 2.4. $\text{NH}_4^+$ -starvation and Cs uptake by plants

*L. perenne* var S23 was grown for 47 days in the greenhouse as above but with average day/night at 24 °C/20 °C, in 68 gauze-bottomed plastic tubs suspended into 2  $\times$  50 L tanks of the following  $\text{NH}_4^+$ -N solution—0.5 mM  $(\text{NH}_4)_2\text{SO}_4$ , 1.0 mM KCl, 0.5 mM  $\text{CaCl}_2$ , 0.15 mM  $\text{MgSO}_4$ , 0.1 mM  $\text{KH}_2\text{PO}_4$  plus FeDTA and micronutrients which was circulated every 20 min and aerated continually. Solution pH was constantly adjusted to 6.5 by the controlled addition of 0.5 mM  $\text{NH}_3$  triggered by a peristaltic pump linked to a pH probe and threshold activator. The solutions were replaced approximately weekly throughout the growth period. On Day 43, the solution in tank 1 was changed to an N-free solution in which  $(\text{NH}_4)_2\text{SO}_4$  was replaced by  $\text{CaSO}_4$ , whilst tank 2 (controls) were refreshed with the  $\text{NH}_4^+$ -N solution. In tank 1, pH adjustment ceased on Day 43 and pH remained at 6.5 until the end of the experiment on Day 47. On Days 44, 45 and 47 (i.e. after 1, 2 and 4 days  $\text{NH}_4^+$ -starvation in tank 1) nine randomly selected tubs were removed from both tanks. Three of the tubs from tank 1 (N-starved) and three from tank 2 (N sufficient control) were immersed in each of three aerated treatment solutions. Each treatment solution had 10  $\mu\text{M}$   $\text{CsCl}$  and the nutrient solution buffered to 6.5 with TRIS-MES but with 0  $\mu\text{M}$  (treatment 1), 30  $\mu\text{M}$  (treatment 2) or 500  $\mu\text{M}$   $(\text{NH}_4)_2\text{SO}_4$  (treatment 3).  $\text{CsCl}$  was labelled with 1110 kBq  $^{134}\text{Cs L}^{-1}$  and  $(\text{NH}_4)_2\text{SO}_4$  was 30%  $^{15}\text{N}$ . Tubs of plants in treatments 1 and 3 were placed individually in 275 mL conical flasks containing 275 mL of treatment solution but those of treatment 2 were placed together in a single 5 L beaker containing 3 L of treatment solution. After 2 h in treatment solutions, plants were transferred to fresh  $\text{NH}_4^+$ -nutrient solution for 30 min. Plants were then harvested, freeze-dried and homogenised. Samples (100 mg) of all root and shoot material was digested in 2 mL  $\text{HNO}_3$  at

170 °C until  $\text{NO}_2$  production ceased, 0.66 mL  $\text{HClO}_4$  (60%) was then added and the samples refluxed at 230 °C until clear. Samples were analysed for  $^{134}\text{Cs}$   $\beta$ -emissions with appropriate blanks and background corrections and for  $^{15}\text{N}$  by mass spectrometry.  $\text{NH}_4^+$  and  $^{134}\text{Cs}$  uptake rates were statistically analysed using two-way ANOVA as above with N starvation and time as factors.

### 2.5. Inhibition of $\text{NH}_4^+$ assimilation and Cs uptake by plants

*L. perenne* var S23 was grown in four tubs in each of 18 beakers for 36 days in 600 mL of  $\text{NH}_4^+$ -N nutrient solution (as used in experiment in Section 2.4). The solution was changed every 5 days and contained 1.5 M  $\text{NH}_4^+$ . Beakers were placed in a growth room 16 h/8 h day/night at ca. 25 °C/20 °C with supplementary lighting to ca. 300  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . On Day 36, the solution in nine beakers was changed to a solution containing 0.4 mM methionine sulphoximine (MSX) and then 1 h later one containing 0.4 mM MSX, 0.05 mM  $\text{NH}_4^+$  and 4  $\mu\text{M}$  Cs. The other nine were treated identically except that MSX was not added. Samples of all treatment solutions were taken before root immersion and then after 1, 3, 6 and 10 h.  $\text{NH}_4^+$  was measured by ion chromatography and  $^{133}\text{Cs}$  by electrothermal atomic absorption spectrophotometry (AAS). One plant from each beaker was harvested after 1, 3, 6 and 10 h and analysed for  $^{133}\text{Cs}$  by AAS.  $\text{NH}_4^+$  and  $^{133}\text{Cs}$  in treatment solutions were statistically analysed using two-way ANOVA as above with MSX regime and time as factors.

## 3. Results

In the nutrient solutions of experiment in Section 2.1 there was no detectable change in nitrogen form or concentration in the N-free,  $\text{NO}_3^-$  or  $\text{NH}_4^+$  solutions at any time and nitrifying bacteria were undetectable. This indicates that the plants were subjected to excess N from different sources. Fig. 1A shows that of the plant species in experiment in Section 2.1, the Caryophyllids (*A. cruentus*, *C. album*) grew well only on nitrate solution but that *H. vulgare* from the Poaceae and *Carthamus tinctoria* and *Z. elegans* (Asteraceae) produced, at  $P=0.05$ , statistically indistinguishable amounts of biomass on either  $\text{NO}_3^-$ , glycine or  $\text{NH}_4^+$ -based solutions. There were significant effects of N-source ( $F=7.857$ ,  $P<0.01$ ), and species ( $F=13.588$ ,  $P<0.01$ ) on  $^{137}\text{Cs}$  concentrations in shoots and a significant interaction between the two ( $F=4.96$ ,  $P<0.01$ ). For the factor N-source there were numerous significant pairwise comparisons in  $^{137}\text{Cs}$  concentration, with the concentration of  $^{137}\text{Cs}$  in  $\text{NO}_3^-$ -grown *A. cruentus* and *C. album* being significantly greater than that in  $\text{NO}_3^-$ -grown plants of *H. vulgare* and *C. tinctorius*, and greater than that of all *L. perenne* and  $\text{NH}_4^+$ -, glycine- and control-grown *Z. elegans* (Fig. 1B). All three N-sources produced uptake significantly different to the controls in at least one species

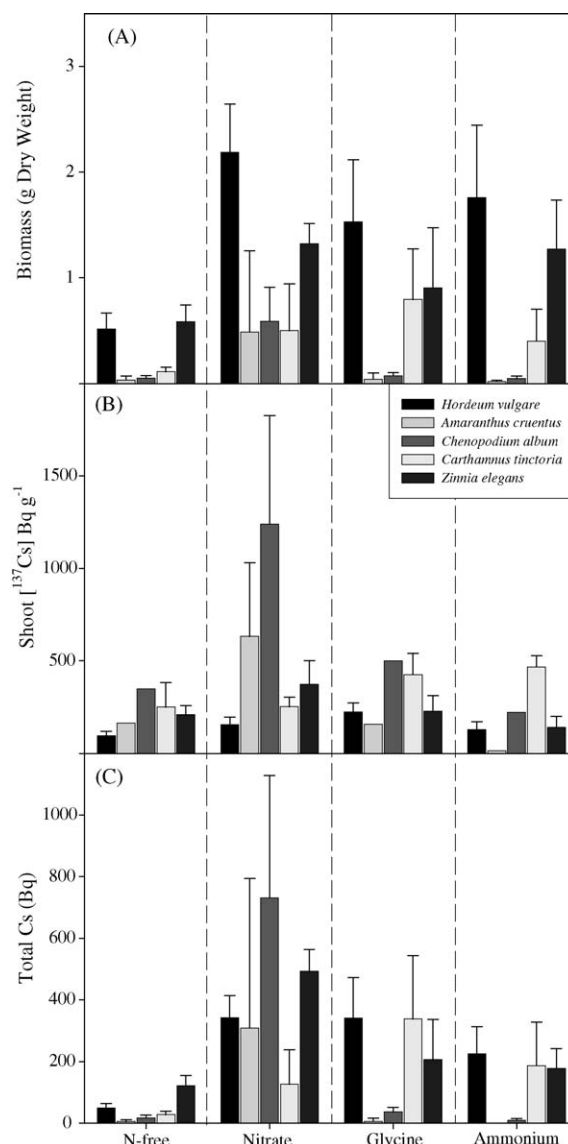


Fig. 1. Biomass dry weight (A), shoot  $^{137}\text{Cs}$  concentrations (B) and total shoot  $^{137}\text{Cs}$  (C) in five plant taxa grown for 21 days in hydroponics with Hoagland's solutions modified to either nitrate, glycine or ammonium as the sole nitrogen source at 10 mg  $\text{N L}^{-1}$ . (Means  $\pm$  S.E.,  $n=9$ , except where no S.E. then  $n=1$ ).

(Fig. 1B). Overall, the greatest total amount of  $^{137}\text{Cs}$  was removed by *C. album* grown on nitrate. *H. vulgare*, *C. tinctorius* and *Z. elegans* grown on any of the N sources removed, at  $P=0.05$ , statistically indistinguishable total amounts of  $^{137}\text{Cs}$  (Fig. 1C).

In  $\text{NO}_3^-$  and  $\text{NH}_4^+$  nutrient solutions in experiment in Section 2.2 the maximum detectable  $\text{NO}_3^-$  concentration in  $\text{NH}_4^+$  solutions was 4 mg  $\text{L}^{-1}$  (0.015 mM N) and the minimum  $\text{NH}_4^+$  32 mg  $\text{L}^{-1}$  (1.4 mM N), whilst in  $\text{NO}_3^-$  solutions the maximum  $\text{NH}_4^+$  was 3 mg  $\text{L}^{-1}$  (0.131 mM N) and the minimum  $\text{NO}_3^-$  28 mg  $\text{L}^{-1}$  (0.105 mM N). *L. perenne* plants grown on either  $\text{NO}_3^-$  or  $\text{NH}_4^+$  as an N source did not produce significantly different biomass totals but  $\text{NH}_4^+$ -grown plants had significantly greater shoot:root ratios ( $P<0.05$ ;

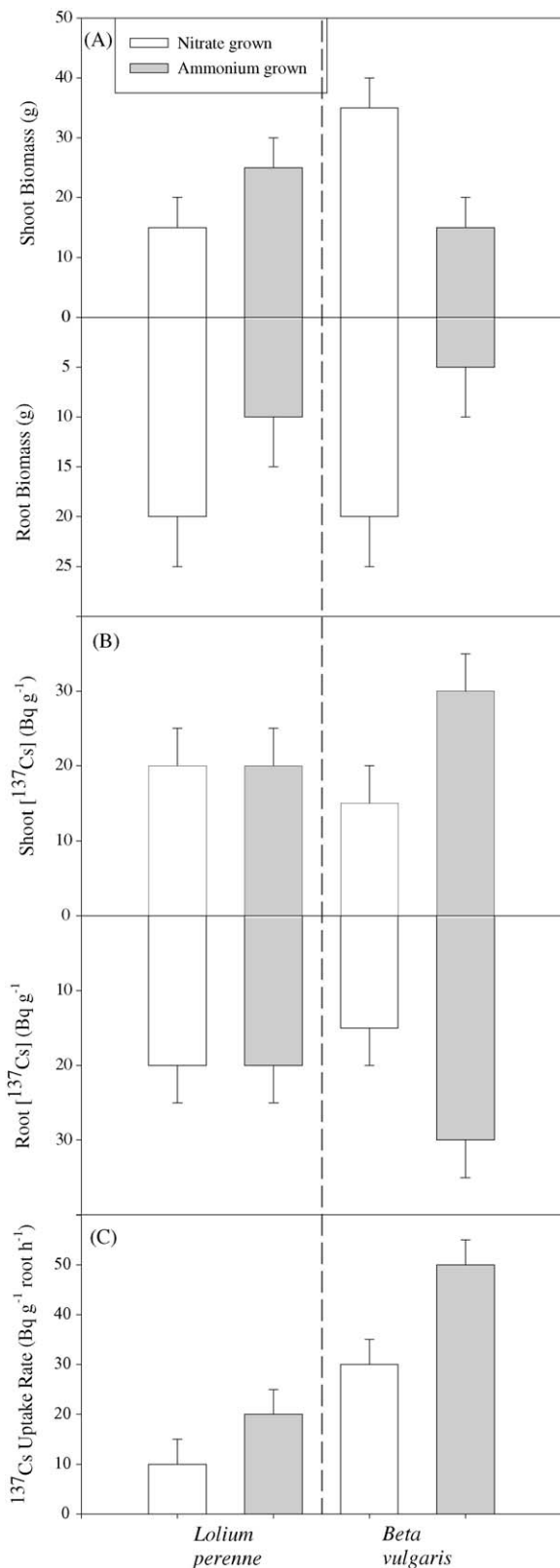


Fig. 2. Biomass dry weight (A) and <sup>137</sup>Cs concentrations (B) of roots and shoots, and <sup>137</sup>Cs uptake rates g<sup>-1</sup> root (C) by *L. perenne* and *B. vulgaris* grown for 28 days in hydroponics with nitrate and ammonium solutions based on Padgett and Leonard (1993) at 10 mM N. (Means ± S.E., n = 8).

Fig. 2A). *B. vulgaris* plants produced significantly greater biomass in NO<sub>3</sub><sup>-</sup> solutions ( $P=0.006$ ; Fig. 2A). The Cs concentration in roots and shoots of *L. perenne* were not significantly different between NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>-grown plants (Fig. 2B) and the total amount of Cs they accumulated was not therefore different, but due to the differences in shoot: root biomass ratios, NH<sub>4</sub><sup>+</sup>-grown plants accumulated significantly more Cs in total in their shoots than NO<sub>3</sub><sup>-</sup>-grown plants (not shown,  $P=0.014$ ). Cs concentrations in the roots and shoots of NH<sub>4</sub><sup>+</sup>-grown *B. vulgaris* were significantly greater than NO<sub>3</sub><sup>-</sup>-grown plants ( $P=0.014$  and  $0.011$ , respectively; Fig. 2B) but they accumulated significantly less Cs in total than NO<sub>3</sub><sup>-</sup>-grown plants (not shown,  $P=0.05$ ). In both *L. perenne* and *B. vulgaris*, NH<sub>4</sub><sup>+</sup>-grown plants had significantly greater uptake rates g<sup>-1</sup> root and, after growth in either NO<sub>3</sub><sup>-</sup> or NH<sub>4</sub><sup>+</sup>, *B. vulgaris* had a greater Cs uptake rate than *L. perenne* (Fig. 2C). Root concentrations, however, need to be interpreted in the knowledge that not all Cs will have been internalised. The root wash in cold 5 mM CsCl will have removed much <sup>137</sup>Cs from the outside of the

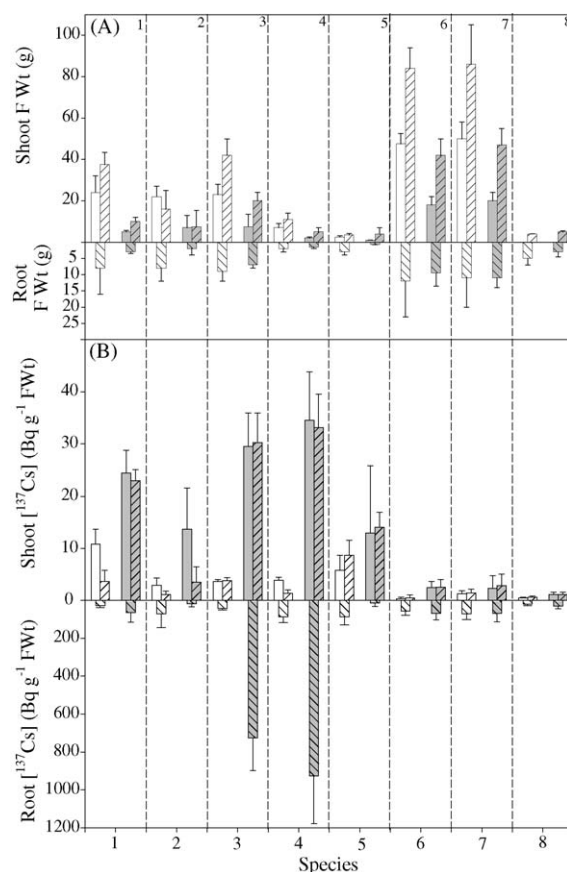


Fig. 3. Fresh weights (A) and <sup>137</sup>Cs concentrations (B) in eight Caryophyllid species grown for 42 days in nitrate and ammonium solutions based on Padgett and Leonard (1993) at 10 mM N (1, *C. album*; 2, *C. bonus-henricus*; 3, *A. cruentus*; 4, *A. giganteum*; 5, *P. fagopyrum*; 6, *R. rhaponticum*; 7, *C. mexicanum*; 8, *R. tataricum*). (Means ± S.E., n = 9. Above axis unshaded bar, stem; shaded bar, leaf. Below axis shaded bar, root. White bars, NO<sub>3</sub><sup>-</sup>-grown, grey bars, NH<sub>4</sub><sup>+</sup>-grown).

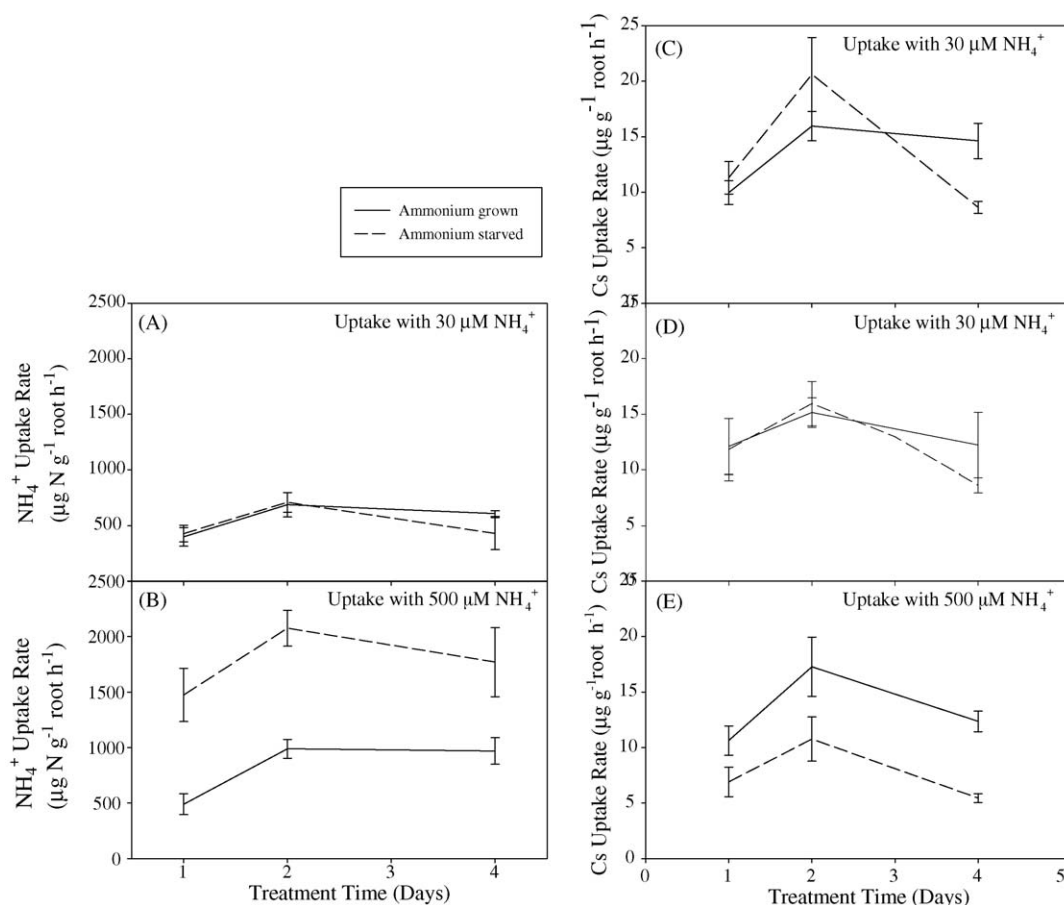


Fig. 4.  $\text{NH}_4^+$  uptake rates (A + B) and  $^{134}\text{Cs}$  uptake rates (C, D + E) of 43 days old  $\text{NH}_4^+$ -grown *L. perenne* plants starved of  $\text{NH}_4^+$  for 1, 2 and 4 days from solutions with 0 mM  $\text{NH}_4^+$  (C), 30  $\mu\text{M}$   $\text{NH}_4^+$  (A + D) and 500  $\mu\text{M}$   $\text{NH}_4^+$  (B + E) compared to control N-supplied plants. (Means  $\pm$  S.E.,  $n=9$ ).

root by swamping ion exchange sites with  $^{133}\text{Cs}$  whilst slowing physiological transfers in the root, but complete removal of  $^{137}\text{Cs}$  from the complex apoplastic cell wall matrix surrounding root cells is difficult to demonstrate and was not attempted.

Fig. 3A shows that there were significant differences in biomass production between plant taxa on the Caryophyllid clade and that all the species in experiment in Section 2.3 produced more biomass and smaller root:shoot ratios when grown on nitrate solutions ( $P < 0.05$ ). In four out of eight species there were significantly higher concentrations of Cs in roots, stems and/or leaves in  $\text{NH}_4^+$ -grown plants ( $P < 0.05$ ; Fig. 3B) and significantly greater shoot:root ratios (see Fig. 3B). Cs concentrations were higher in roots than in shoots in all species but, with the exception of nitrate grown *C. album*, there were no significant differences in Cs concentrations between leaves and stems in any of the species (Fig. 3B). In seven out of eight species the total amount of Cs removed to shoots by  $\text{NH}_4^+$ -grown plants was greater than by  $\text{NO}_3^-$ -grown plants (*C. album*, 395 Bq/352 Bq  $\text{NH}_4^+/\text{NO}_3^-$  ( $n=9$ ,  $P < 0.05$ ); *C. bonus-henricus*, 121 Bq/80 Bq ( $n=9$ ,  $P < 0.05$ ); *A. cruentus*, 824 Bq/239 Bq ( $n=9$ ,  $P < 0.05$ ); *A. giganteum*, 234 Bq/40 Bq ( $n=9$ ,  $P < 0.05$ ); *P. fagopyron*, 69 Bq/44 Bq; *R. rhaponticum*, 149 Bq/50 Bq ( $n=9$ ,  $P < 0.05$ );

*C. mexicanum*, 177 Bq/186 Bq; *R. tartaricum*, 54 Bq/23 Bq ( $n=9$ ,  $P < 0.05$ )).

*L. perenne* plants that have been starved of  $\text{NH}_4^+$  for 1, 2 or 4 days have increased uptake of  $\text{NH}_4^+$  from 500  $\mu\text{M}$  solutions (Fig. 4B) but not from 30  $\mu\text{M}$  solutions (Fig. 4A). The uptake rate of  $\text{NH}_4^+$  by  $\text{NH}_4^+$ -grown plants from 30  $\mu\text{M}$  (Fig. 4A) and 500  $\mu\text{M}$  (Fig. 4B) solutions was statistically indistinguishable. The  $^{134}\text{Cs}$  uptake rates of  $\text{NH}_4^+$ -grown *L. perenne* plants in treatment solutions with 0  $\mu\text{M}$  N, 30  $\mu\text{M}$  N or 500  $\mu\text{M}$  N were statistically indistinguishable (Fig. 4C–E).  $^{134}\text{Cs}$  uptake rates by  $\text{NH}_4^+$ -starved *L. perenne* plants in 0  $\mu\text{M}$  N or 30  $\mu\text{M}$  N were statistically indistinguishable from each other and from uptake by any  $\text{NH}_4^+$ -grown plants but  $^{134}\text{Cs}$  uptake by  $\text{NH}_4^+$ -grown plants from 500  $\mu\text{M}$  treatment solution was significantly lower than for any other plants ( $P < 0.05$ ; Fig. 4E). Uptake rates of  $^{134}\text{Cs}$  and  $\text{NH}_4^+$  were lower on Day 1 than on Days 2 and 4, but this effect was only statistically significant for  $\text{NH}_4^+$ -grown plants treated with 500  $\mu\text{M}$  solution. *L. perenne* plants treated with MSX removed significantly less  $\text{NH}_4^+$  from solution than did untreated plants (Fig. 5A). Cs concentrations in solutions with plants treated with MSX were significantly less than those with untreated plants (Fig. 5B).

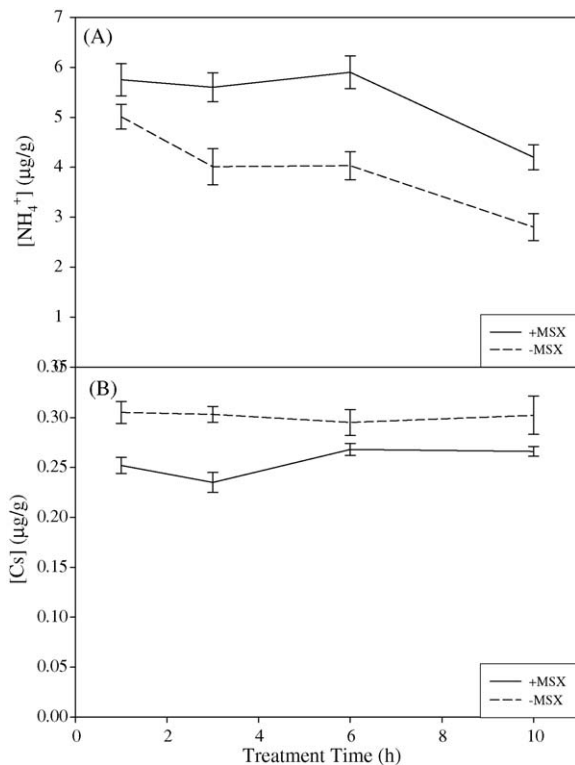


Fig. 5. Solution concentrations of  $\text{NH}_4^+$  (A) and  $^{133}\text{Cs}$  (B) after 1, 3, 6 and 10 days contact with 36 days old  $\text{NH}_4^+$ -grown *L. perenne* treated with MSX (methylsulphoxamine). (Means  $\pm$  S.E.,  $n=9$ ).

#### 4. Discussion and conclusions

It has long been known that both the N form fed to plants and the presence of  $\text{NH}_4^+$  in the solution surrounding plant roots can affect the uptake of Cs by plants. The experiments reported here were not designed as a single coherent set of experiments, and the details of their experimental conditions vary and make inter-comparisons difficult, but they do confirm that there can be some statistically significant effects of N-nutrition on Cs uptake by plants. Recent advances in understanding the N-nutrition of plants not only facilitated the design of many of the experiments outlined here but, together with the results in Figs. 1–5, also allow fresh interpretations of the effects of N nutrition on Cs uptake by plants.

The *H. vulgare* in experiment in Section 2.1 is in the same family (Poaceae) as the wheat used by Minotti et al. (1965) and Jackson et al. (1968) but there is no evidence in Fig. 1 that it had higher uptake of  $^{137}\text{Cs}$  when grown on  $\text{NH}_4^+$  rather than  $\text{NO}_3^-$ . The data from experiment in Section 2.2 which included measurements of root concentrations and calculations of Cs uptake rates per g root, shows that *L. perenne*, also in the Poaceae, did not have different root:shoot Cs ratios nor higher Cs concentrations in shoots and roots but did have higher Cs uptake rate when grown on  $\text{NH}_4^+$  rather than  $\text{NO}_3^-$  (Fig. 2B and C). Overall, Fig. 2 supports the assertion that  $^{137}\text{Cs}$  uptake rates can be affected by growth in  $\text{NH}_4^+$  in at least some members of the Poaceae, although because of the

different hydroponic and treatment conditions used, both in the experiments reported here and in previous experiments, the details of the phenomenon remain obscure.

Interestingly, when *H. vulgare* was grown with glycine as its sole nitrogen source (Fig. 1), it had biomass production and  $^{137}\text{Cs}$  uptake that was indistinguishable to those of  $\text{NO}_3^-$  or  $\text{NH}_4^+$ -grown plants. It is only relatively recently that it has become clear that many plants are capable of utilising simple organic sources of nitrogen, such as amino acids (Persson and Nasholm, 2001). It now seems, however, that there are probably a significant number of plants in soils with organic rooting zones, such as the podsol and histosols widely contaminated with  $^{137}\text{Cs}$  after the Chernobyl accident and by weapons test fall-out at high latitude, respectively, that use amino acids extensively. The Poaceae is a large family of plants (ca. 10,000 species), with important members in many  $^{137}\text{Cs}$  contaminated environments, that is often perceived as flexible in its utilisation of different nitrogen sources. Results in Figs. 1 and 2 are consistent with this and Fig. 1 shows, for the first time, that plants in the Poaceae grown with an amino acid as a sole nitrogen source have  $^{137}\text{Cs}$  uptake indistinguishable from plants grown on other sources. Species in the Asteraceae were shown to be similarly flexible in N source and Cs uptake.

There is evidence that plants in the Caryophyllid clade take Cs up, on average, to higher concentrations than plant taxa on other important clades (Broadley et al., 1999a,b). The Caryophyllid clade is distinguished by a number of morphological, genetic and physiological traits. Experiments in Sections 2.1–2.3 included 11 different taxa on the Caryophyllid clade, all of them closely related, and they all produced more biomass when grown on  $\text{NO}_3^-$ . However, although  $\text{NH}_4^+$  nutrition reduced Caryophyllid biomass production, it increased the Cs uptake rate  $\text{g}^{-1}$  root (Fig. 2C) and in some instances Cs concentration (Fig. 3B). These results indicate that, in attempts to maximise  $^{137}\text{Cs}$  extraction from soil, it might be interesting to investigate the effects of N nutrition on  $^{137}\text{Cs}$  uptake in Caryophyllids that prefer  $\text{NH}_4^+$  as an N source. In experiment in Section 2.3 we were expecting some species to prefer  $\text{NH}_4^+$  but perhaps Caryophyllid taxa that live in cold or waterlogged environments where  $\text{NH}_4^+$  dominates (Armstrong et al., 1994) need to be specifically targeted.

Experiments in Sections 2.1–2.3, used species in the Poaceae (which are numerous in many  $^{137}\text{Cs}$  contaminated environments and provide all cereal crops), and the Caryophyllidae and Asteraceae (which have previously been reported to have high Cs uptake and have been investigated for phytoremediation, e.g. Dushenkov et al., 1999; Willey et al., 2001; Tang et al., 2003). The results show, for some taxa for the first time, that the uptake rate and total Cs removal by these radioecologically important plant groups can, at least in hydroponics, be affected by N nutrition. In those instances in which it is unaffected, e.g. *H. vulgare*, the results demonstrate that  $^{137}\text{Cs}$  uptake occurs under a wider range of N sources than has previously been investigated. N uptake and metabolism has widespread effects on the physiology of

plants (Crawford et al., 2000), so it is perhaps unsurprising that N nutrition can affect Cs uptake. It is notable that in some instances the changes in Cs concentrations in plant parts are at least double (e.g. *C. album*, *C. bonus-henricus* and *A. cruentus* in Fig. 3B). So, although with none of the N forms was Cs uptake eliminated or hyperaccumulation induced, there were some quite significant changes in Cs activity concentrations and totals accumulated.

Since previous experiments on the effects of nitrogen regime on  $^{137}\text{Cs}$  uptake it has become clear that plants have specific  $\text{NH}_4^+$  transport proteins that are expressed in roots primarily in the presence of  $\text{NH}_4^+$ . In general, nutrient starvation leads to short-term increases in the expression of transport proteins. This phenomenon has provided the route to identifying many nutrient transporters because transporter-specific mRNA can be isolated from root cells during this transient period of increased gene expression. Fig. 4B certainly confirms that  $\text{NH}_4^+$ -grown *L. perenne* plants starved of  $\text{NH}_4^+$  for 1, 2 or 4 days have increased  $\text{NH}_4^+$  uptake rates from solutions with approximately soil solution concentrations of  $500\ \mu\text{M}\ \text{NH}_4^+$ . Although it is possible that the same transporters are taking up  $\text{NH}_4^+$  more quickly than in non-starved plants, the increases in uptake are most likely to have occurred because of increased expression of  $\text{NH}_4^+$  transport proteins in the roots, as has been observed with many other nutrient ions.  $^{134}\text{Cs}$  uptake from either 0 or  $30\ \mu\text{M}$  solutions was unaffected by  $\text{NH}_4^+$  starvation but, interestingly,  $\text{NH}_4^+$ -starvation decreased  $^{134}\text{Cs}$  uptake from  $500\ \mu\text{M}$  solutions, i.e. in instances where actual  $\text{NH}_4^+$  uptake was increased,  $^{134}\text{Cs}$  uptake decreased. It is, perhaps, notable that the phenomenon of  $\text{NH}_4^+$ -grown plants having increased Cs uptake might only apply from solutions in which  $\text{NH}_4^+$  is absent, as it was in all treatment solutions, including those used here in experiments in Sections 2.1–2.3, from which increased Cs uptake by  $\text{NH}_4^+$ -grown plants has been reported. Fig. 5 confirms this inverse relationship—decreases in  $\text{NH}_4^+$  uptake induced by exposure to MSX increased  $^{134}\text{Cs}$  uptake. MSX decreases the assimilation of  $\text{NH}_3$  in plants (Fentem et al., 1983a), increasing cytoplasmic  $\text{NH}_4^+$  concentrations and decreasing  $\text{NH}_4^+$  uptake rates (Fentem et al., 1983b; Britto et al., 2001). Only a molecular biological dissection of this phenomenon could describe its mechanism but  $\text{NH}_4^+$  uptake can certainly affect Cs uptake. The results in Figs. 4 and 5 are consistent with a link between  $\text{NH}_4^+$  and Cs uptake that is more complex than competition for ion transport sites, and perhaps involves both influx and efflux. Usefully, a number of *A. thaliana* N transport mutants exist that might provide enlightening results if fed with Cs (Kaiser et al., 2002).

The results reported here indicate plant N ecophysiology might affect Cs transfer from soils to plants. There has been much recent progress in understanding the pedological controls on N forms in soils, primarily driven by concern about emissions of nitrogenous greenhouse gases, and manipulations of soil N forms is becoming increasingly refined (Zerulle et al., 2001). The results reported here indicate that this knowledge might be useful in understanding,

managing and manipulating Cs transfer from soils to plants. Overall, however, given the central importance of N nutrition to whole plant physiology, its effects on Cs uptake by plants, though statistically significant, seem most likely to be radioecologically important in soil–plant systems of contrasting N-ecophysiology. Given that some variability in soil-to-plant transfer might be caused by differences in N nutrition, researchers interested in managing soil-to-plant transfer of  $^{137}\text{Cs}$  to minimise ecosystem contamination or manipulating uptake rates for phytoremediation, might consider links between soil N form and plant uptake of  $^{137}\text{Cs}$ . For example, given current knowledge, we hypothesise that plants on the Caryophyllid clade with a preference for  $\text{NH}_4^+$  as an N source will have the highest  $^{137}\text{Cs}$  uptake of any plants. Much recent research into N in the soil–plant system, including the availability of plant N transport mutants, the variety of mechanisms for manipulating soil N forms, and the existence of metabolic inhibitors of N-metabolism might aid the testing of such hypotheses and hence the management and manipulation of Cs transfer from soils to plants.

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