

# Interspecific competition between a non-native metal-hyperaccumulating plant (*Noccaea caerulescens*, Brassicaceae) and a native congener across a soil-metal gradient

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**Abstract.** Adaptive traits are hypothesised to incur fitness trade-offs, and a classical example is metal-tolerant plants that exhibit reduced competitive ability when grown on low-metal substrates. In the present study, we examined whether metal-hyperaccumulating plants exhibit a similar trade-off, by assessing competition across a soil-metal gradient in the context of phytoremediation. We studied the cadmium- and zinc-hyperaccumulator *Noccaea caerulescens* (J. Presl & C. Presl) F.K. Mey., which has been introduced to potential remediation sites contaminated with those metals, and the nickel- and zinc-hyperaccumulator *Noccaea fendleri* subsp. *glauca* (A. Nelson) Al-Shehbaz & M. Koch, which is native to our study sites. We performed a greenhouse experiment with a response-surface design to quantify their competitive interactions on higher- and lower-metal substrates from each of three mine sites. Overall competitive effects between the species were weak, but we did find evidence of competition on the substrates that supported the highest rates of plant growth and reproduction. Abiotic factors were more limiting than competitive interactions for both species, and both performed better on substrates containing higher zinc, lower cadmium and lower copper concentrations. A complementary field trial supported these findings. Our results also showed that substrates outside of contaminated mine tailings can still contain sufficiently high zinc concentrations to support *N. caerulescens*, suggesting that the use of these plants for phytoremediation should be monitored to prevent the unintentional establishment of non-native species.

**Additional keywords:** fitness trade-off, metal hyperaccumulators, non-native species, phytoextraction.

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## Introduction

Adaptive traits are hypothesised to incur fitness trade-offs by diverting limited resources away from other traits that also contribute to survival, reproduction or growth (Stearns 1989; Agrawal *et al.* 2010). Adaptation to soils with high concentrations of heavy metals has been shown to incur such trade-offs, and these physiological costs may explain why metal-tolerant plants are rarely found on non-metalliferous soils (Kruckeberg 1954; Baker 1987; Kazakou *et al.* 2008). Plants that are tolerant of high-metal soils have slower growth rates than do non-tolerant plants (Cox and Hutchinson 1981; Wilson 1988), exhibit reduced competitive ability against non-tolerant plants (Cook *et al.* 1972; Hickey and McNeilly 1975; also see Anacker 2014), and may be more susceptible to herbivory (Lau *et al.* 2008) when grown on low-metal soils. Interestingly, studies using independent genetic lines of the copper-tolerant *Mimulus guttatus* Fischer ex DC. did not find evidence of slower growth (Harper *et al.* 1997b) or micronutrient deficiency (Harper *et al.* 1997a; Harper *et al.* 1998) in the plants selected for high metal tolerance. This suggested that

adaptation to other environmental factors at the high-metal sites, and not the high metal concentration itself, may have caused the previously observed trade-offs. However, the earlier findings may still be interpreted as being trade-offs that result from adaptation to high-metal soils through metal tolerance.

In contrast to metal-tolerant plants, whether metal-hyperaccumulating species exhibit fitness trade-offs has not been as thoroughly examined (El Mehdawi and Pilon-Smits 2012; Boyd 2013). Metal accumulation and tolerance are independent genetic traits (Pollard *et al.* 2002; Assunção *et al.* 2003b; Macnair 2007) and may not incur the same trade-offs. Thus far, studies of the ecological trade-offs of hyperaccumulation have focussed on the growth of these plants across metal gradients. One study found no evidence of reduced growth or reproduction when plants from metalliferous populations of the cadmium (Cd)- and zinc (Zn)-hyperaccumulating plant *Noccaea caerulescens* (J. Presl & C. Presl) F.K. Mey. were grown in low-Zn soil compared with high-Zn soil (Dechamps *et al.* 2007). Another study found that metalliferous populations of *N. caerulescens* had

lower reproductive fitness than did non-metalliferous populations on low-metal soils under field conditions because of increased herbivory (Dechamps *et al.* 2008). Whether the competitiveness of hyperaccumulators differs between high- and low-metal soils has not been tested, even though reduced competitive ability has been suggested as a mechanism for limiting the distribution of metal hyperaccumulators (El Mehdawi and Pilon-Smits 2012).

The competitive ability of metal-hyperaccumulating plants has important implications for phytoremediation, or the use of plants to remove, contain or degrade contaminants from soils (Chaney 1983; Arthur *et al.* 2005; Pilon-Smits 2005; Pilon-Smits and Freeman 2006). One form of phytoremediation is phytoextraction, in which a metal-hyperaccumulating species is planted and subsequently harvested to remove contaminants from a substrate (Lasat 2002). In most cases, this requires deliberately introducing a non-native hyperaccumulator because no native species is able to hyperaccumulate the specific contaminants, leading to the potential risk of non-native establishment and spread. An important biotic factor determining whether an introduced plant may become invasive is the degree of competitive interactions between it and native species at the site of introduction (Crawley 1990; Alpert *et al.* 2000; Vilà and Weiner 2004). If metal-hyperaccumulating plants have weak competitive strength on non-contaminated soils, they may not pose a significant threat of becoming invasive.

In the present study, we assessed the competitive interactions of the hyperaccumulator *Noccaea caerulescens* in the context of phytoremediation. *Noccaea caerulescens* has been shown in field trials to extract Cd and Zn successfully from moderately contaminated soils (Brown *et al.* 1995; Hammer and Keller 2003; McGrath 2006). We chose three sites in south-western Colorado containing Cd- and Zn-contaminated silver-mine tailings similar to substrates where phytoremediation by *N. caerulescens* may be implemented. The congener *Noccaea fendleri* (A. Nelson) Al-Shehbaz & M. Koch is native to our study region and can be found at our study sites growing adjacent to the mine tailings. Although it has not colonised the high-metal tailings at these sites, *N. fendleri* is also a metal-tolerant species and a known Ni- and Zn-hyperaccumulator on serpentine soils (Reeves *et al.* 1983; Boyd and Martens 1998). We chose to examine the potential competitive interactions between the two congeners because competition is hypothesised to be most intense among closely related species because of their similar ecological requirements (Rejmánek 1996; Burns and Strauss 2011; but see Thuiller *et al.* 2010). Comparing the congeners also controlled for phylogenetic history and growth form, which are both factors that can affect the outcome of competition and the likelihood of invasive success (Vilà and Weiner 2004; Garcia-Serrano *et al.* 2007; van Kleunen *et al.* 2010).

To test for competitive interactions between the native *N. fendleri* and non-native *N. caerulescens*, we performed a greenhouse experiment with a response-surface design, so as to examine independently the effects of intra- and inter-specific competition on plant performance (Damgaard 1998; Inouye 2001). At each of the three sites, we focussed on two locations where native *N. fendleri* occurred: at the edge of the mine tailings (higher-metal soil), and 25 m away from the tailings (lower-metal soil) where native vegetation cover did not appear affected by the contaminated tailings. We examined (1) whether *N. caerulescens*

and *N. fendleri* performed better (i.e. had higher growth and reproduction) on higher-metal soil than on lower-metal soil, because both species were metal-tolerant and should exhibit reduced fitness on lower-metal soil, and (2) whether competitive interactions between the species were stronger on higher-metal than on lower-metal soils. In addition to the greenhouse study, a small-scale complementary field experiment was conducted at one of our three study sites. Findings from this experiment can improve our understanding of the ecological interactions of metal-hyperaccumulating plants, and also provide an assessment of the potential ecological impacts of phytoremediation.

## Materials and methods

### Study site and species

We chose three small abandoned mine sites (Woods: 39°1'46.39"N, -107°4'0.31"W; Copper: 39°1'24.19"N, -107°4'5.10"W; and Cinnamon: 38°59'52.23"N, -107°3'38.84"W) near the Rocky Mountain Biological Laboratory in the central Rocky Mountains, Colorado. Mine tailings at these sites contain moderately high concentrations of Cd ( $18.7 \pm 5.24 \mu\text{g g}^{-1}$ ) and Zn ( $2860 \pm 636 \mu\text{g g}^{-1}$ ) (Che-Castaldo and Inouye 2014), which is similar to sites where phytoremediation by *N. caerulescens* may be implemented. At each site, we collected substrate from two locations where native *N. fendleri* naturally occurs, namely, at the edge of the tailings (higher-metal soil) and 25 m away from the tailings (lower-metal soil). We sampled substrate to ~30-cm depth at three spots per location per site, and mixed and sieved these samples before use. We analysed substrate pH in a 1 : 2 soil to water (by volume) slurry after 1 h of equilibration, and total metal concentrations of relevant metals by using Aqua regia extraction (McGrath and Cunliffe 1985) and flame atomic absorption spectrometry (AAS; Table 1). We also analysed concentrations of plant-available metals by using strontium nitrate extraction and AAS, a method that has been used successfully to predict phytoavailability of Cd, Zn and nickel (Ni) (Li *et al.* 2000; Siebielec *et al.* 2000; Table 1).

*Noccaea caerulescens* (formerly *Thlaspi caerulescens* J. & C. Presl; Brassicaceae; alpine pennycress) is native to central and western Europe and is a model species for studying metal hyperaccumulation (Assunção *et al.* 2003a). It is a constitutive hyperaccumulator of Zn (Escarré *et al.* 2000), and requires Zn for optimal growth (Mathys 1977; Li *et al.* 1995; Shen *et al.* 1997). Populations differ in their ability to hyperaccumulate Cd (Chaney *et al.* 2005), which is not an essential nutrient, but has been shown to stimulate growth in one ecotype of *N. caerulescens* from southern France (Pongrac *et al.* 2009). Cadmium hyperaccumulation is defined as  $>100 \mu\text{g g}^{-1}$  of plant dry mass, and Zn hyperaccumulation as  $>10\,000 \mu\text{g g}^{-1}$  (Reeves 1988), although, more recently,  $3000 \mu\text{g g}^{-1}$  has been proposed as a less conservative threshold for Zn (van der Ent *et al.* 2013). *Noccaea fendleri* subsp. *glauca* (formerly *Thlaspi montanum* var. *montanum*) is a long-lived perennial, native to the mountains of the western USA (Holmgren 1971). It is a known Ni hyperaccumulator on serpentine soils and has been shown to accumulate Zn at concentrations up to  $3000 \mu\text{g g}^{-1}$  leaf dry mass (Reeves *et al.* 1983); therefore, it is also a Zn hyperaccumulator, depending on the definition used; however, it does not accumulate Cd.

**Table 1. Mean  $\pm$ s.d. values (in  $\mu\text{g g}^{-1}$ , except for pH) for soil pH, plant-available (Av) and total concentrations of zinc (Zn), cadmium (Cd), copper (Cu) and nickel (Ni) for three study sites (Cinnamon, Copper, Woods)**

Site	Location	pH	Field trial, soil from the field experiment; higher, higher-metal soils collected adjacent to tailings; lower, lower-metal soils collected 25 m away from tailings			Field trial, soil from the field experiment; higher, higher-metal soils collected adjacent to tailings; lower, lower-metal soils collected 25 m away from tailings				
			Av Zn	Zn	Av Cd	Cd	Av Cu	Cu	Av Ni	Ni
Cinnamon	Higher	4.67 $\pm$ 0.04	6.19 $\pm$ 4.65	451.0 $\pm$ 7.22	6.19 $\pm$ 4.650	1.45 $\pm$ 0.104	0.070 $\pm$ 0.090	92.3 $\pm$ 1.12	0.055 $\pm$ 0.031	6.41 $\pm$ 0.229
Cinnamon	Lower	4.39 <sup>A</sup>	1.24 $\pm$ 0.94	167.0 $\pm$ 5.66	1.24 $\pm$ 0.940	0.403 $\pm$ 0.026	0.010 $\pm$ 0.009	37.3 $\pm$ 1.62	0.080 $\pm$ 0.023	6.17 $\pm$ 0.202
Copper	Higher	4.74 $\pm$ 0.02	7.58 $\pm$ 8.53	667.0 $\pm$ 36.20	7.58 $\pm$ 8.530	2.96 $\pm$ 0.033	1.190 $\pm$ 0.874	1320.0 $\pm$ 43.5	0.107 $\pm$ 0.061	7.40 $\pm$ 0.065
Copper	Lower	4.81 $\pm$ 0.00	2.08 $\pm$ 1.02	604.0 $\pm$ 63.60	2.08 $\pm$ 1.020	1.13 $\pm$ 0.133	0.038 $\pm$ 0.024	63.5 $\pm$ 3.02	0.025 $\pm$ 0.000	8.01 $\pm$ 0.371
Woods	Higher	5.35 $\pm$ 0.06	2.38 $\pm$ 0.95	745.0 $\pm$ 13.50	2.38 $\pm$ 0.949	2.83 $\pm$ 0.051	0.441 $\pm$ 0.151	1950.0 $\pm$ 26.20	0.025 $\pm$ 0.000	6.25 $\pm$ 0.089
Woods	Lower	4.66 $\pm$ 0.03	2.45 $\pm$ 1.27	348.0 $\pm$ 7.79	2.45 $\pm$ 1.270	0.915 $\pm$ 0.027	0.018 $\pm$ 0.010	33.8 $\pm$ 1.26	0.033 $\pm$ 0.015	6.02 $\pm$ 0.189
Woods – field trial	Higher	5.88 $\pm$ 0.49	2.44 $\pm$ 0.71	764.0 $\pm$ 38.90	0.037 $\pm$ 0.019	2.67 $\pm$ 0.153	0.487 $\pm$ 0.127	2000.0 $\pm$ 62.6	0.025 $\pm$ 0.000	6.72 $\pm$ 0.413
Woods – field trial	Lower	5.06 $\pm$ 0.32	2.82 $\pm$ 0.35	325.0 $\pm$ 20.20	0.032 $\pm$ 0.012	0.881 $\pm$ 0.226	0.020 $\pm$ 0.006	30.6 $\pm$ 2.60	0.025 $\pm$ 0.000	6.57 $\pm$ 0.337

<sup>A</sup>Value without s.d. indicates that the measurement was only taken from one sample.

### Greenhouse experiment

We conducted the competition experiment from September 2007 to December 2008 in a research greenhouse at the University of Maryland, College Park, MD, USA. The greenhouse allowed for greater control of ambient temperature and soil moisture, higher replication and a longer continuous growing period than in the field. We collected *N. fendleri* seeds haphazardly from at least 15 fruiting plants per location at our study sites in 2007. We obtained *N. caerulescens* seeds at Palmerton, PA, a former field-trial location (Li *et al.* 1997). These seeds contained a mix of ecotypes commonly used in hyperaccumulation studies, including the southern France and Prayon populations (Chaney *et al.* 2005). We stratified *N. fendleri* seeds at 3°C for 3 months, then germinated these and *N. caerulescens* seeds on potting soil. We recorded germination dates for each individual, and matched seedlings of the same age in each pot to avoid size and age bias.

We transplanted seedlings into 10.16-cm square pots containing field substrate (weighing 425.2–544.3 g for the same volume, depending on substrate source). We implemented a response-surface design in which the total density and proportion of the species were varied independently, so that the effects of inter- and intra-specific competition could be examined separately (Damgaard 1998). Each pot contained 0, 1, 2 or 3 plants of each species, for a total of nine competition treatments, with the ratio of *N. fendleri* to *N. caerulescens* being as follows: 1 : 0, 0 : 1; 2 : 0, 1 : 1, 0 : 2; 3 : 0, 2 : 1, 1 : 2 or 0 : 3. These densities were within the range of observed densities of *N. fendleri* at our field sites. These treatments were repeated at every level of the three sites and two locations for 54 treatment combinations. We replicated each treatment combination four to six times, depending on the availability of germinated seedlings and field substrate from each location, for a total of 267 pots and 597 plants. We checked plants daily and watered them as needed with deionised water. After 20 weeks, we vernalised all pots at 4°C for 12 weeks, so as to simulate field conditions and induce flowering. We measured the initial size, growth (length of longest leaf and number of leaves) and reproductive effort (number of buds, flowers, stalks, and mature fruits per stalk) of the plants weekly. Because *N. fendleri* plants did not set seed in the greenhouse, likely because of self-incompatibility (Peer *et al.* 2006), we used flower number as our measure of reproductive effort. We collected, washed and dried the plant-shoot biomass after reproduction, and analysed the nutrient and metal concentrations (calcium (Ca), Cd, copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), Ni, phosphorus (P), Zn) using hot nitric digestion and inductively coupled plasma atomic emission spectroscopy.

### Competition coefficient estimates

We estimated competition coefficients for each species by using Damgaard's (1998) hyperbolic competition model, as follows:

$$y_i = D_i(a_i + b_i(D_i + c_{ij}D_j + e_iD_iD_j)^{d_i})^{-1/f_i}$$

where  $y_i$  is the yield of plant Species  $i$ ,  $D_i$  is the density (in plants  $\text{cm}^{-2}$ ) of Species  $i$ ,  $D_j$  is the density of the competing Species  $j$ , and  $a_i$ ,  $b_i$ ,  $d_i$ , and  $f_i$  are shape parameters. The parameter  $e_i$  represents the interaction effect between the densities of the two species. We used maximum leaf number as the measure of yield for both species. The competition coefficient of Species  $j$

on Species  $i$  ( $c_{ij}$ ) indicates the relative effects of intra- and inter-specific competition on the yield of Species  $i$ . If  $c_{ij} > 1$ , then interspecific competition from Species  $j$  is greater than intraspecific competition in Species  $i$ , and Species  $j$  may outcompete Species  $i$ . If  $0 < c_{ij} < 1$ , then interspecific competition is present, but has a smaller effect than does intraspecific competition. If  $c_{ij} < 0$ , then interspecific competition is not present, and the presence of the other species actually increases the fitness of Species  $i$ . We also fitted a simplified competition model, which assumed that the shape Parameters  $d_i$  and  $f_i$  equal 1 (Campbell and Snow 2007). We calculated maximum-likelihood parameter estimates by using the normal, negative binomial, and Poisson residual distribution, using the `mle2` function in the `bbmle` package in R (Bolker 2008; R Development Core Team 2012). For both species, the simplified model with a negative binomial error distribution, and including site and location effects, had the best fit to the data on the basis of dAIC values (Table S1, available as Supplementary material for this paper), and we present results from this model. We tested whether each competition coefficient estimate was significantly different from zero or from one, by using one-sample Student's  $t$ -tests.

#### Plant growth and reproduction

To test for differences in plant performance between species across sites, locations and density treatments, we first performed a factor analysis on the growth variables (maximum number of leaves, length of longest leaf, and number of rosettes recorded for each plant) to create a composite response variable. The first resulting factor was significant and positively related to all three original variables (Table S2, available as Supplementary material for this paper), and we used it as the response in an ANOVA, to test the effects of species, sites, locations, density treatments, and their interactions. We examined reproduction in a separate ANOVA, using square-root-transformed flower number as the response. We included overall density rather than density of each species as an explanatory variable in the analysis because interspecific effects from the competition analysis were small (see Results). To account for effects of initial plant size, we included the first factor from a factor analysis of the initial leaf length, leaf number and seedling age as a covariate in these models.

To examine whether soil characteristics affected plant performance, we performed canonical correlations analyses using the R package CCA (González *et al.* 2008), so as to quantify correlations between the set of soil metal concentrations and pH to the set of plant growth-response variables (maximum number of leaves, length of longest leaf and number of rosettes) and also to a set of reproductive variables (number of buds, number of flowers). Because we expected these relations to differ among plant species, we performed separate analyses for each species.

#### Field experiment

In the summer of 2007, we germinated *N. caerulescens* seeds on flats of potting soil in field conditions. Low germination rates allowed us to perform this field trial at only one of three study sites (Woods). We planted paired plots at the same higher- and

lower-metal locations as where substrate was collected for the greenhouse experiment. At each location and in each of three replicates, we planted 15 germinated seedlings interspersed with 15 similarly sized *N. fendleri* seedlings transplanted from the field into  $0.5 \times 0.5$ -m half-plots. Paired half-plots were each planted with a monoculture of 30 *N. fendleri* seedlings to examine its growth in the absence of the non-native species. We measured the number of leaves, length of the longest leaf, and any reproductive effort (number of flowering stalks, buds, flowers and fruits) weekly over two growing seasons.

We performed a factor analysis of these measurements, and the resulting two significant factors were used as response variables in analyses. The first factor was positively related to all original variables, and the second was positively related only to the vegetative growth variables (Table S3, available as Supplementary material for this paper). We analysed these data in two ways. The first ANOVA used only native *N. fendleri* data to test whether location and presence of *N. caerulescens* affected plant performance. The second ANOVA used only data from mixed subplots to test the effects of location and species. Initial leaf number and leaf length were included as covariates. We performed all analyses, except the hyperbolic competition model and canonical correlation analyses, by using SAS software (ver. 9.2, SAS Institute Inc., Cary, NC, USA).

## Results

### Competition-coefficient estimates

In all but two cases, estimates of competition coefficient for the two species did not differ significantly from zero or one, indicating that effects of interspecific competition were absent or not different from the effects of intraspecific competition (Table 2). The two exceptions were both on the lower-metal soils. First, native *N. fendleri* exhibited a competitive effect but it was equal to that of intraspecific competition among non-native *N. caerulescens* plants on the lower-metal soil from Woods site. Second, *N. caerulescens* exhibited a significant competitive effect that was greater than intraspecific competition among *N. fendleri* plants on the lower-metal soil from Copper site (Table 2).

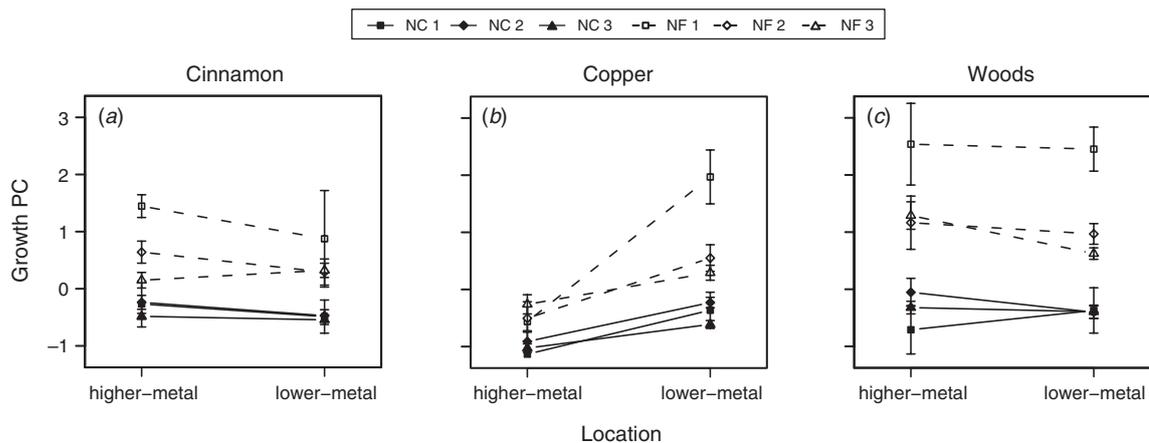
### Plant growth and reproduction

Overall, native *N. fendleri* had greater vegetative growth than did *N. caerulescens*, whereas *N. caerulescens* produced more flowers than did *N. fendleri* (Figs 1, 2). Site was a significant factor in the analysis of both growth and flower number; thus, we then analysed each site separately (Table 3). The effect of location (i.e. higher- vs lower-metal soil) on plant performance differed between sites but was similar between species. The expected pattern of better plant performance on higher-metal soil was found only on soils from Cinnamon site; both species had better growth (significant effect of location on growth; Table 3, Fig. 1a) and *N. caerulescens* also had higher reproduction (significant location  $\times$  species effect on flower number; Table 3, Fig. 1c) on higher-metal than on lower-metal soil. For Woods site, there was no difference in growth between locations, but *N. caerulescens* in the lowest-density treatment actually produced more flowers on the lower-metal soil (significant location  $\times$  species  $\times$  density effect on flower

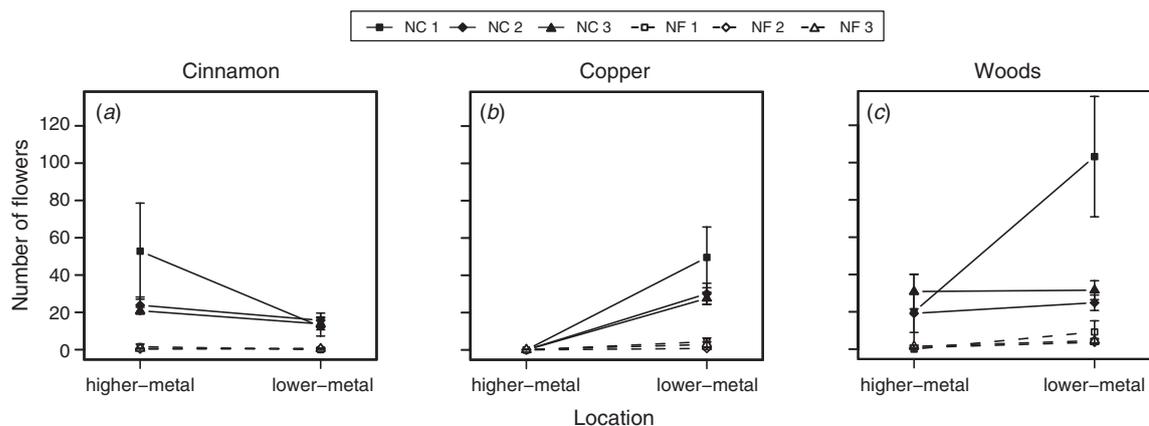
**Table 2. Estimates of competition coefficient, quantifying the competitive effects of native *Noccaea fendleri* on non-native *N. caerulea* (left columns) and effects of *N. caerulea* on *N. fendleri* (right columns) when grown on substrate from three sites (Cinnamon, Copper, Woods) and at two locations (higher- and lower-metal soils) per site**

Significant effects of interspecific competition were observed only on the lower-metal soils from Copper and Woods sites. Values presented are the maximum-likelihood estimates from the best-fitting model, standard error of the estimates, and the calculated one-sample *t*-values testing whether estimates differed from zero or from one. For all tests, the critical *t*-value ( $n = 30$ ,  $P = 0.05$ ) is 2.045, and an asterisk indicates a significant *t*-value. Competition coefficients that are significantly greater than zero indicate the presence of interspecific effects, and coefficients significantly greater than one indicate an effect of interspecific competition that is greater than intraspecific competition

Site	Location	NF (native) on NC				NC (non-native) on NF			
		Estimate	s.e.	<i>t</i> -value (from 0)	<i>t</i> -value (from 1)	Estimate	s.e.	<i>t</i> -value (from 0)	<i>t</i> -value (from 1)
Cinnamon	Higher	0.326	0.437	0.747	1.542	2.253	1.303	1.729	0.962
Cinnamon	Lower	4.073	25.481	0.160	0.121	0.837	1.419	0.590	0.115
Copper	Higher	230.39	365.350	0.631	0.628	-11.585	54.454	0.213	0.231
Copper	Lower	0.421	0.440	0.958	1.315	5.906	2.336	2.528*	2.100*
Woods	Higher	2.572	2.970	0.866	0.529	0.277	1.798	0.154	0.402
Woods	Lower	1.896	0.802	2.363*	1.117	1.174	0.626	1.875	0.278



**Fig. 1.** Growth in the greenhouse experiment, shown by location (higher-metal and lower-metal soils), plant species (non-native *Noccaea caerulea* (NC) and native *N. fendleri* (NF)), and total plant density (1, 2, and 3 plants per pot) for sites (a) Cinnamon, (b) Copper and (c) Woods. Data shown are the values for the first principal component from a factor analysis of the growth-response variables (maximum number of leaves, length of longest leaf, and number of rosettes recorded for each plant). Overall, *N. fendleri* had better vegetative growth than did *N. caerulea*, and both species grew as well or better on the lower-metal than on higher-metal soil, contrary to expectations.



**Fig. 2.** Reproduction (measured as flower number) in the greenhouse experiment, shown by location (higher-metal and lower-metal soils), plant species (non-native *Noccaea caerulea* (NC) and native *N. fendleri* (NF)) and total plant density (1, 2, and 3 plants per pot) for sites (a) Cinnamon, (b) Copper and (c) Woods. Overall, *N. caerulea* produced more flowers than *N. fendleri*, and the expected pattern of higher reproduction on higher-metal soil was observed only at the Cinnamon site.

**Table 3.** ANOVA results from the greenhouse experiment examining effects of location (higher and lower-metal soils), plant species (non-native *Noccaea caerulescens* and native *N. fendleri*), density (1, 2, and 3 plants per pot), and the covariate of initial plant size on plant performance at three sites (Cinnamon, Copper, and Woods)

\*\*\* $P < 0.001$ , \*\* $P < 0.01$ , \* $P < 0.05$ , + $P < 0.1$

Effect	Cinnamon			Copper			Woods		
	NDF	DDF	F	NDF	DDF	F	NDF	DDF	F
Results for composite growth response									
Location	1	197	10.40**	1	146	81.12***	1	160	0.24
Species	1	197	31.88***	1	146	40.62***	1	160	77.00***
Location × species	1	197	0.80	1	146	0.42	1	160	5.40*
Density	2	197	8.33**	2	146	4.72*	2	160	8.95**
Location × density	2	197	2.98 <sup>+</sup>	2	146	5.72**	2	160	1.28
Species × density	2	197	1.22	2	146	0.78	2	160	6.62**
Location × species × density	2	197	0.84	2	146	3.65*	2	160	0.51
Covariate	1	197	1.56	1	146	3.35 <sup>+</sup>	1	160	5.35*
Results for flower number									
Location	1	197	16.98***	1	146	112.1***	1	160	22.37***
Species	1	197	149.2***	1	146	56.92***	1	160	66.06***
Location × species	1	197	10.51**	1	146	63.65***	1	160	4.35*
Density	2	197	1.16	2	146	2.71 <sup>+</sup>	2	160	3.60*
Location × density	2	197	2.08	2	146	2.59 <sup>+</sup>	2	160	5.70**
Species × density	2	197	0.86	2	146	0.27	2	160	2.11
Location × species × density	2	197	0.78	2	146	0.12	2	160	3.05*
Covariate	1	197	0.00	1	146	0.00	1	160	0.09

number; Table 3, Fig. 2c). For Copper site, both species performed very poorly on the higher-metal soil, but had better growth on the lower-metal soil, with especially high growth occurring for *N. fendleri* in the lowest-density treatment (significant location × species × density effect on growth; Table 3, Fig. 1b). *Noccaea caerulescens* also produced more flowers on the lower-metal soil than on higher-metal soil from Copper site (significant location × species effect on flower number; Table 3, Fig. 2b). Density treatment had a significant effect on plant growth for all three sites (Table 3), being likely driven by *N. fendleri*, which tended to grow better in pots with one plant than with two or three plants (Fig. 1). Density effects on reproduction were significant only for Woods site (significant location × species × density effect on flower number; Table 3), where *N. caerulescens* in the lowest-density treatment and on the lower-metal soil tended to produce many flowers (Fig. 2c).

We used canonical correlation analysis to quantify how soil characteristics affected plant growth and reproduction (Table 4). For both species, higher vegetative growth was correlated with higher concentrations of soil Zn and lower concentrations of soil Cd on the basis of the standardised canonical correlation coefficients (bold values in Table 4). For *N. fendleri*, the first significant variate indicated that shorter leaf length (−0.644) and fewer rosettes (−0.500) were most correlated with lower total Zn (−1.969), higher plant-available Cd (1.126) and higher total Ni (1.046). The second significant variate indicated that longer leaf length (1.982) but fewer rosettes (−1.984) were most correlated with higher available Cd (3.490), higher total Cu (1.737), lower available Zn (−2.088) and lower total Zn (−1.362). For the non-native *N. caerulescens*, only the first canonical variate was statistically significant, and it showed that having more leaves (0.860) was most correlated with lower available Cd (−1.588), lower total Cd (−1.479), higher total Zn (1.408) and higher available Zn (1.269).

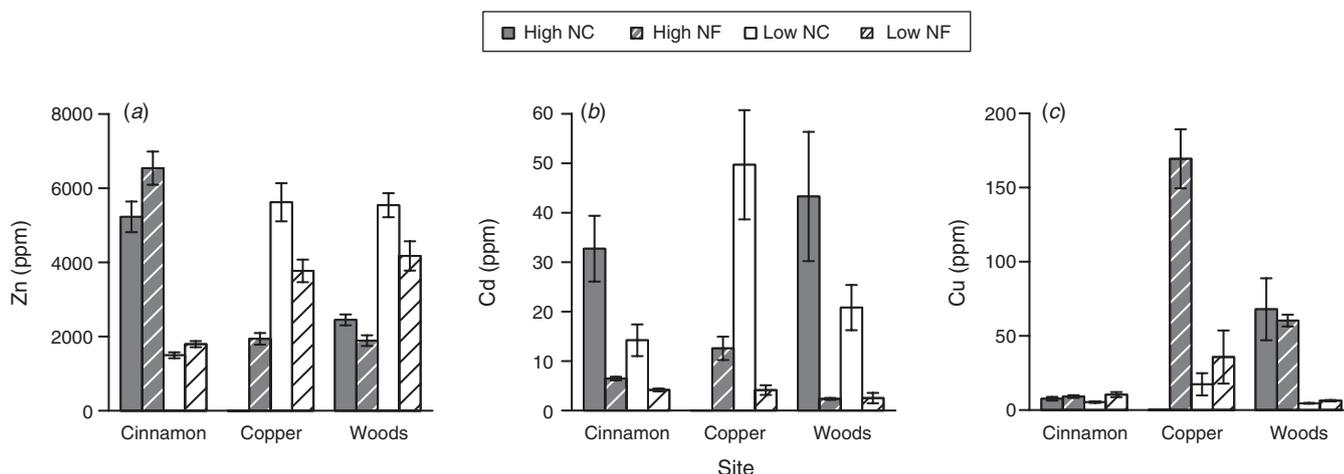
The relationship between soil variables and reproduction was also similar for the two species, with better reproduction being correlated with higher concentrations of soil Zn and lower concentrations of soil Cd and Cu (Table 4). For native *N. fendleri*, the significant variate indicated that fewer buds (−0.845) were most correlated with higher available Cd (2.927), higher total Cu (1.957), lower available Zn (−1.521) and lower total Zn (−1.489). For *N. caerulescens*, the significant variate indicated that fewer flowers (−0.708) were also most correlated with higher available Cd (4.303), higher total Cu (2.667), lower available Zn (−2.209) and lower total Zn (−2.158).

#### Plant metal uptake

Because of low survival on the higher-metal soil from Copper site, the biomass of *N. caerulescens* for that location was too small to allow analysis of metal accumulation. On the basis of biomass that was analysed, Zn accumulation was higher on the higher-metal soil from one site (Cinnamon) but higher on the lower-metal soil from the other two sites (site × location interaction,  $F_{2,93} = 33.55$ ,  $P < 0.0001$ , Fig. 3a). As expected, *N. caerulescens* accumulated more Cd than did *N. fendleri*, although the amount of Cd accumulated differed by site (site × species interaction,  $F_{2,93} = 4.38$ ,  $P = 0.015$ ; Fig. 3b). Plant Cu was elevated on the higher-metal soils from Copper and Woods sites (site × location interaction,  $F_{2,93} = 9.34$ ,  $P = 0.0002$ ; Fig. 3c). Density treatments did not affect plant metal concentrations. *Noccaea fendleri* did not hyperaccumulate Ni at these sites, but *N. caerulescens* did hyperaccumulate Cd in some replicates; plants in the 1:2 treatment on the lower-metal soil from Copper site had on average (mean ± s.e.)  $123.0 \pm 19.2 \mu\text{g g}^{-1}$  Cd. No plants exhibited Zn hyperaccumulation at  $>10\,000 \mu\text{g g}^{-1}$ , but both species accumulated to  $>3000 \mu\text{g g}^{-1}$  at locations where the plants grew well (i.e. higher-metal soil from Cinnamon and

**Table 4.** Significant canonical variates from the canonical correlation analyses examining how soil metal concentrations (total and plant-available concentrations of zinc (Zn), cadmium (Cd), copper (Cu) and nickel (Ni)) affect the growth (maximum leaf number, leaf length and rosette number) and reproduction (maximum bud number and flower number) of native *Noccaea fendleri* and non-native *N. caerulescens* in the greenhouse experiment. Values shown for each variable are the standardised canonical correlation coefficients, which describe the standardised unique contribution of each original variable to the canonical variate when all other variables are held constant. Bold values highlight the substrate characteristics with the largest independent contributions to each canonical relationship.

Parameter	Native <i>Noccaea fendleri</i>			Non-native <i>Noccaea caerulescens</i>	
	Growth – Variate 1	Growth – Variate 2	Reproduction – Variate 1	Growth – Variate 1	Reproduction – Variate 1
Canonical correlation ( <i>r</i> )	0.433	0.34	0.367	0.619	0.492
<i>F</i> (ndf, ddf)	2.50(27, 478)	193(16, 328)	1.77(18, 328)	3.87(27, 447)	2.72(18, 308)
<i>P</i> -value	<0.001	0.017	0.028	<0.001	<0.001
Leaf number	0.300	0.116		0.860	
Leaf length	-0.644	1.982		0.096	
Rosette number	-0.500	-1.984		0.128	
Bud number			-0.845		-0.369
Flower number			-0.212		-0.708
pH	-0.174	0.549	-0.618	0.989	-0.423
Total Zn	<b>-1.969</b>	<b>-1.362</b>	<b>-1.489</b>	<b>1.408</b>	<b>-2.158</b>
Total Cd	0.573	-0.064	0.238	<b>-1.479</b>	-0.421
Total Cu	0.688	<b>1.737</b>	<b>1.957</b>	-1.077	<b>2.667</b>
Total Ni	<b>1.046</b>	0.858	1.104	-0.851	1.260
Available Zn	<b>-0.698</b>	<b>-2.088</b>	<b>-1.521</b>	<b>1.269</b>	<b>-2.209</b>
Available Cd	<b>1.126</b>	<b>3.490</b>	<b>2.927</b>	<b>-1.588</b>	<b>4.303</b>
Available Cu	0.033	-0.750	-0.999	0.584	-0.546
Available Ni	-0.346	-0.557	-0.448	0.460	-1.411



**Fig. 3.** Concentration of metal in the shoot for (a) zinc, (b) cadmium and (c) copper, shown by site (Cinnamon, Copper, Woods), location (higher- and lower-metal soils) and plant species (non-native *Noccaea caerulescens* (NC) and native *N. fendleri* (NF)). Biomass of *N. caerulescens* for the higher-metal soil from Copper site was too low to analyse. *Noccaea caerulescens* did not hyperaccumulate cadmium (b), although both species hyperaccumulated zinc (at >3000  $\mu\text{g g}^{-1}$ ) on some of the substrates examined (a). Both species accumulated less zinc on the higher-metal than the lower-metal soil from Copper and Woods sites, perhaps owing to interactions with copper (c).

lower-metal soils from Copper and Woods); *N. caerulescens* biomass contained on average 5460 (range 1780–8800)  $\mu\text{g g}^{-1}$  Zn, and *N. fendleri* biomass contained on average 4950 (range 1950–8900)  $\mu\text{g g}^{-1}$  Zn. Considering the low contamination levels of our soils (<1000  $\mu\text{g g}^{-1}$  Zn for all substrates), these values supported preferential uptake of Zn by these plants.

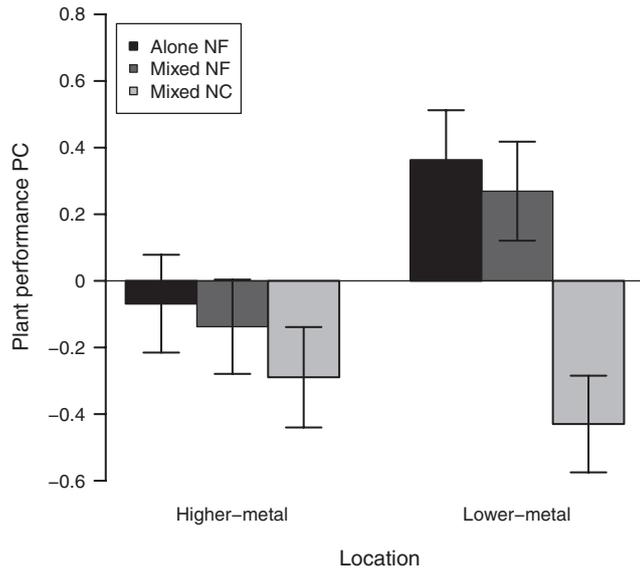
#### Field experiment

Results from the field plots at Woods site were similar to those from the greenhouse experiment; native *N. fendleri* had higher

vegetative growth than did *N. caerulescens* overall (species effect on Factor 2:  $F_{1,178} = 73.99$ ,  $P < 0.001$ ) and its growth was not affected by the presence of *N. caerulescens*. However, native *N. fendleri* grew better on the lower-metal than on higher-metal soil (Factor 1:  $F_{1,262} = 11.2$ ,  $P = 0.001$ ; Fig. 4), whereas, in the greenhouse, it grew equally well at the two locations.

#### Discussion

Our examination of the performance and competitive interactions of the Cd- and Zn- hyperaccumulator *Noccaea caerulescens*



**Fig. 4.** Performance of non-native *Noccaea caerulescens* (NC) and native *N. fendleri* (NF) in the field plots at Woods site, shown by location (higher-metal and lower-metal soils) and competition treatment (NF alone, or NC and NF mixed in a 1 : 1 ratio). Data shown are the values for the first principal component of all reproductive and growth responses (number of leaves, length of the longest leaf, and the number of flowering stalks, buds, flowers, and fruits, if any). Native *N. fendleri* performed better in the field than did *N. caerulescens* overall, and also grew better on the lower-metal soil than the higher-metal soil.

and the Ni- and Zn-hyperaccumulator *N. fendleri* on higher- and lower-metal soils yielded results that did not conform to our original expectations. First, we expected both species to perform better on the higher-metal soils than on the lower-metal soils; however, we found this pattern only for one of the three sites (Cinnamon) from which we collected substrates. Second, we expected to find stronger competitive interactions between the two species on the higher-metal soils than the lower-metal soils, but this was also not supported. The only instances of significant interspecific competition effects were on the lower-metal soils from Copper and Woods sites.

The observed unexpected pattern in plant performance was likely caused by the specific substrate characteristics for our study sites. The canonical correlation analysis indicated that both species exhibited better growth and reproduction on substrates with higher Zn but lower Cd and lower Cu concentrations. The positive correlation with soil Zn was consistent with the previously demonstrated Zn requirement for *N. caerulescens* (Mathys 1977; Li *et al.* 1995; Shen *et al.* 1997), and suggested that *N. fendleri* may also have such a requirement. Metal requirement has generally been demonstrated in terms of growth, whereas positive relationships between flower production and metal concentrations have been found only one other time in the Ni-hyperaccumulator *Alyssum inflatum* Nyár. (Ghasemi *et al.* 2014). High soil Cu has been previously shown to reduce the growth and Zn uptake of *N. caerulescens*, limiting its effectiveness for phytoremediation of sites contaminated with Cu as well as Zn (Walker and Bernal 2004), which we found was the case for two of our sites. *Noccaea fendleri* has been documented

to occur on substrates contaminated with Cu (Hobbs and Streit 1986), although that study did not report on plant growth. It was surprising to find that soil Cd was negatively correlated with the growth and reproduction of *N. caerulescens* and that soil Ni was negatively correlated with the growth of *N. fendleri* because these two species are hyperaccumulators of those metals; perhaps those metals were correlated with other unmeasured soil characteristics that reduced plant fitness.

These canonical relationships represented the independent effects of each metal, while holding other effects constant, whereas the observed pattern of plant performance across locations would depend on the specific combinations of metals in our field-collected substrates. For example, although the higher-metal soils from Copper and Woods sites contained the highest concentrations of Zn in our study (Table 1), they also contained high concentrations of Cu that reduced plant performance at those locations. In contrast, the lower-metal soils from those sites had lower concentrations of Cu as well as elevated concentrations of Zn compared to normal soil (Table 1). Together, these led to the unexpected result of better reproduction by *N. caerulescens* and better growth of *N. fendleri* on the lower-metal soil than on the higher-metal soil from Copper and Woods sites.

Thus, our experiment demonstrated that both species performed better on substrates with higher Zn content, but these were represented by the lower-metal soil at two of our study sites. In other words, our lower-metal soils were not sufficiently low in metals to observe the expected fitness trade-off, even though they were collected at some distance away from the tailings where native vegetation cover appeared normal. It is possible that we would find the expected trade-off if we compared plant performance on our higher- and lower-metal soils with that on completely uncontaminated substrates, such as potting soil. Additionally, our finding has direct implications for phytoremediation in that *N. caerulescens* may not always be prevented from establishing outside of the target remediation area (i.e. the tailings) by its Zn requirement, because the surrounding substrates may still contain elevated concentrations of Zn that would support its growth. We found similar results when examining the establishment potential of *N. caerulescens* in a related field experiment (Che-Castaldo and Inouye 2014).

The unexpected characteristics of some of our lower-metal soils may also help explain our results regarding the competitive interactions between *N. caerulescens* and *N. fendleri*. The two instances in which we observed significant interspecific effects were also on the lower-metal soils from Copper and Woods sites, which still had elevated Zn but low Cu. Thus, competition effects were observed only where plants grew well, suggesting that substrate characteristics posed greater limitations on the performance of these species than did competitive effects. However, the higher-metal soil from Cinnamon site had similar concentrations of Zn and Cu (Table 3) and supported similar rates of growth and reproduction (Figs 1, 2) to the lower-metal soils from Copper and Woods sites, but competitive interactions on that location were weak or absent. This suggested that the competitive ability of these species was promoted by characteristics of the lower-metal soils other than their Zn and Cu levels (e.g. other

unexamined contaminants and nutrients, soil texture and structure), as long as they contained sufficiently high concentrations of Zn to support the growth of *N. caerulescens* and *N. fendleri*.

We found *N. caerulescens* to be the better competitor in the one case in which the effect of interspecific competition was significantly greater than intraspecific competition (on the lower-metal soils from Copper site). However, this is likely to have represented the maximum potential competitive effect that *N. caerulescens* would have on the native community because it would be expected to compete less strongly against other native species that are more distantly related and also not metal-tolerant. Additionally, both *N. caerulescens* and *N. fendleri* grew more slowly in our field plots than in the greenhouse, suggesting more stressful conditions (e.g. temperature fluctuations, moisture availability) in the field that would further favour native species relative to non-natives in competition (Alpert *et al.* 2000; Daehler 2003; Garcia-Serrano *et al.* 2007; Burns 2008). Unfortunately, we were unable to identify the specific substrate characteristics that enhanced the competitive ability of *N. caerulescens* on the lower-metal soil from Copper site, because both *N. caerulescens* and *N. fendleri* responded similarly to the metal concentrations that we measured on the basis of the results from the canonical correlation analysis. Perhaps *N. caerulescens* was more tolerant than *N. fendleri* of the slightly higher concentrations of Cd and Cu in the lower-metal soils from Copper site than those at Woods site. It is also likely that other substrate attributes that we did not measure, such as other metal and nutrient concentrations (e.g. nitrogen and phosphorus), porosity and texture, may better explain the differences we observed in plant performance and competition.

Our overall finding of weak competitive effects between the two species was consistent with the theory of life-history strategy predicting that species adapted to tolerating abiotic stress tend not to be competitive (Hickey and McNeilly 1975; Grime 1977; Linhart and Grant 1996). However, the weak competitive interactions and functional similarity of the species also made it difficult to detect fitness trade-offs. An interesting extension to our study would be to examine the competitive ability of both *N. caerulescens* and *N. fendleri* against a non-hyperaccumulating or non-tolerant native species across a soil-metal gradient. Such an experiment would be more likely to demonstrate the expected trade-off patterns, and also allow a comparison of whether non-native *N. caerulescens* would be a better competitor than native *N. fendleri* against other native species. Findings from this would further provide insight into the invasion potential of *N. caerulescens* at introduction sites. Based on our current results, we would expect *N. caerulescens* to be a poor competitor on truly low-metal or metal-free soils, and it should also be less competitive against other native species than it was against *N. fendleri*.

Finally, characteristics other than competitive ability may also contribute to the potential invasive success of *N. caerulescens* if it were introduced to non-native sites for phytoremediation. For example, we found that *N. caerulescens* tended to invest into reproduction rather than into vegetative growth, and the production of many small seeds and persistent reproduction have been shown to be key traits of invasive plants (Alpert *et al.* 2000; Daehler 2003; Lockwood *et al.* 2005). Moreover,

elevated concentrations of foliar Cd in *N. caerulescens* may present a novel defence mechanism (Boyd 2007; Vesik and Reichman 2009) against native herbivores in the introduced community, resulting in higher invasive potential through the evasion of new enemies (Levine *et al.* 2004; Mitchell *et al.* 2006). The longer-term implications of high reproductive investment and the effectiveness of elemental defence against native herbivores both warrant further examination.

Our goal in the present study was to investigate the competitive ability of *N. caerulescens* against *N. fendleri* across a soil-metal gradient in the context of phytoremediation. Because of the specific characteristics of the substrates at our study sites, we did not find the expected patterns of better plant performance and stronger competitive interactions on lower-metal soils than on higher-metal soils. Our findings indicated that interspecific competition had a smaller effect on the growth and reproduction of *N. caerulescens* than did abiotic factors such as soil metal concentrations. We also found that substrates that did not appear contaminated at the potential introduction sites in fact contained elevated concentrations of Zn and other factors that favoured the performance and competitive ability of *N. caerulescens*. Thus, the establishment and invasive potential of *N. caerulescens* should be further tested, and we suggest that introductions of *N. caerulescens* to non-native sites for phytoremediation should be carefully monitored.

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