

## Species specific plant-soil interactions influence plant distribution on serpentine soils

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**Abstract** Where serpentine soils exist, variation in soil properties affects plant species distribution at both coarse and fine spatial scales. The New Idria (California, USA) serpentine mass has barren areas, supporting only sparse shrub and tree islands, adjacent to areas of densely-vegetated serpentine chaparral. To identify factors limiting growth on barren relative to vegetated serpentine soils, we analyzed soils from barren, shrub-island within barren, and vegetated areas and foliage from shrub-island and vegetated areas. We also grew *Ceanothus cuneatus* (native evergreen shrub), *Achillea millefo-*

*lium* (native perennial forb), and *Bromus madritensis* ssp. *rubens* (invasive annual grass) in soils from barren and vegetated areas amended factorially with N, K, and Ca in a pot study. In well-watered pots, biomass was greater by 5-, 14-, and 33-fold for *Ceanothus*, *Achillea*, and *Bromus*, respectively, on vegetated-area-collected soils than on barren-collected soils, indicating a strong soil chemistry effect. Although field soil data suggested nutrient deficiency and not heavy metal toxicity, pot study plant data indicated otherwise for two of the three species. On barren-collected soils, only *Ceanothus* responded positively to added N and Ca and did not show greater foliar Mg or heavy metal (Fe, Ni, Cr, Co, Zn) concentrations than on vegetated-area-collected soils. *Ceanothus* maintained lower root Mg and heavy metal (Fe, Ni, Cr, Co) concentrations on barren soils and translocated less heavy metal (Fe, Ni, Cr, Co, Mn, Cu) from roots to foliage than *Achillea* and *Bromus*. *Achillea* and *Bromus* showed significant log-log biomass relationships with foliar Ca:Mg (+), Mg (-), and heavy metals (Fe, Ni, Cr, Co, Mn, Cu, Zn) (-), while *Ceanothus* showed relationships only with Ca: Mg (+) and Mg (-). The New Idria barren-vegetated pattern appears to be maintained by different factors for different species or functional types— low Ca:Mg ratios on barrens for all species tested, high heavy metal concentrations for *Achillea* and *Bromus*, and low macronutrient (N) concentrations for *Ceanothus*. Combined data from this and other studies suggest high heavy metal concentrations more strongly affect

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herbaceous than woody species, contributing to variation in species distribution on serpentine soils.

**Keywords** Ultramafic soils · Low nutrient adaptation · Calcium:magnesium ratio · Metal toxicity · Nickel

## Introduction

Much variation exists among soils derived from ultramafic parent materials, generally (and hereafter) referred to as serpentine soils. At global and regional scales, different temperature and precipitation regimes lead to different weathering and leaching rates as well as various serpentine plant communities that differentially influence soil properties (e.g., forests and grassland create different types and spatial distributions of soil organic matter). Topographic differences create variation at a local scale (Rajakaruna and Bohm 1999; Alexander et al. 2007). At multiple scales, differences in parent material (e.g., degree of serpentinization, mineral composition, presence of accessory minerals) influence soil chemistry, erosion resistance, and weathering rates (Alexander et al. 2007; McGahan et al. 2008, 2009). The New Idria serpentine mass (San Benito and Fresno Counties, California, USA) shows striking within-site variation, with dense chaparral giving way to barrens that are nearly devoid of vegetation except for scattered shrub and tree islands. Though some of California's broad scale serpentine vegetation patterns are well understood (e.g., the distribution of forest vs. grassland corresponds with precipitation), other differences (such as the distribution of grassland vs. chaparral) are not yet clear (Grace et al. 2007), and there has been little study of what causes barrens, though hypotheses include lack of soil formation resulting from geologic and topographic instability (Coleman 1996; Kruckeberg 1999).

It is also clear that plant species respond differently to serpentine soils, with some excluded altogether, others “indifferent” (bodenvag), and still others endemic (found only on serpentine soils) (Kruckeberg 1984; Safford et al. 2005). Many bodenvag species have serpentine edaphic ecotypes that perform better on serpentine soils than nonserpentine ecotypes (Kruckeberg 1951; O'Dell and Claassen 2006; O'Dell and Rajakaruna 2011). Different species growing together on the same soils often have different foliar

elemental concentrations, indicating different nutrient selectivities (Lyon et al. 1971; Johnston and Proctor 1977; Koenigs et al. 1982; Alexander et al. 1989; Pope et al. 2010). Different chemical elements may limit different species on serpentine, resulting in species-specific responses to substrate amendments (e.g., Koide and Mooney 1987; Huenneke et al. 1990; Nagy and Proctor 1997).

Plant ecologists and evolutionary biologists have long been fascinated by the common attribute of serpentine soils—uniquely adapted and often endemic plant species and communities that differ markedly from those of adjacent, non-serpentine soils as a result of the relative “infertility” of serpentine soil. This infertility is commonly attributed to a “syndrome” of chemical and physical features—low macronutrient content, low calcium (Ca) to magnesium (Mg) molar ratios, high heavy metal content, micronutrient deficiency, and poor water-holding properties (Proctor and Woodell 1975). While experimental evidence supports the role of each factor of the serpentine syndrome in causing serpentine infertility in one case or another, no single factor has emerged as growth-limiting in every instance, and few studies have simultaneously considered more than one facet of the serpentine syndrome for more than one species or functional type or on more than one serpentine soil. The most logical explanation for differing results is that the factors of greatest importance causing serpentine infertility vary not only from one serpentine soil to another, but also from one plant species or functional group to another.

In this study, we considered multiple hypotheses of serpentine infertility (macronutrients nitrogen (N) and potassium (K), low Ca:Mg molar ratios, and high heavy metal concentrations) for multiple species. Our objective was to better understand the New Idria barren-vegetated pattern and whether it varied with species. We hypothesized that this pattern is maintained by differences in soil chemical and/or fine scale physical properties that limit plant growth on barren relative to vegetated soils. To test this hypothesis we 1) analyzed field collected samples of soils and foliage and 2) conducted an experimental study with three serpentine-collected species representing three plant functional groups (native evergreen shrub, native perennial forb, and invasive annual grass) grown in soils collected from barren and vegetated areas amended with factorial nutrient combinations (N, K, Ca).

Sub-hypotheses and the data that addressed each were as follows:

- 1) Plant growth is limited on barren relative to vegetated soils by fine scale soil physical properties. This was tested by comparing texture and water holding properties on field-collected barren, shrub-island within barren, and vegetated soils. Significant differences among soil types would indicate possible limitation by physical factors.
- 2) Plant growth is limited on barren relative to vegetated soils by soil chemical properties. This was tested by comparisons of plant growth on both soil types in well-watered pots, which eliminated possible physical limitations. Significant differences obtained under these conditions would indicate the importance of chemical factors, which can be further divided into two categories: A) essential nutrients and B) potentially toxic elements. We tested the following sub-hypotheses:
  - A. Low levels of important macronutrients or ratios (N, K, Ca, Ca:Mg) limit plant growth on barren relative to vegetated soils. Support for this hypothesis would come from i) lower concentrations of these elements or ratios in soils and foliage collected from barren than from vegetated areas in the field, ii) experimental study plant biomass increases accompanying N, K, or Ca additions, and iii) significant positive correlations between foliar element or ratio concentrations and biomass across soil types and treatments in the experimental study
  - B. High levels of toxic elements (Mg, heavy metals such as Ni, Co, Cr) limit plant growth on barren relative to vegetated soils. Support for this hypothesis would come from i) higher concentrations of Mg or heavy metals in soils and foliage collected from barren than from vegetated areas in the field, ii) higher concentrations of these elements in roots or shoots of plants grown in barren than vegetated soils in the experimental study, and iii) significant negative correlations between potentially toxic foliar elements and biomass across soil types and treatments in the experimental study

Understanding the barren-vegetated pattern is necessary for better understanding of the serpentine syndrome in general and for successful restoration of vegetation or shrub and tree islands on serpentine soil areas degraded by logging, mining, and off-highway vehicle use on the New Idria serpentine mass. Improved understanding is also important for activities that might facilitate conservation of endemic serpentine species. Understanding variation in species or functional group responses to different serpentine soils is of use to restoration practitioners, who need to amend degraded serpentine substrates to promote native plant growth without stimulating invasive species. Serpentine restoration and revegetation are of increasing importance because these soils, which support very high levels of biodiversity and endemic species relative to the extent of the area they occupy globally, are frequently heavily disturbed by recreational activities or by mercury, asbestos, nickel, and chromium mining (O'Dell and Claassen 2009).

## Methods

### Field soil and foliage collection and analyses

The New Idria serpentine mass is located at the southern end of the Diablo Range in the California Coast Ranges (36.3°N, 120.6°W). The area experiences a Mediterranean-type climate (cool wet winters and hot dry summers) with mean annual precipitation of 40–60 cm (Alexander et al. 2007). Hydrothermal alteration has nearly completely transformed the original peridotite protolith to serpentinite which contains a high concentration of chrysotile asbestos (Coleman 1986). The serpentinite has been intensely sheared and crushed, resulting in a terrain of low, rounded hills with a strong tendency to slide when slopes become oversteepened. Barrens with shallow, soft, powdery soils are widely interspersed with densely vegetated areas of chaparral displaying more advanced soil development. Herbaceous cover is very sparse to non-existent in both barrens and chaparral.

We selected three pairs of barren and vegetated areas within the New Idria serpentine mass that met criteria of close physical proximity with similar slope, aspect, and elevation (Table 1, Online Resource Fig. 1). Within each barren area, we collected soils at 20–30 cm depth (rooting zone) on three randomly chosen mid-slope

**Table 1** Slope, elevation and aspect of three sites where soils and vegetation were sampled. Potential rooting depth was measured at four random locations within each of the three sites for each soil type (except off serpentine where only one site

was sampled). Depth range is given because the soil depth probe could only measure to 1 m, and soils were often deeper than that maximum. Cover was estimated for a 10 m radius around each site (mean  $\pm$  SE)

Soil type	Slope (%)	Elevation (m)	Aspect (°)	Rooting depth range (m)	% Cover
Barren	25, 25, 24	1070, 1466, 1341	80, 245, 345	0.15->1.00	0 $\pm$ 0
Shrub Island				0.20->1.00	33 $\pm$ 5
Vegetated	13, 22, 22	1067, 1467, 1292	165, 260, 290	0.15->1.00	66 $\pm$ 11
Off serpentine	35	810	260	0.12->1.00	56

barren locations and within three shrub/tree islands. On vegetated slopes, we collected soils at the same depth in three randomly-selected canopy gaps (though the steepness of the slopes and the high vegetative cover overall made canopy gaps similar in their surface properties to under-shrub areas). Coarse fragment volume was estimated in the field and added to the volume of any coarse fragments not passing the sieve during preparation of the soil for analyses.

Within each barren area, we collected mature evergreen foliage that grew the previous spring (collection was in early March 2008) from one *Pinus sabiniana* D. Don Pinaceae (foothill pine) and one *Arctostaphylos glauca* Lindl. Ericaceae (bigberry manzanita) in each of three shrub islands. Hereafter these species are referred to as *Pinus* and *Arctostaphylos*. Within each vegetated area, we located three *Pinus* individuals and collected foliage from these as well as from the nearest *Arctostaphylos*. For each individual, sampling occurred at random and included all sides of the canopy.

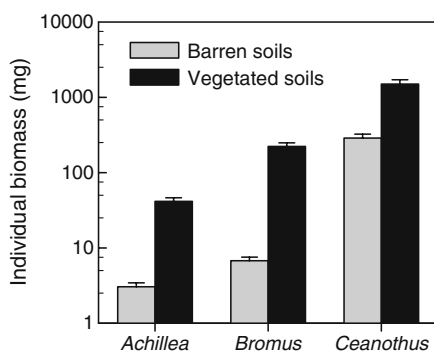
Soil was dried, sieved to 2 mm, and submitted to A&L Western Analytical Laboratories (Modesto, CA,

USA) for analysis of organic matter (loss on ignition at 360°C), pH (saturated paste), cation exchange capacity (CEC), extractable Ca, Mg, and K (ammonium acetate at pH 7), bicarbonate extractable phosphorus (P), nitrate N (2M KCl extraction), and DTPA extractable iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), molybdenum (Mo), nickel (Ni), cobalt (Co), and chromium (Cr). We measured total N by combustion on ball mill ground samples using a CHNSO analyzer (Costech Analytical Technologies, Valencia, CA, USA, Model ECS 4010). We also measured texture by fractionation and moisture release properties using weighing and a dewpoint water potential meter (Decagon Devices, Inc., Pullman, WA, USA; Model WP4).

We analyzed soil data by performing 18 univariate ANOVA's using a sequential Bonferroni correction (Rice 1990) to control family-wise type I error. Dependent variables were soil properties (e.g., pH, organic matter, Ni content) and independent variables were soil type, collection location (random), and their interaction. We performed Tukey-Kramer comparisons on soil type (barren, shrub island, vegetated) for soil properties with significant univariate models.

Foliage was washed with deionized water, dried for 48 h at 60°C, and ground to 40 mesh in a Wiley mill. Samples were microwave digested with nitric acid and peroxide (Sah and Miller 1992) and analyzed for Ca, Mg, K, P, Fe, Mn, Cu, Mo, Ni, Co, and Cr concentrations on an ICP-MS (Agilent Technologies, Santa Clara, CA, USA, Model 7500a). Samples were further ground to a fine powder, packed in tin capsules, and analyzed for foliar N levels using the CHNSO analyzer.

We performed principal components analysis (PCA) of foliar elemental concentrations (Ca:Mg, K, P, N, Fe, Mn, Cu, Mo, Ni, and Cr; Co was excluded because it was below detection limit for all samples)



**Fig. 1** Least squares means and SE ( $n=16-24$ ) for total plant biomass (root plus shoot) of the three study species grown in serpentine soils collected from barren and vegetated areas. Note log scale

for *Pinus* and *Arctostaphylos*. Data were log transformed prior to analysis. We performed ANOVA on the first three principal components (PC's), dependent variables, with soil type, collection location (random), species, and interactions as independent variables.

## Plant growth on barren and vegetated soils

### Plant culture

For the experimental study of plant growth on soils collected from barren and vegetated areas we chose plants of three functional groups commonly found at the field site and that could be effectively grown in pots. The field sampled *Pinus* and *Arctostaphylos* were not suitable for the pot study because of their large stature and slow growth. We grew three species— a native shrub (*Ceanothus cuneatus* (Hook.) Nutt.; Rhamnaceae; buckbrush), a native perennial forb (*Achillea millefolium* (L.); Asteraceae; common yarrow), and an invasive annual grass (*Bromus madritensis* ssp. *rubens* (L.) Husn.; Poaceae; red brome) in 650 mL pots using soils collected from the same paired barren and vegetated areas in which soil sampling occurred. Soils were from barren areas, not from shrub islands within barrens. Hereafter these three study species are referred to as *Ceanothus*, *Achillea*, and *Bromus*. Soil was sieved to 5 mm prior to planting. *Ceanothus* and *Achillea* seeds were collected from serpentine populations (at least 20 and 500 mothers, respectively) in the New Idria serpentine mass and pooled. *Bromus* seed was collected from at least 200 mothers in a single population growing on a serpentine outcrop near Colusa, California. *Ceanothus* seed was treated with hot water to break dormancy (Emery 1988), germinated in potting soil, and bare-rooted before planting into experimental pots. Seedlings were approximately 2 cm tall and had no branches at the time of repotting. *Achillea* and *Bromus* seeds were sown directly into experimental pots.

During the entire experiment plants grew outside on the University of California, Davis, CA, USA campus starting in April of 2008, and they were kept well-watered (twice daily with deionized water) to eliminate water deficit as a possible stressor and to remove potential soil type differences in physical properties. Excess water was not captured but allowed to drain away to prevent soil saturation effects. Pots were arranged in a completely randomized design,

with two rows of empty edge pots ensuring that no experimental pot was heated by direct solar radiation from the side. Fertilization treatments applied weekly were doses of nutrient solution containing factorial combinations of dissolved N, K, and Ca (i.e., control, +N, +K, +Ca, +NK, +Nca, +KCa, +NKCa) in the following amounts: 2.69 mMol N as  $\text{NH}_4\text{NO}_3$ , 0.93 mMol K as KCl, and 9.42 mMol Ca as  $\text{CaCl}_2$ . Ratios of Ca to other nutrients were based on literature (Chiarucci et al. 1999), and solution concentrations were adjusted to keep conductivities low enough for plants to tolerate. The choice of these elements (N, K, Ca) for the factorial fertilization experiment was based on apparent deficiency revealed by soil analyses (see Results).

Above and belowground parts were harvested, washed, dried and weighed at 8, 9, and 16 weeks for *Bromus*, *Achillea*, and *Ceanothus*, respectively. A portion of the soil in each pot was washed to extract fine roots and another portion was dried for soil analyses. Roots were stored briefly in cold, deionized water and then scanned and root length measured with WinRHIZO (Regent Instruments Inc., Québec City, QC, Canada) prior to drying.

Biomass and root length density (km of root per cubic meter of soil) data were analyzed on a per individual basis and include only biomass produced during the course of the experiment for *Ceanothus*, which was transplanted as a seedling. We used a mixed model in JMP version 5.0.1 (SAS Institute, Cary, NC, USA) to perform ANOVA's with treatment, species (*Achillea*, *Bromus*, *Ceanothus*), soil type (barren/vegetated), field soil collection location (random), and all possible 3-way interactions (the 4-way interaction was by definition the residual error). Data were log transformed prior to analysis to meet homogeneity of variance assumptions. Rather than including a general "treatment" factor in our models and then performing a large number of orthogonal contrasts after the fact, we included the factors N (+/-), K (+/-), and Ca (+/-) and their interactions with each other and with species, soil type and collection location in the initial model (Online Resource Tables 1, 2). Due to a logistical issue leading to the early demise of all *Ceanothus* in vegetated soil treated with both Ca and K, it was not possible to analyze the experiment as an NKC full factorial in a single ANOVA. Because preliminary analyses showed no significant effect of K addition on biomass or root length density, we removed the K factor from the

**Table 2** Mean  $\pm$  SE for chemical properties of field collected soils. Nonserpentine soil data are provided for comparison only and were not included in the statistical analyses. Soil typesconnected by the same letter did not differ significantly at the 5% level. Mo was below detection limit (BDL; 0.1 mg kg<sup>-1</sup>) in all soils

	Barren	Shrub island within barren	Vegetated	Nonserpentine
n locations	3	3	3	1
n samples per location	3	3	3	6
pH	7.09 $\pm$ 0.16	7.39 $\pm$ 0.05	7.29 $\pm$ 0.03	7.48
Cation exchange capacity (cmol/kg)	2.94 <sup>b</sup> $\pm$ 0.44	8.21 <sup>a</sup> $\pm$ 0.36	10.84 <sup>a</sup> $\pm$ 0.42	23.40
Organic matter (%)	1.21 <sup>b</sup> $\pm$ 0.09	3.39 <sup>a</sup> $\pm$ 0.51	3.79 <sup>a</sup> $\pm$ 0.59	2.58
Total N (%)	0.009 <sup>c</sup> $\pm$ 0.003	0.064 <sup>b</sup> $\pm$ 0.016	0.104 <sup>a</sup> $\pm$ 0.030	0.096
Nitrate N (mg kg <sup>-1</sup> )	5.90 $\pm$ 0.99	3.73 $\pm$ 0.86	5.38 $\pm$ 1.15	6.73
Bicarbonate extractable P (mg kg <sup>-1</sup> )	5.28 $\pm$ 0.93	6.06 $\pm$ 0.51	9.38 $\pm$ 0.93	4.10
K (mg kg <sup>-1</sup> )*	9.3 <sup>b</sup> $\pm$ 5.9	25.4 <sup>a</sup> $\pm$ 0.6	39.5 <sup>a</sup> $\pm$ 3.3	294.7
Ca (mg kg <sup>-1</sup> )*	68.7 <sup>c</sup> $\pm$ 11.6	233.9 <sup>b</sup> $\pm$ 34.2	514.6 <sup>a</sup> $\pm$ 8.4	3924.2
Mg (mg kg <sup>-1</sup> )*	303.4 <sup>b</sup> $\pm$ 49.1	842.5 <sup>a</sup> $\pm$ 25.6	988.1 <sup>a</sup> $\pm$ 49.2	363.0
Ca:Mg (molar ratio)	0.16 <sup>b</sup> $\pm$ 0.03	0.16 <sup>b</sup> $\pm$ 0.02	0.32 <sup>a</sup> $\pm$ 0.01	9.2
Fe (mg kg <sup>-1</sup> )**	2.28 <sup>b</sup> $\pm$ 1.19	6.42 <sup>a</sup> $\pm$ 2.96	8.30 <sup>a</sup> $\pm$ 3.37	5.25
Mn (mg kg <sup>-1</sup> )**	0.86 <sup>c</sup> $\pm$ 0.06	3.89 <sup>b</sup> $\pm$ 1.08	5.80 <sup>a</sup> $\pm$ 0.45	3.33
Zn (mg kg <sup>-1</sup> )**	0.11 $\pm$ 0.01	0.10 $\pm$ 0.00	0.16 $\pm$ 0.01	0.37
Cu (mg kg <sup>-1</sup> )**	0.16 $\pm$ 0.04	0.14 $\pm$ 0.03	0.19 $\pm$ 0.03	0.90
B (mg kg <sup>-1</sup> )	0.21 $\pm$ 0.03	0.41 $\pm$ 0.07	0.39 $\pm$ 0.05	0.83
Mo (mg kg <sup>-1</sup> )**	BDL	BDL	BDL	BDL
Ni (mg kg <sup>-1</sup> )**	1.77 <sup>b</sup> $\pm$ 0.50	13.70 <sup>a</sup> $\pm$ 1.06	21.29 <sup>a</sup> $\pm$ 2.28	0.27
Co (mg kg <sup>-1</sup> )**	0.10 $\pm$ 0.00	0.24 $\pm$ 0.06	0.32 $\pm$ 0.02	0.10
Cr (mg kg <sup>-1</sup> )**	1.51 <sup>a</sup> $\pm$ 0.14	0.21 <sup>b</sup> $\pm$ 0.08	0.24 <sup>b</sup> $\pm$ 0.13	0.22

\*Exchangeable

\*\*DTPA extractable

model and present an NCa full factorial. We performed orthogonal linear contrasts on least squares means to better understand soil type differences within species or treatment differences within soil type and species.

### Tissue analyses

Leaf tissues from all plants and root tissues from a subset of plants (control and +Ca treatments) were rinsed well in distilled deionized water, dry ashed for 6 h at 450°C and re-suspended in 3% nitric acid. Solution Ca, Mg, K, Fe, Mn, Zn, Cu, Mo Ni, Co, and Cr concentrations were measured on the ICP-MS. Leaf tissue from a subset of plants (control and +N treatments) was ground, packed in tin capsules, and analyzed for foliar N levels using the CHNSO analyzer.

Foliar elemental data (other than N) were analyzed using the same mixed model ANOVA described for biomass for each of the foliar elemental concentrations

with a sequential Bonferroni correction on each whole model fit to control for experiment-wide type I error (note—*P*-values all <0.0001 or >0.05). Data were log transformed prior to analysis for homogeneity of variance. Because treatments had no impact on foliar elements other than the ones we added (i.e., N, K, and Ca), we report least squares means of the N x Ca full factorial analyses for all elements except K (for which we report the results of an N x K full factorial). Foliar N concentrations were analyzed separately, as this variable was measured only for control and +N treatments. This ANOVA included species, treatment (control or +N), soil type, collection location, and all possible 3-way interactions that did not include collection location.

For roots, where elemental concentration measurements were made only on control and +Ca treatments, we analyzed Mg and heavy metal (Fe, Mn, Zn, Cu, Ni, Co, Cr) concentrations using individual univariate mixed model ANOVA's with a sequential Bonferroni



correction to control for experiment-wide type I error. Data were log transformed prior to analysis for homogeneity of variance. Because preliminary results showed that Ca addition did not significantly affect the concentrations of these elements, we removed the treatment factor from the model for these analyses, which had dependent variable concentration and independent variables soil type, species, collection location (random), and their interactions. We compared least squares means for the species  $\times$  soil type interaction using Tukey-Kramer comparisons. We also calculated foliage:root concentration ratios to represent patterns of translocation from roots to aboveground parts and performed the same set of analyses. Foliage:root values  $>1$  suggest preferential net translocation to leaves, whereas values  $<1$  suggest root sequestration or poor translocation to leaves. Data were log transformed prior to analysis for homogeneity of variance. We analyzed root Ca, Ca:Mg, and foliage:root Ca with an ANOVA that included species, soil type (barren/vegetated), treatment ( $-/+$  Ca), and their interactions (collection location was treated as a replicate). Log transformations were not needed for these data.

We calculated  $r^2$  values and growth response coefficients (GRC's) for the logarithmic relationships between foliar elemental concentrations and biomass for each species. GRC's (slopes of log-log plots of a variable vs. growth, Poorter and van der Werf 1998) measure the proportional change in a variable that accompanies a proportional change in growth rate. Poorter and van der Werf, who developed this concept, used it to distinguish between competing hypotheses with respect to the most important explanatory variables for growth rate (i.e., Net Assimilation Rate and Leaf Area Ratio), interpreting the variable with the largest GRC as the one of greatest importance. They favored GRC's over correlation coefficients for this purpose because correlation coefficients measure relative variation around the mean but do not take the absolute size of that variation into account. We follow their interpretation here.

## Results

### Rooting depth and soil physical properties

Across the New Idria serpentine mass, barrens occurred on all aspects and on slopes that were flat

enough for vegetation establishment, based on comparison to adjacent vegetated slopes (Table 1; Online Resource Fig. 1). Soil pits show that true soils on the New Idria barrens are quite shallow (18–30 cm) but overlie 30–50 cm of highly weathered and fractured bedrock (Soil Survey Staff 2010). This agrees well with our soil probe measurements, showing potential rooting depths for all three soil types were quite deep ( $>1$  m) and did not differ among the three types (Table 1). Barren soil (bare area) textures were sands (% sand/silt/clay=87/8/5), shrub-island soils within barrens were loamy sands (79/13/8), and vegetated soils were sandy loams (69/5/26). Vegetated soils had more clay ( $P=0.039$ ) and less sand ( $P=0.011$ ) than soils collected on barrens (both shrub islands and bare areas—orthogonal contrasts). Within barrens, the trends for more clay and less sand in shrub islands than bare areas were not statistically significant ( $P=0.78$  and  $P=0.12$ , respectively).

Mean ( $\pm$  SE;  $n=3$ ) available soil water of the  $<2$  mm fraction of these sandy soils between  $-0.01$  and  $-1.5$  MPa, calculated from moisture release curves, was not significantly different (paired  $t$ -test,  $P=0.87$ ) between barren ( $0.140\pm 0.021$  m<sup>3</sup> m<sup>-3</sup>) and vegetated ( $0.153\pm 0.067$  m<sup>3</sup> m<sup>-3</sup>) soils (Online Resource Table 3). Extending the low water potential cutoff to  $-5.0$  MPa, because of drought tolerant native species, did not result in any significant difference (Online Resource Table 3). Including the coarse fraction ( $>2$  mm diameter) also showed no significant difference (paired  $t$ -test,  $P=0.77$ ) in available water between  $-0.01$  and  $-1.5$  MPa between barren ( $0.096\pm 0.015$  m<sup>3</sup> m<sup>-3</sup>) and vegetated ( $0.114\pm 0.049$  m<sup>3</sup> m<sup>-3</sup>) soils.

### Soil chemistry

Vegetated and shrub-island soils had greater CEC, organic matter, K, Mg, Fe, and Ni and less Cr than barren soils (Table 2). Barren and shrub-island soils had lower Ca:Mg than vegetated soils. Mn and Ca were greatest on vegetated soils, least on barren soils, and intermediate on shrub-island soils. Many soil properties and elements showed strong correlations (Online Resource Table 4). The highest concentrations of Ca, Mg, K, Ni, and Mn were measured on soils with the greatest CEC, organic matter, and total carbon, all of which were closely related.

**Table 3** Mean  $\pm$  SE elemental concentrations in field-collected foliage of *Arctostaphylos glauca* and *Pinus sabiniana* on serpentine soils ( $n=3$  locations with 3 samples per location)

	<i>Arctostaphylos glauca</i>		<i>Pinus sabiniana</i>	
	Shrub island	Vegetated	Shrub island	Vegetated
N (%)	0.724 $\pm$ 0.098	0.829 $\pm$ 0.015	1.058 $\pm$ 0.064	1.106 $\pm$ 0.016
P (g kg <sup>-1</sup> )	0.551 $\pm$ 0.082	0.674 $\pm$ 0.165	0.923 $\pm$ 0.076	0.969 $\pm$ 0.070
K (g kg <sup>-1</sup> )	3.54 $\pm$ 0.25	4.45 $\pm$ 1.59	3.74 $\pm$ 0.37	4.71 $\pm$ 1.01
Ca (g kg <sup>-1</sup> )	5.88 $\pm$ 0.75	9.40 $\pm$ 2.31	1.99 $\pm$ 0.41	1.98 $\pm$ 0.69
Mg (g kg <sup>-1</sup> )	3.92 $\pm$ 0.23	3.75 $\pm$ 0.62	2.61 $\pm$ 0.18	2.01 $\pm$ 0.32
Ca:Mg	0.90 $\pm$ 0.03	1.42 $\pm$ 0.29	0.46 $\pm$ 0.10	0.60 $\pm$ 0.14
Fe (mg kg <sup>-1</sup> )	51.07 $\pm$ 8.42	29.85 $\pm$ 3.06	93.63 $\pm$ 16.58	56.06 $\pm$ 6.57
Mn (mg kg <sup>-1</sup> )	15.16 $\pm$ 0.60	22.22 $\pm$ 3.70	64.24 $\pm$ 7.75	56.60 $\pm$ 15.19
Cu (mg kg <sup>-1</sup> )	0.88 $\pm$ 0.10	1.37 $\pm$ 0.50	1.32 $\pm$ 0.19	1.24 $\pm$ 0.34
Mo ( $\mu$ g kg <sup>-1</sup> )	24.67 $\pm$ 1.79	15.09 $\pm$ 6.80	43.51 $\pm$ 10.3	34.98 $\pm$ 7.46
Ni (mg kg <sup>-1</sup> )	3.03 $\pm$ 0.75	4.35 $\pm$ 1.00	2.15 $\pm$ 0.14	2.56 $\pm$ 0.37
Co (mg kg <sup>-1</sup> )	BDL	BDL	BDL	BDL
Cr (mg kg <sup>-1</sup> )	3.95 $\pm$ 0.75	6.10 $\pm$ 2.17	2.53 $\pm$ 0.53	2.75 $\pm$ 0.88

#### Field-collected foliage

Measured foliar concentrations of *Arctostaphylos* and *Pinus* are presented in Table 3. Overall 67.1% of the variation in elemental composition between these two species on the two soil types was captured with the PCA (Table 4). PC1 explained 37.5% of the variation and was related most strongly to species differences. Positive loadings were elements for which *Pinus* concentrations were greater than *Arctostaphylos* concentrations (N, P, K, Fe, Mn, Mo), and negative loadings were elements for which the reverse was true (Ca:Mg, Ni, Cr).

PC2 explained 16.3% of the total variation. ANOVA with PC2 as the dependent variable was not quite significant ( $P=0.095$ ), and soil type  $\times$  genus ( $P=0.131$ ), was the most nearly significant effect. PC2 and the elements with positive loadings (N, Ca: Mg, Cu, Ni, Cr) all showed a soil type difference (vegetated > barren) for *Arctostaphylos*, and no difference or a smaller difference for *Pinus*.

PC3 explained 13.3% of the total variation. ANOVA with PC3 as the dependent variable showed a nearly significant ( $P=0.073$ ) effect of soil type. Elements with strong positive loadings (Fe, Mo) had higher concentrations in foliage of both species collected on barrens. K, and P, with weaker negative loadings showed slightly higher concentrations in foliage collected on vegetated soils for both species.

#### Plant growth on barren and vegetated soils

Biomass was substantially greater, by 5-, 14-, and 33-fold for *Ceanothus*, *Achillea*, and *Bromus*, respectively, when grown on soil collected from vegetated areas than on soil collected from adjacent barren areas (significant soil type  $\times$  species interaction, Fig. 1, Online Resource Table 1). Biomass differences due to fertilizer treatments were small compared to the large species and soil type differences (Fig. 2). In single or factorial nutrient addition studies, increases in biomass that accompany increases in foliar concentration of a particular element with fertilization are interpreted as evidence of limitation by that element for that species in the unfertilized soil (James et al. 2005; model in Fig. 2). *Ceanothus* grown on barren soils responded positively to N and Ca addition, and *Bromus* grown on barren soils responded negatively to N addition (Fig. 2, orthogonal linear contrasts on significant soil type  $\times$  species  $\times$  N  $\times$  Ca interaction LS means for NCa model, Online Resource Table 1). No other significant fertilizer treatment effects on biomass were detected. Lack of growth response to treatments, at least on barren soils, was not due to lack of treatment element availability, particularly in the case of Ca. In general, foliar concentrations increased with treatments, though sometimes more on barren than vegetated soils (Fig. 2, orthogonal linear contrasts for species  $\times$  soil type  $\times$  N for N concentration, species  $\times$  soil type  $\times$  Ca with NCa



**Table 4** Principal components analysis (PCA) and probability values for ANOVA on PCA scores for field-collected foliar elemental concentrations. These data are for *Arctostaphylos* and *Pinus* collected at three locations on two serpentine soil types (vegetated and shrub island within barren). Strongest loadings for eigenvectors and lowest probabilities for ANOVA's are indicated in bold text. CL = collection location

	PC1	PC2	PC3
Percent variance explained	37.5	16.3	13.3
Cumulative percent variance explained	37.5	53.8	67.1
	Eigenvectors		
log N	<b>0.35</b>	<b>0.42</b>	-0.09
log P	<b>0.37</b>	-0.11	<b>-0.34</b>
log K	<b>0.28</b>	0.05	<b>-0.43</b>
log Ca:Mg	<b>-0.32</b>	<b>0.46</b>	-0.09
log Fe	<b>0.34</b>	-0.07	<b>0.57</b>
log Mn	<b>0.34</b>	0.22	-0.22
log Cu	<b>0.25</b>	<b>0.53</b>	0.04
log Mo	<b>0.33</b>	0.14	<b>0.53</b>
log Ni	<b>-0.21</b>	<b>0.31</b>	-0.05
log Cr	<b>-0.35</b>	<b>0.38</b>	0.18
	ANOVA probabilities		
Whole model	<0.0001	0.095	0.046
Soil type	0.376	0.321	<b>0.073</b>
CL (random)	0.107	0.552	0.302
Soil type*CL (random)	0.277	0.259	0.296
Species	<b>0.008</b>	0.335	0.856
Soil type*Species	0.282	<b>0.131</b>	0.911
CL*Species (random)	0.356	0.659	0.689
Soil type*CL*Species (random)	0.603	0.460	0.619

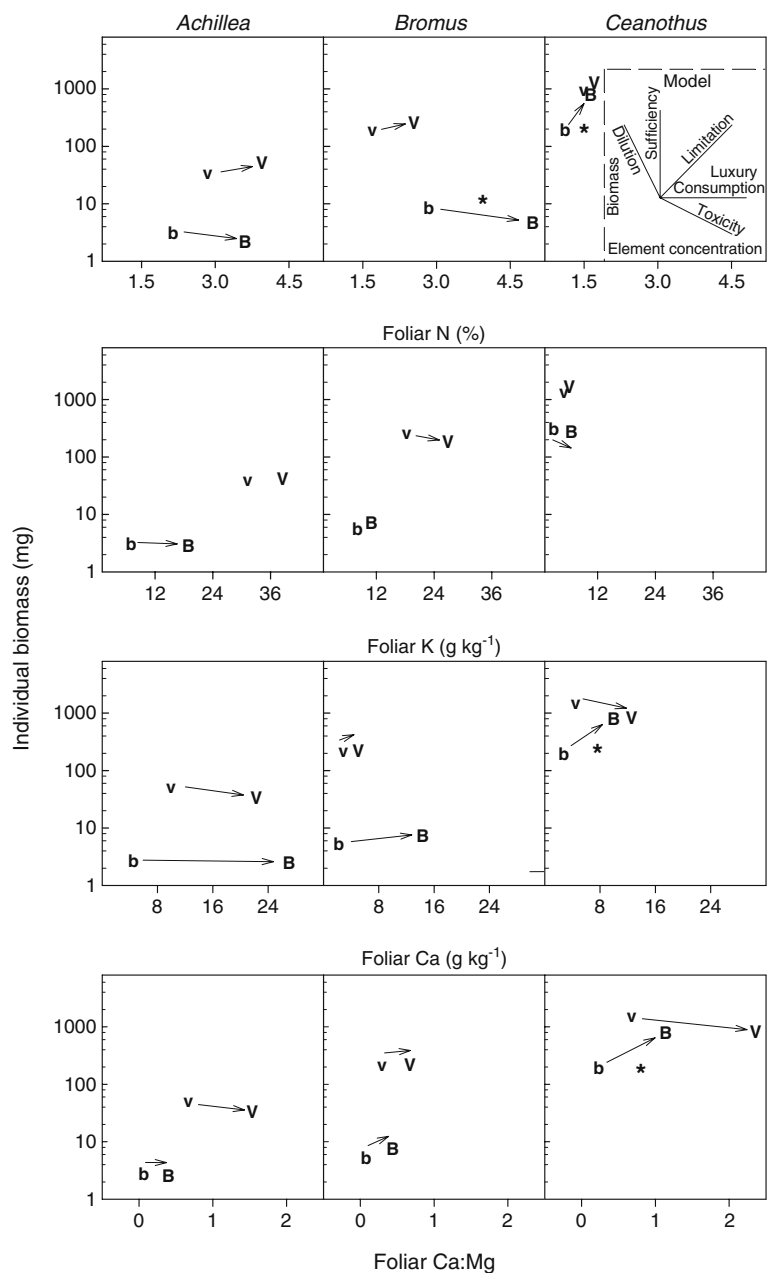
model for Ca concentration, species  $\times$  soil type  $\times$  K with NK model for K concentration). Root length density was greater on vegetated than barren soils and increased slightly but significantly with added Ca on barren soils only (Fig. 3, Online Resource Table 2).

Foliar Mg and heavy metal (Fe, Mn, Zn, Cu, Mo, Ni, Co, and Cr) concentrations were significantly higher on barren than vegetated soils for *Achillea* and *Bromus* (except Mn and Mo for *Achillea*). *Ceanothus* differences were non-significant, except in the case of Cu, where vegetated was greater than barren (Fig. 4, orthogonal contrasts for species  $\times$  soil type interactions, least squares means obtained from NCa models). Without added Ca, all species showed significantly higher foliar Ca and Ca:Mg ratios for vegetated than barren soils (Fig. 2, linear contrasts for species  $\times$  soil type  $\times$  Ca least squares means for NCa model). With added Ca, foliar Ca concentrations on barren soils surpassed those on vegetated soils for *Achillea* and *Bromus*. Foliar Ca:Mg showed a greater proportional increase on barren than vegetated soils with added Ca, but

absolute levels did not surpass those of vegetated soils with added Ca (Fig. 2).

On barren soils *Achillea* and *Bromus* roots had significantly higher concentrations of potentially toxic elements (Mg, Fe, Ni, Co, Cr) than *Ceanothus*, suggesting better avoidance of toxicity in the shrub. On vegetated soils where metal toxicity was less apparent, the difference between the species was not present (Fig. 5). For *Achillea* and *Bromus* but not for *Ceanothus*, root concentrations of potentially toxic elements (Mg, Fe, Mn, Ni, Co, Cr) were higher for plants grown on barren versus vegetated soil, although these differences were not always significant (Fig. 5). This again is consistent with *Ceanothus* avoiding metal toxicity on barren soils better than *Achillea* and *Bromus*. In all but one case, shoot concentrations of Mg and heavy metals increased more than root concentrations on barren relative to vegetated soils, suggesting limited capacity to sequester potentially toxic elements in roots on these soils for all three species. Nevertheless, on both soils *Ceanothus* protected shoots from high concentrations

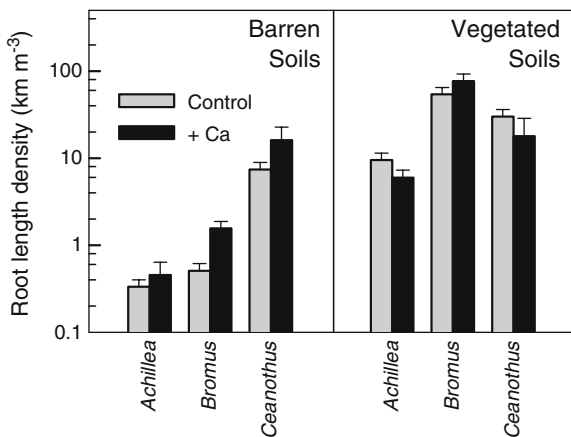
**Fig. 2** Least squares means for biomass plotted against foliar elemental concentrations for the three study species grown on two types of serpentine soils (barren = b, vegetated = v). Uppercase letters represent all treatments for which the element on the x-axis was added, while lowercase letters represent all treatments for which it was not added. Arrows denote significant concentration increases ( $P < 0.05$ , based on orthogonal linear contrasts for species  $\times$  soil type  $\times$  element). Asterisks denote significant biomass increases or decreases. The plotted locations of the upper or lower-case letters are the locations of the data values (arrows were drawn in manually). Model in upper right after James et al. (2005). Note log scales



of these potentially toxic elements better than the other two species (especially Fe, Mn, Cu, Ni, Co, Cr, Fig. 6). Although we measured heavy metal concentrations in *Ceanothus* foliage only and not in stems, O'Dell et al. (2006) found low Ni and Mn concentrations in *Ceanothus* stems relative to foliage. Thus, we are confident that *Ceanothus* does not sequester heavy metals in stem tissue. *Ceanothus* maintained the highest root Ca:Mg ratio of the three species on

barren soil (Online Resource Fig. 2), which it achieved not by taking up more Ca, but by maintaining lower Mg levels (Fig. 5). It also showed the most homeostatic translocation of Ca from roots to foliage between the two soils types (Online Resource Fig. 2).

For *Achillea* and *Bromus*, log biomass was strongly negatively correlated with log of foliar Mg and heavy metal concentrations (particularly Fe, Co, Ni, and Cr), though the relative strengths of the correlations varied



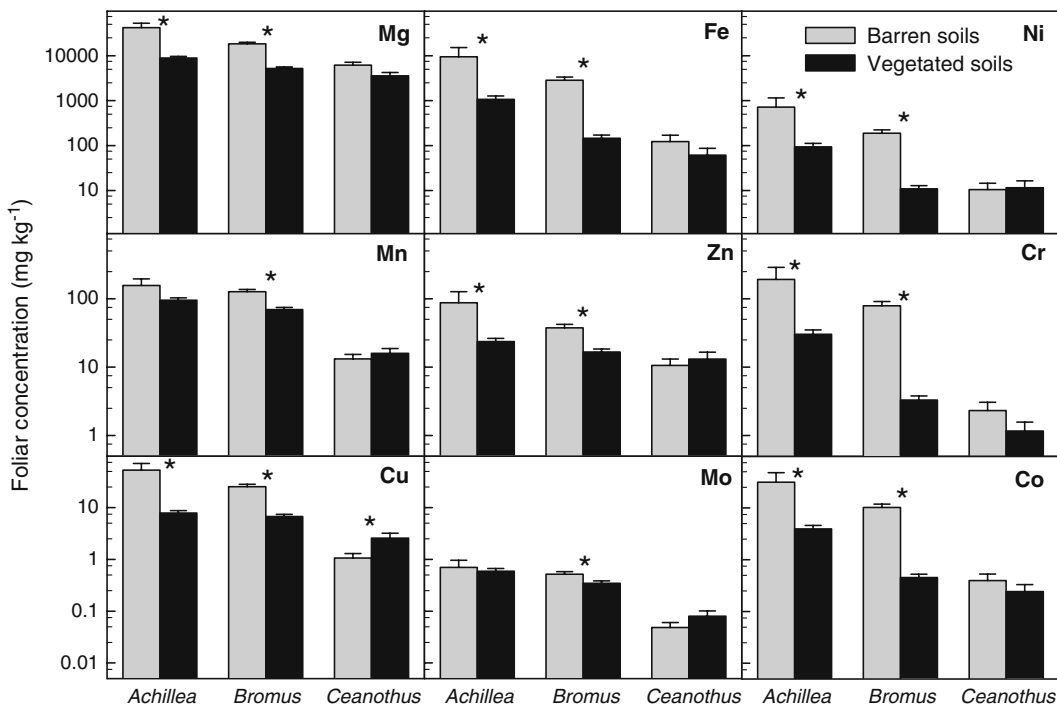
**Fig. 3** Least squares means and SE ( $n=5-12$ ) for root length density of the three study species grown in serpentine soils collected from barren and vegetated areas. Note log scales

with species (Fig. 7). Log biomass for *Ceanothus* was correlated only with Mg (negatively) and Ca:Mg (positively). This suggests that heavy metals may play an important role in limiting growth on these soils for *Achillea* and *Bromus* but not for *Ceanothus*, while Ca and/or Mg (or Ca:Mg) limit growth for all three species.

### Discussion

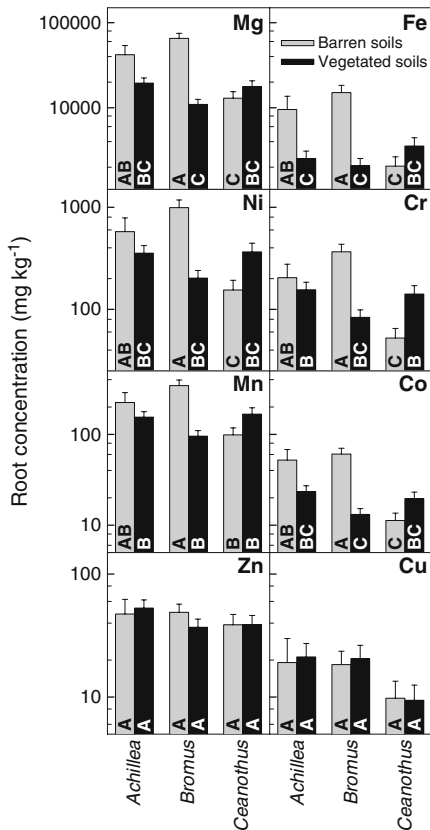
Large differences observed in plant growth under well-watered conditions indicate that soil chemistry is an important factor maintaining the barren-vegetated pattern. While the fine-scale physical differences we measured may also play a role, they were relatively small, leading us to focus on the larger differences we observed in soil chemistry. More work could be done in the laboratory or the field to better ascertain the relative importance of the small but significant soil texture differences we measured.

While soil chemistry is of clear importance in maintaining the barren-vegetated pattern at New Idria, the relative importance of the various soil chemical properties varied from one species or functional group to another. In particular, *Achillea* and *Bromus* were strongly affected by heavy metal availability on barren soils, while *Ceanothus* was able to protect its shoots from high metal concentrations by exclusion and limited root-to-shoot translocation. Supporting evidence for this includes 1) the lesser degree of growth reduction for *Ceanothus* than for the other two



**Fig. 4** Least squares means and SE ( $n=14-24$ ) for foliar elemental concentrations of the three study species grown in serpentine soils collected from barren and vegetated areas.

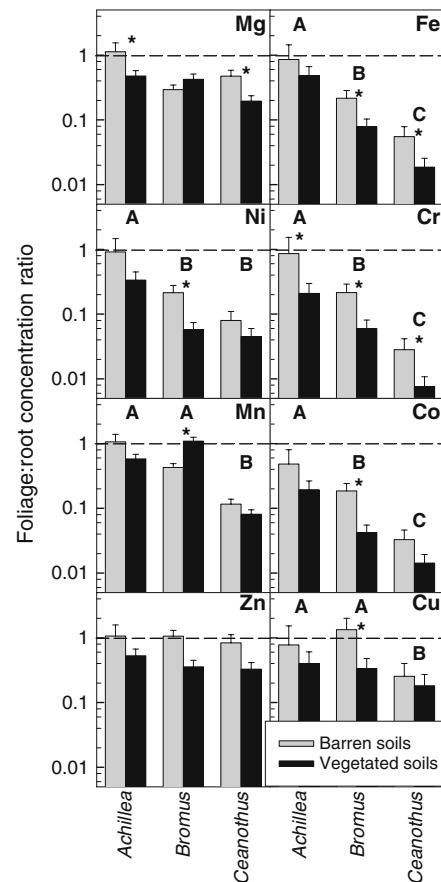
Asterisks denote significant soil type difference within species ( $P<0.05$  in orthogonal linear contrasts). Note log scales



**Fig. 5** Least squares means and SE ( $n=4-6$ ) for root Mg and heavy metal concentrations for the three study species grown in serpentine soils collected from barren and vegetated areas. Letters denote results of Tukey-Kramer comparisons for species  $\times$  soil type LS means— values not connected by the same letter were significantly different ( $P<0.05$ ). Note log scales

species on barren relative to vegetated soils; 2) the positive response of *Ceanothus* to N and Ca on barren soils, which was lacking for the other two species; and 3) the highly significant log-log relationships between biomass and foliar heavy metal concentrations for *Achillea* and *Bromus* combined with the lack of this relationship in *Ceanothus*. The fact that these relationships extended to vegetated serpentine soils indicates that heavy metals limited the growth of these species, albeit to a lesser degree, on these soils as well. The root metal concentration pattern suggests a mechanism for this difference in metal tolerance, indicating that *Ceanothus* may both take up and translocate lesser quantities of these metals from roots to shoots.

While it is clear that Mg and heavy metals limited growth of *Achillea* and *Bromus*, particularly on barren soils, neither Mg nor any one heavy metal distin-



**Fig. 6** Least squares means and SE ( $n=4-6$ ) for foliage:root concentration ratios for Mg and heavy metals for the three study species grown in serpentine soils collected from barren and vegetated areas. Reference line at 1 represents equal root and shoot concentrations. Asterisks indicate significant soil type differences within a species ( $P<0.05$ , orthogonal linear contrasts within species  $\times$  soil type interaction), and letters show overall species differences (Tukey-Kramer— species not connected by the same letter were significantly different). Note log scale

guished itself from the others as the strongest cause of growth limitation. A principal components analysis on pot study foliar data yielded a first principal component that explained 68% of the total variation and had relatively even loadings for Mg, Fe, Mn, Cu, Ni, Co, and Cr (0.32–0.35). Absolute values of growth response coefficients (slopes of log-log plots between foliar elements and biomass) were greatest for Cr, Co, Ni, and Fe for *Bromus* and Ca:Mg, Fe, Co, and Cr for *Achillea* (coefficients listed in order of magnitude, all coefficients were negative except Ca:Mg, Fig. 7). In addition, foliar concentrations of Mg and many heavy metals were highly positively correlated in *Achillea* and *Bromus* foliage, but only

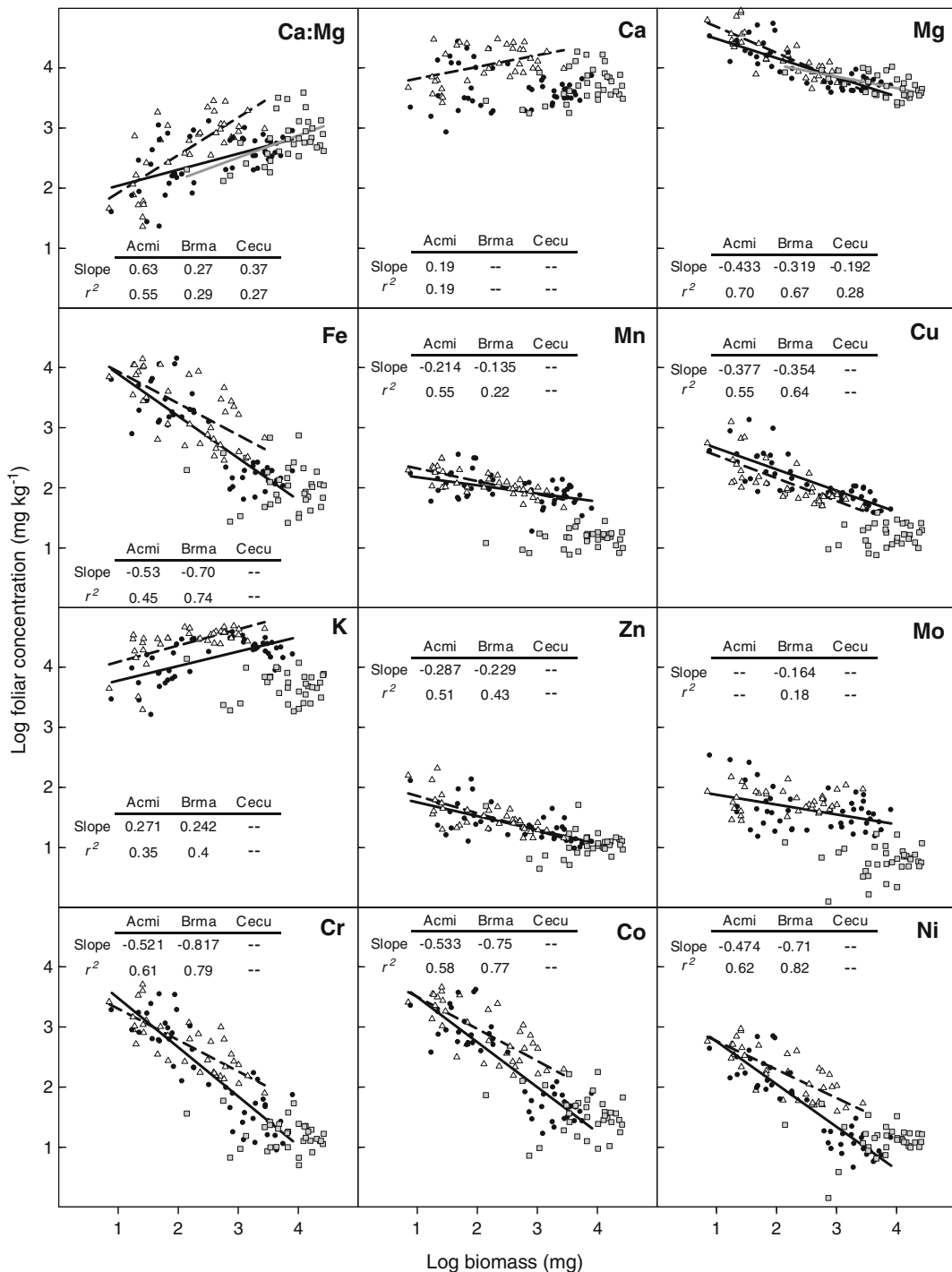
moderately correlated in *Ceanothus* foliage (Online Resource Table 5). Lyon et al. (1971) also reported significant positive correlations between Mg and some heavy metals in New Zealand serpentine species, and Shewry and Peterson (1976) observed positive correlations between shoot Cr and Ni for 5 of 8 species growing in UK serpentine soils. Yang et al. (1985) found positive correlations among Fe, Mn, Ni, Co, and Cr concentrations in foliage of woody species growing on New Caledonia serpentine soils. We hypothesize that uptake of Mg and heavy metals may be correlated. While some transporters are specific to a particular metal, others have been shown to transport multiple metals (Hall and Williams 2003). In addition, plant-released reducing or chelating agents needed for Fe uptake may also reduce Cu or mobilize Zn (Palmer and Gueriot 2009).

Interestingly, we measured higher Fe, Mn, and Ni concentrations on vegetated than barren soils, but found the opposite pattern in foliage and roots of *Achillea* and *Bromus* grown on these soils. Vegetated soils were also significantly higher in organic matter, which has been shown to chelate heavy metals, reducing their uptake by plants (Halstead 1968; O'Dell and Claassen 2006). Fernandez et al. (1999) measured increased available Mn and Ni with organic matter (peat moss) addition to a serpentine soil (perhaps because of a reduction in pH that made these metals more soluble) but decreased concentrations in plant tissue and increased biomass relative to plants on untreated serpentine soil. They also measured relatively high Cr in plants growing on these soils despite low levels of available soil Cr. Robinson et al. (1999) found that adding chelators (NTA, DTPA, EDTA) to a serpentine soil decreased plant Ni uptake but increased (ammonium acetate) extractable Ni and Co. Together these studies suggest that chelated heavy metals (whether chelated by organic matter or some other means) may be relatively unavailable for plant uptake but still detectable by soil extraction methods, and this provides a possible explanation for our seemingly opposite soil and foliar heavy metal patterns (i.e., the higher concentrations of heavy metals we measured on vegetated soils may have been chelated by the greater organic matter present in these soils and unavailable for uptake). These results and ours highlight the difficulty of measuring truly plant-available heavy metals on serpentine soils and emphasize the importance of

measuring plants grown on these soils as well. Our results also point out the need to base conclusions on multiple species if possible, as species clearly deal differently with metals.

Despite careful washing of foliage and roots, we considered the possible effects of soil contamination, particularly on 1) small herbaceous plants growing close to the surface on barren soils and 2) roots in general. While some contamination of roots with soil particles is likely in any study of this kind, we rejected the hypothesis that contamination drove the foliar or root element concentration patterns we observed. For foliage, strong relationships between biomass and foliar elemental concentrations (including heavy metals) existed for the herbaceous species, and these relationships extended into the vegetated soils, where the herbaceous species were not small in stature and hence were very unlikely to have been contaminated. For roots, if *Ceanothus* roots had lower metal concentrations on barren soils than the other species due only to less contamination conferred by their woodier character (smaller surface area relative to total biomass), we would have expected the same pattern on vegetated soils. However, we observed comparable root heavy metal concentrations for all three species on vegetated soils. Finally, the relative distribution of metallic elements in soils was quite different from those of roots and foliage, leading us to conclude that the foliar and root concentrations we measured were the result of plant processes and not of contamination by soil particles. We were, however, unable to determine what portion of the heavy metal we measured was contained in the root symplast vs. the apoplast and whether this varied with species or soil type. Nevertheless, sequestration in either of these compartments is a valid method for minimizing metal concentrations in foliage.

Elemental concentrations in foliage collected from *Pinus sabiniana* and *Arctostaphylos glauca* growing both in vegetated serpentine soils and in shrub islands within barren serpentine soil areas provided further evidence that plant species respond differently to serpentine soils but did not strongly implicate any specific factor(s) limiting plants on barren relative to vegetated soils. The greatest variation in elemental concentrations occurred between species. Soil type differences accounted for only a small proportion of the total variation in the data and were not always consistent between species (further evidence that



**Fig. 7** Log log plots of foliar concentrations vs. biomass. Slopes are growth response coefficients sensu Poorter and van der Werf (1998). Open triangles and dashed lines are *Achillea* (Acmi), solid black circles and lines are *Bromus* (Brma), and gray squares and lines are *Ceanothus* (Cecu). Least squares fit lines, slopes,

and  $r^2$  values are provided for statistically significant correlations. All  $P$ -values  $< 0.0001$  except as follows: Ca:Mg *Ceanothus*  $P=0.0018$ ; Ca *Achillea*  $P=0.0084$ ; Mg *Ceanothus*  $P=0.0014$ ; Mn *Bromus*  $P=0.0009$ ; Mo *Bromus*  $P=0.0033$



species responses to serpentine soils are vastly different). Inconsistencies between species generally occurred when there was a barren-vegetated difference for *Arctostaphylos* but not for *Pinus*, indicating that *Pinus* may be the more homeostatic of the two species. The strongest and most consistent differences between soil types were in Fe and Mo (higher concentrations in shrub-island foliage). It should also be emphasized, however, that it was not possible to measure foliar elements in plants growing on truly barren soils in the field (where no plants grew), and soil measurements showed many differences in shrub islands relative to truly barren soils. As such, it was also important to measure foliar concentrations in the plants we grew on soil collected in truly barren areas, as these may best reflect the chemical stresses such soils present.

Others have found ameliorative effects of Ca on metal toxicity (Gabbrielli & Pandolfini 1984; Gabbrielli et al. 1990). Although we observed an increase in root length density and higher foliar and root Ca concentrations when Ca was added to barren soils, these were not accompanied by a biomass increase for *Achillea* or *Bromus*. Halstead (1968) also observed an increase in foliar Ca concentration without any accompanying growth increase in oats grown on a Guatemalan serpentine soil fertilized with  $\text{CaSO}_4$ . Adding organic matter or  $\text{Ca(OH)}_2$  lowered foliar Ni concentrations and increased foliar Ca and total biomass, leading the author to conclude that Ni toxicity was the limiting factor for this plant in this soil and that it was overcome not by increasing Ca availability but by chelating Ni, making it less available (organic matter) or raising soil pH and thus making Ni less soluble ( $\text{Ca(OH)}_2$ ).

It is curious that none of our soil amendments were effective in significantly increasing growth for any species on vegetated serpentine soils. One possible explanation is continued metal toxicity on vegetated soils for *Achillea* and *Bromus* (see Fig. 7). Another possible explanation is that some other nutrient (such as P) limited or co-limited growth on these soils. P levels on vegetated soils (9.4 mg/kg, bicarbonate extractable) were relatively low, though not uncharacteristically low for serpentine soils. It is also possible that the amounts of N, K, and Ca we added were not large relative to the pools of these elements already available on vegetated serpentine soils. Indeed, N levels in our fertilizer solutions were low relative to those measured in soil solution by Proctor

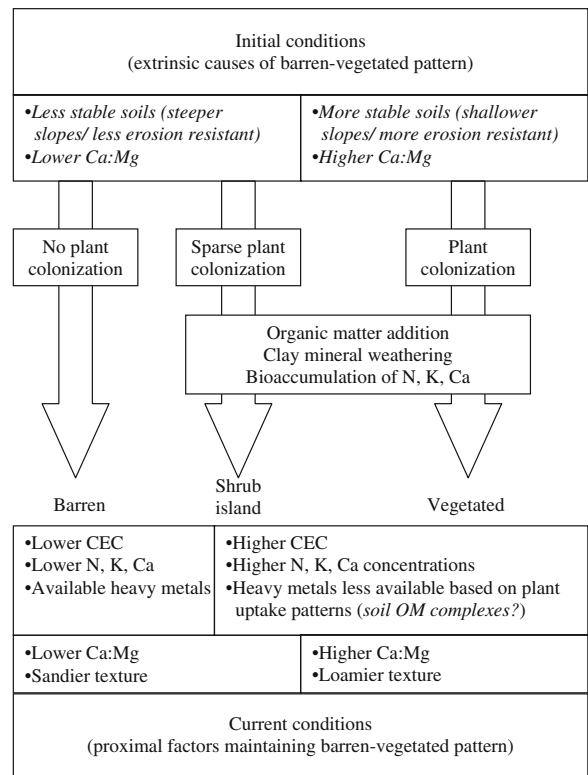
et al. (1981), though K and Ca levels were somewhat higher and much higher than these respectively. Still, given the high degree of variation among serpentine soil solution nutrient contents (28, 66, and 51-fold differences in N, K, and Ca respectively for the seven soils measured by Proctor et al.), the most meaningful comparison would be with soil solutions obtained from vegetated New Idria soils.

The positive growth response of *Ceanothus* to added N on barrens is interesting in light of its ability to fix atmospheric N when growing with the bacterial symbiont *Frankia*. We did not observe root nodulation in any of our specimens growing on barren or vegetated soils. O'Dell et al. (2006) observed nodulation on pot-grown *Ceanothus cuneatus* and *Ceanothus jepsonii* grown in unfertilized serpentine soil and a lack of nodulation when NPKCa fertilizer was added. White (1967) found a near total absence of nodulation in *Ceanothus cuneatus* collected from four serpentine sites in the field, which he attributed to low NPK, low Mo, and toxic heavy metals.

Few studies have simultaneously addressed multiple hypotheses for serpentine infertility (i.e., low Ca:Mg, high heavy metals, low macronutrients), and even fewer have done so for multiple species/functional types on more than one serpentine soil. Our results, taken together with the few other studies that simultaneously addressed multiple infertility hypotheses, suggest that on serpentine soils, herbaceous species may be more strongly affected by high heavy metal concentrations than woody species. Reports of metal toxicity in plants grown on serpentine soils or in soil solutions that approximate these soils' elemental concentrations are generally for herbaceous serpentine species (Nagy and Proctor 1997), non-serpentine ecotypes of herbaceous species (Proctor 1971; Woodell et al. 1975), or agricultural crop plants (Hunter and Vergnano 1952; Halstead 1968; Anderson et al. 1973). Studies of woody vegetation growing on serpentine soils that addressed both Ca:Mg and heavy metal hypotheses of serpentine infertility have found significant positive relationships between growth or cover of woody species and soil (Alexander et al. 1989) or foliar (Koenigs et al. 1982) Ca or Ca:Mg and not heavy metals. O'Dell et al. (2006) showed that the principal difference between serpentine and non-serpentine congeners of woody shrubs growing on a metalliferous California serpentine soil was in their ability to take up and translocate

Ca to foliage and not their heavy metal resistance (serpentine and non-serpentine congeners equally sequestered heavy metals in roots). However, Kayama et al. (2005) showed higher Ni tolerance (possibly mediated through ectomycorrhizal fungi) in a common Japanese serpentine *Picea* (spruce) species than in two *Picea* species (one native, one introduced) that are generally excluded from serpentine soils. Examination of foliar elemental concentrations measured in native species on two British serpentine soils by Johnston and Proctor (1977) generally shows lower heavy metal concentrations (Fe, Ni, Co, and Cr) and higher Ca:Mg ratios in the woody plants (particularly *Juniperus communis*—common juniper) than in the herbaceous plants, which included both grasses and forbs. With the notable exception of Mn, a similar pattern (generally lower heavy metals and higher Ca:Mg in woody plant foliage) was observed for 12 herbaceous and 5 woody species growing on a serpentine soil in Maine, USA (Pope et al. 2010).

While our study identified clear differences in chemistry between barren and vegetated soils that affect plant growth and likely help *maintain* the barren-vegetated landscape pattern, the results also provide some clues as to the initial *causes* of this landscape pattern (Fig. 8). Our study leaves only two possible initial causes—either the explanation for the difference between the two soil types lies with 1) lack of soil development on barrens (perhaps due to instability caused by steeper slopes and/or softer, more erodible parent material, as hypothesized by Coleman 1996 and Kruckeberg 1999) or 2) there are differences in parent material that influence soil chemical properties (e.g., Ca-bearing accessory minerals on vegetated soils – see McGahan et al. 2008) (upper panel Fig. 8). Indeed, Ca:Mg ratios of shrub-island soils more closely matched those of barren than vegetated soils, suggesting that this difference may be related to differing parent material properties (lower panels Fig. 8). This could be verified by analyses of underlying parent material. In contrast to this, shrub island soils were generally intermediate between barren and vegetated soils (total N, Ca, Mn) or more similar to vegetated soils (CEC, organic matter, Mg, K, Fe, Ni, Cr) for most properties for which significant differences were measured, indicating that plants may have influenced these properties (arrows Fig. 8). In shrub-island and vegetated soils, added organic matter and facilitated weathering of clay



**Fig. 8** Diagram depicting extrinsic factors causing the barren-vegetated pattern on the New Idria serpentine mass and proximal causes maintaining the pattern. Factors in italics are hypotheses which can be neither supported nor refuted by the current study

minerals would increase CEC leading to greater retention and bioaccumulation of Ca and K. N also accumulates selectively under the long-lived shrubs in shrub islands and in vegetated serpentine soils. In summary, some of the proximal stresses maintaining the barren-vegetated pattern (e.g., differences in heavy metal availability based on plant uptake in our experiments, N concentrations) are likely feedbacks caused by differential soil development due to differences in plant colonization. In contrast, the extrinsic causes of differential colonization and hence differential soil development appear to be 1) differences in parent material stability and/or 2) differences in parent material Ca:Mg (Fig. 8). Future studies should assess mechanisms affecting heavy metal availability, parent material characteristics, and bioaccumulation rates and patterns.

Our results have some important implications for restoration of vegetation or establishing shrub and tree islands on serpentine soil areas degraded by logging,

mining, and off-highway vehicle use. Perhaps the most important of these is the need to plant metal-tolerant species first on barrens. Once plants have become established and soil organic matter increases, metal availability may be reduced sufficiently for less metal-tolerant species. Alternatively, soil amendments that ameliorate metal toxicity may allow less metal tolerant plants to become established. One interesting possibility would be to use soil collected from vegetated areas, if such areas need to be cleared or disturbed for any reason. Organic amendments such as peat moss or compost have also been shown to ameliorate metal toxicity (Halstead 1968; Fernandez et al. 1999; O'Dell and Claassen 2006). Indeed, some of the most promising results to date in serpentine restoration/revegetation have been obtained using organic amendments (O'Dell and Claassen 2006). Although care should always be taken to minimize the distribution of weed seeds, it appears that the weed of greatest concern for this area, *Bromus madritensis* ssp. *rubens*, is excluded from barren areas by soil chemical properties, and thus the spread of this species is of greater concern for recently disturbed vegetated areas, where it is not excluded by soil chemistry. Our results indicate that adding mineral N or Ca to barrens might assist at least one metal tolerant species (*Ceanothus cuneatus*) to establish more quickly. If this is attempted in the field, it should be done incrementally at a small scale and monitored well, as Ca did increase *Bromus* root growth on barren soils, and because N fertilization has favored non-natives over natives in some cases in previous studies (e.g., Huenneke et al. 1990; Going et al. 2009). In addition, Harrison et al. (2006) found that predictors of exotic species cover on serpentine soils throughout California included higher soil Ca:Mg.

The fact that the factors maintaining the New Idria barren-vegetated pattern appear to be specific to plant species or functional groups (i.e., low Ca/high Mg for all species, low N for some, high heavy metals for others) emphasizes a point made by Alexander et al. (2007): the relative importance of the various facets of the serpentine syndrome in plant growth limitation are specific not only to the soil, but also to the plant species or functional group in question. This more flexible view of the serpentine syndrome reconciles the disparate results of many researchers and eschews generalizations applied to all serpentine plants and soils based on the results of one or a few studies.

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