



Congeneric serpentine and nonserpentine shrubs differ more in leaf Ca:Mg than in tolerance of low N, low P, or heavy metals

Ryan E. O'Dell^{1,3}, Jeremy J. James² & James H. Richards¹

¹Department of Land, Air and Water Resources, University of California, One Shields Avenue, Davis, CA, 95616-8627, USA. ²USDA, Agricultural Research Service, 67826-A Hwy 205, 97720-9394, Burns, OR, USA.

³Corresponding author*

Received 21 March 2005. Accepted in revised form 21 June 2005

Key words: calcium, chaparral, macronutrients, magnesium, nickel, serpentine shrubs

Abstract

Serpentine soils limit plant growth by NPK deficiencies, low Ca availability, excess Mg, and high heavy metal levels. In this study, three congeneric serpentine and nonserpentine evergreen shrub species pairs were grown in metalliferous serpentine soil with or without NPKCa fertilizer to test which soil factors most limit biomass production and mineral nutrition responses. Fertilization increased biomass production and allocation to leaves while decreasing allocation to roots in both serpentine and nonserpentine species. Simultaneous increases in biomass and leaf N:P ratios in fertilized plants of all six species suggest that N is more limiting than P in this serpentine soil. Neither N nor P concentrations, however, nor root to shoot translocation of these nutrients, differed significantly between serpentine and nonserpentine congeners. All six species growing in unfertilized serpentine soil translocated proportionately more P to leaves compared to fertilized plants, thus maintaining foliar P. Leaf Ca:Mg molar ratios of the nonserpentine species were generally equal to that of the soil. The serpentine species, however, maintained significantly higher leaf Ca:Mg than both their nonserpentine counterparts and the soil. Elevated leaf Ca:Mg in the serpentine species was achieved by selective Ca transport and/or Mg exclusion operating at the root-to-shoot translocation level, as root Ca and Mg concentrations did not differ between serpentine and nonserpentine congeners. All six species avoided shoot toxicity of heavy metals by root sequestration. The comparative data on nutrient deficiencies, leaf Ca:Mg, and heavy metal sequestration suggest that the ability to maintain high leaf Ca:Mg is a key evolutionary change needed for survival on serpentine soil and represents the physiological feature distinguishing the serpentine shrub species from their nonserpentine congeners. The results also suggest that high leaf Ca:Mg is achieved in these serpentine species by selective translocation of Ca and/or inhibited transport of Mg from roots, rather than by uptake/exclusion at root surfaces.

Introduction

Serpentine soil habitats have a widespread, but patchy global distribution (Brooks, 1987). In California, USA, serpentine landscapes occupy ~2860 km² (1% of the land area) (Kruckeberg, 1984). Serpentine substrates are primarily

composed of a suite of magnesium-rich minerals including lizardite, antigorite, and chrysotile and are frequently associated with deposits of heavy metals including Ni and Cr (Roberts and Proctor, 1992). Floras rich in endemics have evolved in response to the unusual chemical features associated with serpentine soils globally. In New Caledonia and Cuba, which contain some of the richest serpentine endemic floras in the world, 1176 species (39% of the total endemic taxa in

* FAX No: +530-752-1552.
E-mail: reodell@ucdavis.edu

New Caledonia) and 920 species (31% of the total endemic taxa in Cuba) are serpentine endemics (Kruckeberg, 2002). Serpentine plant communities in the northern California Coast Ranges collectively include an estimated 189 serpentine endemic species (9% of the total endemic taxa in California) (Kruckeberg, 1984). Despite having a rich diversity of endemic species, serpentine soils are generally regarded as low productivity environments with the accompanying flora often exhibiting typical stress tolerance features such as xeromorphic foliage, reduction in stature and density, and high root:shoot biomass ratios. The three main mineral nutrition factors that have been proposed to limit plant productivity and influence speciation on serpentine soils are (1) deficiency of the essential macronutrients NPK, (2) low availability of Ca relative to Mg, and (3) high levels of phytotoxic heavy metals (Brooks, 1987; Kruckeberg, 1984; Roberts and Proctor, 1992). At present, there is no general consensus on which factor is most important or even if one factor is more important than the others.

Plant species tolerant of multiple nutrient stresses imposed by serpentine soils are expected to have slow growth rates, tolerance of low soil N, P, K, and Ca availability, and tolerance of Mg and heavy metal toxicities (Kruckeberg, 1984; Proctor, 1999). In contrast, species growing near, but not on serpentine are expected to lack at least one of these key traits and are thus excluded from such environments. These two species groups have been broadly categorized as serpentine-tolerant and serpentine-intolerant, respectively (Kruckeberg, 1984).

The low macronutrient content of serpentine substrates is attributed to low organic pools (Brooks, 1987) and lack of P and K as significant mineralogical constituents in parent materials (Burt et al., 2001). Documented stress tolerance features of serpentine plants related to low macronutrient availability include high root/shoot biomass ratios and inherently slow growth rates (Freitas and Mooney, 1996; Kruckeberg, 1984; Westerbergh, 1994). The low Ca content and excess Mg availability in serpentine soils are inherited from the magnesium-rich mineralogy of serpentine parent materials (Burt et al., 2001). Low soil Ca:Mg molar ratios are antagonistic to Ca uptake by plants, requiring specialized plant physiological mechanisms to maintain adequate

internal concentrations of Ca (Marschner, 2002). Mechanisms including selective Ca accumulation, Mg exclusion, and Mg sequestration have been identified in diverse serpentine plant species globally (Lyon et al., 1971; Shewry and Peterson, 1975; Wallace et al., 1982). High concentrations of phytotoxic heavy metals contained in serpentine parent materials and soils such as Ni, Cr, and Co, comprise an additional stress that serpentine plants must endure. Physiological features associated with heavy metal tolerance or avoidance in serpentine species include metal exclusion at the root level, sequestration to various plant organs, and toxicity tolerance (Baker, 1987; Shaw, 1990; Westerbergh, 1994).

To examine the relative importance of the major mineral nutrition factors that are limiting on serpentine soils and their interactions, a comparative study of congeneric evergreen serpentine and nonserpentine shrub species that dominate chaparral communities in northern California, USA was used. In this region, extensive serpentine substrates are found adjacent to nonserpentine soils. One species each from serpentine and nonserpentine chaparral communities was selected from the genera *Arctostaphylos* (Ericaceae), *Ceanothus* (Rhamnaceae), and *Quercus* (Fagaceae), producing three congeneric pairs. Within each genus, the serpentine and nonserpentine species were very similar with respect to leaf and canopy morphology, root morphology, leaf life span, and phenology. In this two-year greenhouse study, responses of the serpentine and nonserpentine congeneric pairs grown on a chromite-rich serpentine soil with (fertilized) and without (control) an NPKCa fertilizer were compared and biomass production, allocation, and tissue elemental concentrations were measured. From these measurements, the following three hypotheses were addressed: (1) nonserpentine shrub species will exhibit significantly greater response to fertilization with respect to biomass production, biomass allocation, and tissue nutrient content than their congeneric serpentine counterparts (2) serpentine shrub species will be significantly more tolerant of Ca deficiency and/or Mg toxicity and have higher leaf Ca:Mg molar ratios than their congeneric nonserpentine counterparts, and (3) serpentine shrub species will exhibit significantly greater tolerance or avoid

heavy metal toxicity better than their congeneric nonserpentine counterparts.

Materials and methods

Soil collection and analysis

Soil was collected from a chromite prospect located on serpentine parent material within the Donald and Sylvia McLaughlin UC Natural Reserve (Yolo, Napa, and Lake Counties, California, USA; 38° N, 122° W; 525–800 m elevation; <http://nrs.ucdavis.edu/mclaughlin.html>). This area was chosen as a soil source due to the high concentrations of heavy metals associated with the underlying chromite deposit and the typical serpentine chaparral vegetation present on it. Soil was collected from the O, A, and B horizons on the prospect's undisturbed perimeter. Collected soil was screened to 1.25 cm consistency and mixed 3:1 with Turface® (Profile Products LLC, IL, USA) to improve physical properties for the long-term pot study.

For soil analysis, 3.8 L of the serpentine soil mix was treated with 18 g of Osmocote® 18-6-12 fertilizer (Scotts Company, OH, USA; this fertilizer includes substantial Ca and S in addition to NPK) duplicating the fertilization treatment used for plant growth. Additionally, 3.8 L of soil mix was retained in an untreated state to represent the control treatment. The two soil batches were saturated with distilled water to a paste, spread into separate glass dishes, and covered. Following a two-week incubation period, the soil batches were dried. The soil was then sieved to pass 2 mm, separated into three equal portions, and analyzed for physical properties and available nutrients (A&L Agricultural Labs, Inc., CA, USA). The following methods were used for soil analyses: Weak Bray method for $\text{HPO}_4^{2-}\text{-P}$; ammonium acetate (1 M, neutral pH) extract for K^+ , Ca^{2+} , Mg^{2+} ; hot water extract for $\text{SO}_4^{2-}\text{-S}$; KCl (2 M) extract for $\text{NO}_3^-\text{-N}$; and DTPA (0.1 M) extract for Zn^{2+} , Mn^{2+} , Fe^{2+} , Cu^{2+} , Ni^{2+} , Cr^{2+} , Co^{2+} , and Mo^{6+} .

Study species

Serpentine and nonserpentine chaparral plant communities grow adjacent to each other at the

McLaughlin Reserve and are distinguished by differing substrate characteristics in addition to unique species assemblages. Six sclerophyllous, evergreen shrub species that dominate California serpentine and nonserpentine chaparral were examined in this study. From the mature serpentine chaparral community (Leather Oak Series; Sawyer and Keeler-Wolf, 1995) at the soil collection site, juveniles of *Arctostaphylos viscida* Parry ssp. *pulchella* (T.J. Howell) P.V. Wells (sticky whiteleaf manzanita), *Ceanothus jepsonii* Greene var. *albiflorus* J.T. Howell (Jepson ceanothus, muskbrush), and *Quercus durata* Jepson var. *durata* (leather oak) were collected in winter, 2000. From adjacent mature nonserpentine chaparral (Interior Live Oak Series and Wedgeleaf Ceanothus Series; Sawyer and Keeler-Wolf, 1995) juveniles of *Arctostaphylos manzanita* Parry ssp. *glaucescens* P.V. Wells (whiteleaf manzanita), *Ceanothus cuneatus* (Hook) Nutt. var. *cuneatus* (wedgeleaf ceanothus, buckbrush), and *Quercus berberidifolia* Liebm. (scrub oak) were collected.

Experimental methods and treatments

Collected transplants were potted individually in 3.8 L pots with the prepared soil mix and blocked by size. The pot size chosen had a volume approximately 3-fold greater than that of the initial root volume to provide ample space for root growth during the course of the study. Fertilized and control treatments were randomly assigned and treatments were replicated in five blocks for *Arctostaphylos*, six blocks for *Ceanothus*, and four and five blocks for *Quercus durata* and *Q. berberidifolia*, respectively. Differences in replication were due to early mortality of transplants. The fertilized treatment received 18 g of Osmocote® 18-6-12 slow-release fertilizer thoroughly mixed into the soil. The control received no amendment. Soil moisture was maintained near field capacity throughout the study.

In autumn at the end of the first growing season, the plants were relocated outside the glasshouse during the shrubs' natural winter dormancy cycle. In late winter, 2001, before the second growing season, the plants assigned to receive fertilizer were re-fertilized with the same quantity and type of fertilizer. Fertilizer was applied by insertion through ~1 cm diameter holes

probed 10 cm deep into the soil at multiple, equally-spaced points within the pot. All plants were then returned to the glasshouse for the duration of the second season. During both growing seasons, glasshouse illumination (natural only), temperature, and humidity tracked near ambient in Davis, CA, USA. Cooling pads and fans buffered against high temperatures and prevented maximum air temperature from exceeding 40 °C.

Biomass harvest

At the end of the second growing season, each shrub was clipped at the soil surface, rinsed with distilled water, dried, and leaves and stems separated before weighing and tissue elemental analysis. Fine roots were harvested by sampling a quarter of the soil volume in the pot (0.95 L) and washing the soil sample with a hydropneumatic elutriation device (Smucker et al., 1982). The remaining soil volume was washed to recover the coarse root fraction. Woody debris, including dead roots, was removed from the samples, which were oven-dried before weighing and tissue elemental analysis.

Plant tissue elemental analysis

Dried leaf, stem, coarse root, and fine root samples were ground to a fine powder. N concentration was determined by micro-Dumas dry combustion using a Carlo Erba NA1500 NC elemental analyzer (Fisons Instruments, Milan, Italy). Samples were also microwave digested with nitric acid following the method of Sah and Miller (1992) and analyzed by ICP-AES analysis (Thermo-Jarrell Ash Corp., MA, USA) for all other elements including P, K, S, Ca, Mg, Fe, Cu, Mn, Zn, Ni, Cr, Co, and Mo (Meyer and Keliher, 1992).

Statistical methods

Because tests of the hypotheses required comparisons of multiple responses between groups, a multivariate analysis of variance (MANOVA) was used followed by *a priori* contrasts. When these contrasts indicated significant multivariate differences between groups, the canonical structure was analyzed to evaluate which response variables had the strongest correlation with the canonical axes; variables with stronger correlations contribute

relatively more to potential differences between treatments than variables with weaker correlations (Scheiner, 1993). Response variables with the strongest correlation with the canonical axes were then analyzed with univariate analysis of variance (ANOVA) followed by univariate contrasts (Tabachnick and Fidell, 1996). Normality of residuals was evaluated using Shapiro-Wilk tests on individual univariate models. Because MANOVA is robust against heterogeneity of covariance matrices (Scheiner, 1993), particularly when cell sizes are equal (Tabachnick and Fidell, 1996), no remedial measures were taken to correct heterogeneity of variance in the MANOVA models. However, when univariate approaches were used, the ANOVA models were weighted by the inverse of the variance to correct for heterogeneous variances among treatments (Neter et al., 1990). Sequential Bonferroni corrections were made to maintain an experiment-wise error rate of $\alpha = 0.05$ (Rice, 1989).

To determine if serpentine and nonserpentine congeners differed in nutritional status when grown on a common serpentine soil, response variables in the MANOVA model included leaf concentrations of essential plant nutrients (N, P, K, S, Ca:Mg molar ratio, Fe, Ni, Zn, Cu, and Mn) and Cr. Ca:Mg molar ratios were used based on the prediction that serpentine shrubs would exhibit significantly greater tolerance of Ca deficiency, Mg toxicity, and have higher leaf Ca:Mg molar ratios than their congeneric nonserpentine counterparts. To evaluate if congeners differed in response to fertilization, response variables in the MANOVA model were leaf concentrations of plant macronutrients included in the fertilizer (N, P, K, S, Ca). To evaluate the extent heavy metal toxicity differentially limited congener performance on serpentine soils leaf and root concentrations of Mg, Fe, Cu, Mn, Zn, Ni, Cr, Co, and Mo were included. Data were analyzed with SAS (2001).

Results

Soil nutrients

Availability of NPK and S in the fertilized soil was on average, 5-fold higher than the control (Table 1). The Ca:Mg molar ratio of the fertilized soil was nearly double that of the control,

Table 1. Chemical properties of the serpentine soil planting mix subject to control or fertilized treatments. Means \pm SE (n = 3)

	CEC (cmol _e kg ⁻¹)	Organic C (%)	pH	N (NO ₃ ⁻) (mg kg ⁻¹)	P (HPO ₄ ²⁻) (mg kg ⁻¹)	K ⁺ (mg kg ⁻¹)
Control	12.4 \pm 0.2	1.0 \pm 0.1	7.1 \pm 0.0	136.7 \pm 6.7	9.7 \pm 0.3	136.7 \pm 3.0
Fertilized	14.1 \pm 0.3	1.3 \pm 0.1	7.1 \pm 0.0	765.3 \pm 63.5	48.7 \pm 2.4	701.0 \pm 59.2
	S (SO ₄ ²⁻) (mg kg ⁻¹)	Ca ²⁺ (mg kg ⁻¹)	Mg ²⁺ (mg kg ⁻¹)	Ca:Mg (mol ratio)	Zn ²⁺ (mg kg ⁻¹)	Fe ²⁺ (mg kg ⁻¹)
Control	46.7 \pm 11.5	996.7 \pm 26.4	763.7 \pm 17.8	0.8 \pm 0.2	8.7 \pm 0.2	10.3 \pm 0.3
Fertilized	331.7 \pm 23.0	1367.7 \pm 51.7	593.0 \pm 24.3	1.4 \pm 0.1	6.4 \pm 0.4	8.0 \pm 0.6
	Cu ²⁺ (mg kg ⁻¹)	Mn ²⁺ (mg kg ⁻¹)	Ni ²⁺ (mg kg ⁻¹)	Cr ²⁺ (mg kg ⁻¹)	Co ²⁺ (mg kg ⁻¹)	Mo ⁶⁺ (mg kg ⁻¹)
Control	0.4 \pm 0.0	19.7 \pm 1.7	5.5 \pm 0.3	<0.1 \pm 0.0	0.7 \pm 0.0	0.1 \pm 0.0
Fertilized	0.4 \pm 0.0	26.7 \pm 1.9	4.0 \pm 0.2	<0.1 \pm 0.0	0.8 \pm 0.0	0.1 \pm 0.0

mostly due to a large increase in extractable Ca²⁺ from CaH₂PO₄ in the fertilizer. Micronutrient and heavy metal availability were not significantly different between control and fertilized soils with the exception of Mn, which was higher in the fertilized soil.

Plant biomass

Fertilization of the serpentine soil increased total and aboveground biomass for all six species ($P < 0.05$; Figure 1) but belowground biomass did not increase significantly for *A. manzanita*, *A. viscida*, and *C. jepsonii* ($P \geq 0.1$). Fertilized plants produced 4- to 8-fold more aboveground biomass than control plants in all species (Figure 1). Belowground biomass increased less for fertilized plants, ranging from 1.5- to 4-fold more than control plants. The relative differences in aboveground/belowground biomass allocation resulted mainly from significantly reduced root weight ratios (RWR) and increases in leaf weight ratios (LWR) ($P < 0.0001$) with relatively little change in stem weight ratio (SWR) (Figure 1). Fertilization also influenced the formation of root nodules in both *C. cuneatus* and *C. jepsonii*, which are symbiotic with nitrogen-fixing *Frankia* (Figure 2). Five of six *C. cuneatus* and three of five *C. jepsonii* control plants bore one to several nodule clusters on their roots while none of the fertilized plants of either species contained nodules. It is likely that *Arctostaphylos*, *Ceanothus*, and *Quercus* roots also hosted mycorrhizas due to the use of field-collected soil, but these were not assessed.

Plant elemental concentrations

Leaf nutrients differed significantly between serpentine and nonserpentine groups ($P < 0.05$). The canonical structure indicated that the major variable contributing to this difference was Ca:Mg molar ratio (canonical correlation 0.66). Fertilization, however, reduced differences in leaf nutrient concentrations between serpentine and nonserpentine species. As a result, leaf nutrients did not significantly differ between these groups when plants were fertilized ($P = 0.37$).

Leaf and fine root N, P, K, and S

Leaf N and S concentrations were significantly greater ($P < 0.0001$ and $P < 0.05$, respectively) in fertilized than control treatments in all six species (Figure 3 for N; S not shown) and many control plants of the nonserpentine species displayed uniformly chlorotic leaves, suggesting N deficiency. There was no significant fertilization effect on leaf P or K concentrations ($P \geq 0.05$) (Figure 3 for P; K not shown). Leaf N:P ratios were significantly greater in all fertilized plants compared to controls with the exception of *C. cuneatus*, although the trend was the same. Leaf N in control plants as percent of optimal (calculated as: (control leaf [N])/(fertilized leaf [N])*100) tended to be greater in serpentine species ($47.5\% \pm 6.7$, mean \pm SE, n = 3 genera) compared to their nonserpentine counterparts ($39.8\% \pm 8.6$, mean \pm SE, n = 3 genera), but this difference was not significant ($P = 0.14$, paired *t*-test; Figure 3).

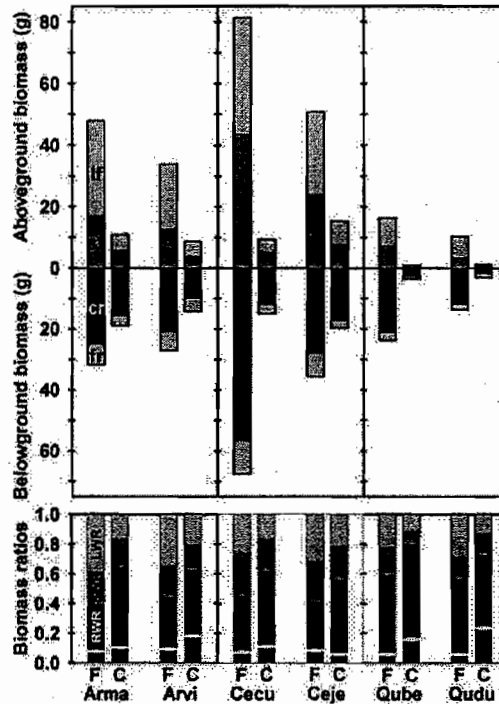


Figure 1. Biomass components (upper panel) and biomass ratios (lower panel) for three nonserpentine shrub species (left species of each pair; *Arctostaphylos manzanita* (Arma), *Ceanothus cuneatus* (Cecu), and *Quercus berberidifolia* (Qube)) and three serpentine shrub species (right species of each pair; *Arctostaphylos viscida* (Arvi), *Ceanothus jepsonii* (Ceje), and *Quercus durata* (Qudu)) in fertilized (F) or control (C) treatments. Aboveground biomass: lf, leaf; st, stem. Belowground biomass: cr, coarse root; fr, fine root. Biomass ratios: RWR, root weight ratio (white bar indicates fine root portion); SWR, stem weight ratio; LWR, leaf weight ratio. All plants within genera were approximately equal size at the beginning of the two growing-season experiment. Bars denote means, $n = 4-6$.

Fine root N concentrations, as for leaves, also were significantly greater ($P < 0.05$) in the fertilized than control treatments in all six species (Figure 3). There was also a fertilization effect on both fine root P and S concentrations ($P < 0.01$) in *Arctostaphylos* and *Ceanothus*, but not *Quercus* ($P \geq 0.2$). The fertilization effect on fine root K concentration was not significant in any of the six species ($P \geq 0.2$). Fine root N concentration in these evergreen shrubs did not differ from leaf N concentration ($P \geq 0.5$; Figure 3). In contrast to leaf N:P ratios, which increased with fertilization, fine root N:P ratios for all six species were slightly lower in fertilized than control plants, but not significantly so ($P \geq 0.2$). Comparison of biomass production (Figure 1) and N and P pools (Figure 4) revealed that both nutrients are distributed nearly proportionately to aboveground and

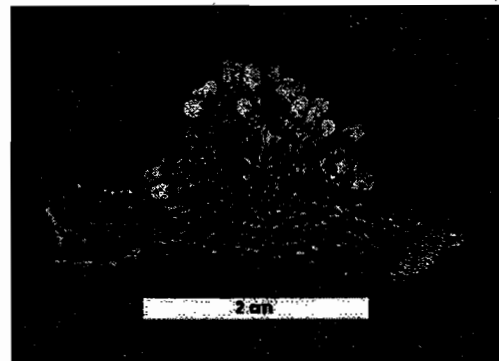


Figure 2. *Frankia* nodule cluster on *Ceanothus jepsonii* root. Identical structures were also observed on *Ceanothus cuneatus*. Nodule sample was collected from a serpentine chaparral community near the McLaughlin Reserve in the California Coast Ranges.

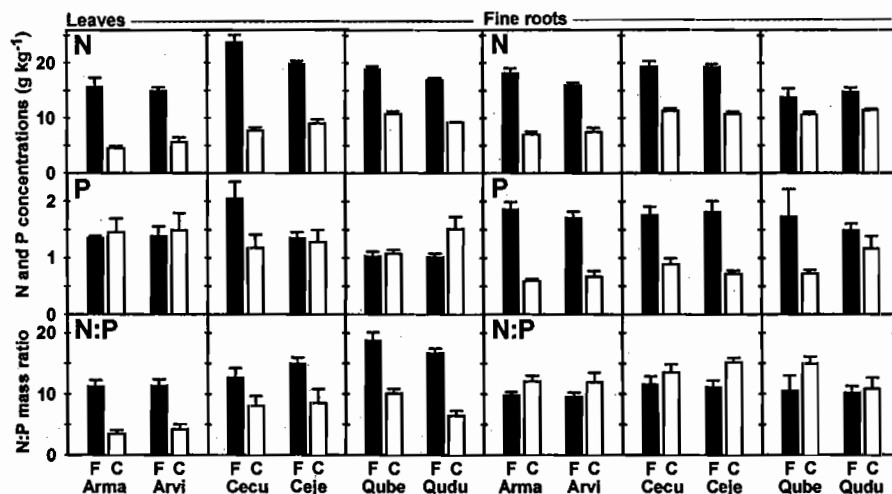


Figure 3. Leaf (left three columns) and fine root (right three columns) N and P tissue concentrations and N:P mass ratio for three nonserpentine shrub species (left species of each pair) and three serpentine shrub species (right species of each pair) subject to fertilized (F) or control (C) treatment. See Figure 1 for species codes. Bars denote mean \pm SE, $n = 4-6$.

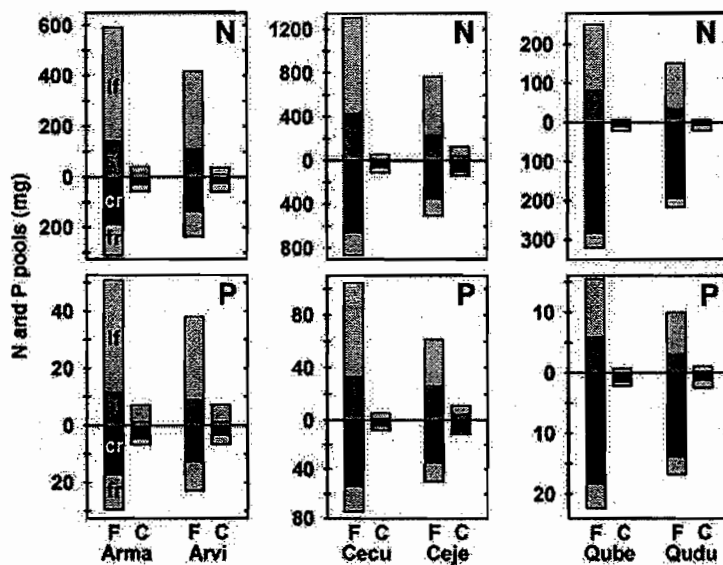


Figure 4. N and P pools for three nonserpentine shrub species (left species of each pair) and three serpentine shrub species (right species of each pair) subject to fertilized (F) or control (C) treatment. See Figure 1 for species and tissue codes. Bars denote means, $n = 4-6$.

belowground biomass allocation when supplied in adequate quantities in the fertilized treatment. Serpentine and nonserpentine shrubs within each genus were similar in total N and P pools in the control treatment.

Leaf and fine root Ca and Mg

Leaf Ca:Mg molar ratios were significantly higher for the serpentine species *A. viscida* ($P < 0.0001$) and *C. jepsonii* ($P < 0.05$) than for

their congeneric nonserpentine counterparts in the control treatment (Figure 5). Leaf Ca:Mg molar ratio was slightly, but not significantly, higher for the serpentine species *Q. durata* than its congeneric nonserpentine counterpart ($P \geq 0.2$). In *A. viscida*, the elevated Ca:Mg molar ratio was due to both higher leaf Ca concentration and lower leaf Mg concentration than *A. manzanita*. In *C. jepsonii* the elevated Ca:Mg molar ratio was due only to substantially higher leaf Ca concentration than in *C. cuneatus*, since leaf Mg concentration was similar for these two species. In *Q. durata*, lower leaf Mg concentration and unchanged leaf Ca concentration resulted in slightly higher Ca:Mg molar ratio than for *Q. berberidifolia*.

Fine root Ca and Mg concentrations did not reflect the same pattern as leaf Ca and Mg concentrations. Fine root Ca concentration in the control serpentine plants was slightly lower and Mg concentration was slightly higher or similar to that of the control nonserpentine plants (Figure 5). Unlike N and P pools, allocation of Ca and Mg pools for all species did not reflect aboveground and belowground biomass allocation (Figure 6). Relative to the nonserpentine species, both the fertilized and control serpentine plants appeared to allocate more Ca to above-

ground biomass, while Mg was primarily retained in the belowground biomass.

Leaf and fine root heavy metals

Fertilization significantly increased leaf Ni and Mn concentration in both serpentine and nonserpentine species with the exception of Ni for the two *Quercus* species ($P < 0.01$; Figure 7). Leaf Ni concentration increased 1- to 3-fold relative to control in the fertilized *Arctostaphylos* and *Ceanothus* plants. Leaf Mn concentration in the fertilized plants for all species increased 3- to 6-fold with the serpentine species having slightly higher concentrations than their nonserpentine counterparts.

Fertilization significantly increased fine root Ni concentrations in all six species by 2- to 4-fold compared to control plants ($P < 0.01$; Figure 7). The concentrations of Ni in the roots were 30- to 40-fold greater than leaf concentrations. Other heavy metals including Fe, Cu, Cr, Co, and Mo had distributions similar to Ni with low leaf concentrations and very high root concentrations. Fine root Mn concentrations were 2- to 3-fold higher in the fertilized plants than controls and unlike leaf Mn concentrations, there was no difference between congeneric

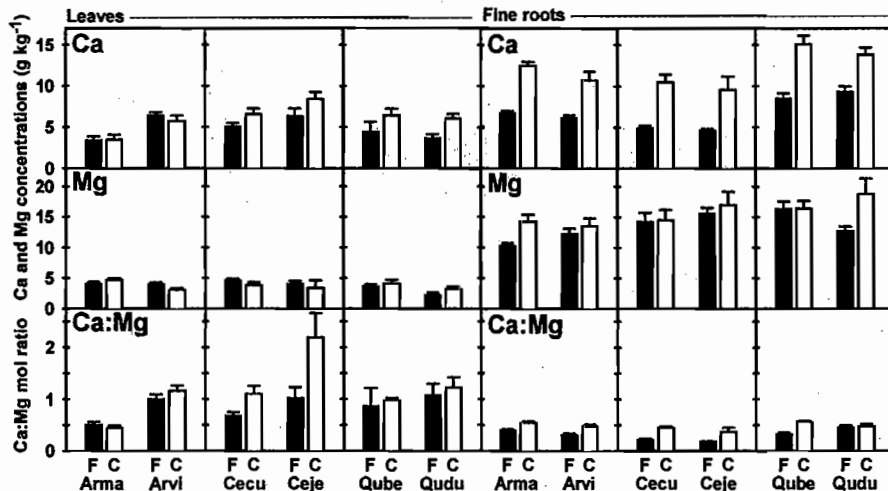


Figure 5. Leaf (left three columns) and fine root (right three columns) Ca and Mg tissue concentration and Ca:Mg molar ratio for three nonserpentine shrubs (left species of each pair) and three serpentine shrubs (right species of each pair) subject to fertilized (F) or control (C) treatment. See Figure 1 for species codes. Bars denote mean \pm SE, $n = 4-6$.

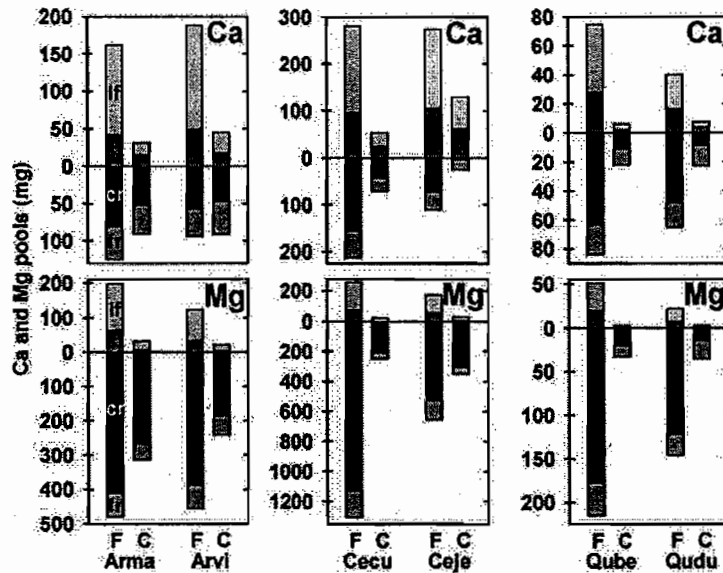


Figure 6. Ca and Mg pools for three nonserpentine shrub species (left species of each pair) and three serpentine shrub species (right species of each pair) subject to fertilized (F) or control (C) treatment. See Figure 1 for species and tissue codes. Bars denote means, $n = 4-6$.

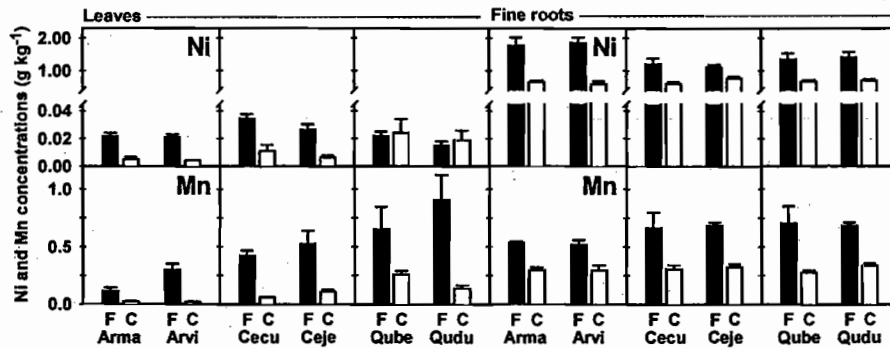


Figure 7. Leaf (left three columns) and fine root (right three columns) Ni and Mn tissue concentration for three nonserpentine shrubs (left species of each pair) and three serpentine shrubs (right species of each pair) subject to fertilized (F) or control (C) treatment. See Figure 1 for species codes. Bars denote mean \pm SE, $n = 4-6$.

serpentine and nonserpentine species with respect to root Mn concentration. The close similarity between root and shoot Mn concentrations within each species was unlike that of any of the other heavy metals examined. Ni (and the other heavy metals) is largely sequestered belowground in all six species (Figure 8). Up to half of the Mn pool in each species, however, was contained in the aboveground biomass (Figure 8). Zn had root to shoot translocation intermediate between Ni and Mn (data not shown).

Discussion

NPK plant nutrition and biomass production

Increased biomass production and higher leaf N:P ratios in fertilized plants of all six shrub species suggests that N is more limiting than P in this serpentine soil. Although the unfertilized serpentine species were N limited, they maintained somewhat greater N concentrations than the nonserpentine species. These differences are likely

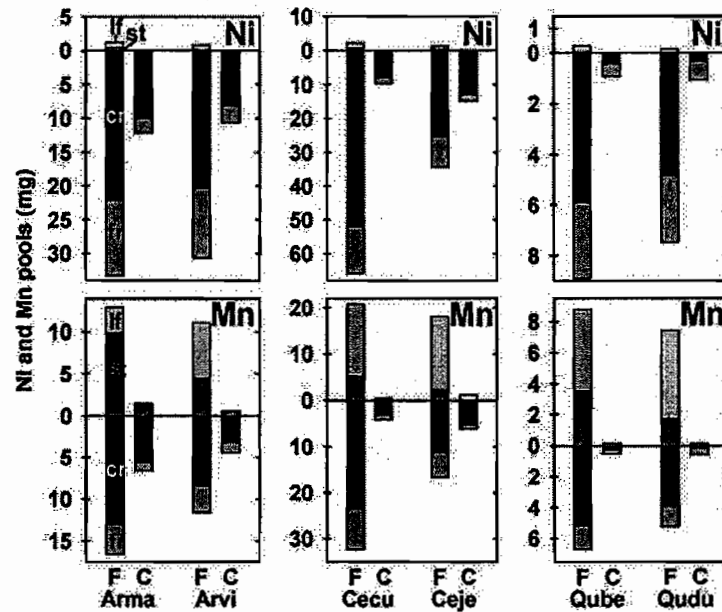


Figure 8. Ni and Mn pools for three nonserpentine shrub species (left species of each pair) and three serpentine shrub species (right species of each pair) subject to fertilized (F) or control (C) treatment. See Figure 1 for species and tissue codes. Bars denote means, $n = 4-6$.

due to the inherently slower growth rates of the serpentine species. Macronutrient deficiency has been diagnosed as limiting plant growth on serpentine soils of several continents, however, the limiting nutrient has varied between N, P, and Ca with apparently no collective agreement on which nutrient is most limiting (Carter et al., 1988; Chiarucci et al., 1999; Huenneke et al., 1990; Nagy and Proctor, 1997). Due to the additive plant growth responses observed with multiple macronutrient treatments in many studies, it is likely that multiple soil macronutrient deficiencies rather than any single nutrient deficiency are responsible for poor plant productivity on serpentine soils globally (Chiarucci et al., 1999; Huenneke et al., 1990; Nagy and Proctor, 1997; Turitzin 1982).

The variation in leaf and fine root P concentration between fertilized and control plants indicates that P translocation from root to shoot is physiologically regulated and influenced by plant nutrient status in these species. Although soil P concentration of the control soil was 5-fold lower than that of the fertilized soil, plants growing in the control soil were able to maintain leaf P concen-

trations close to that of plants growing in the fertilized soil, regardless of serpentine/nonserpentine habitat of origin (Figure 3). Translocation of P, a phloem-mobile element, from shoots to roots can be greatly reduced in P-deficient plants (Jeschke et al., 1997). Greater translocation of P from roots to leaves and/or reduced translocation of P from leaves to roots in the unfertilized plants may account for the maintenance of adequate leaf P concentrations. This pattern for translocation of P was not observed for N, although N availability was concluded to be more limiting than P for plant productivity in this serpentine soil. Although the presence of mycorrhizas was not verified in this study, mycorrhizas are known to buffer plants against P deficiency (Marschner, 2002). The similar leaf P concentration between fertilized and control plants, suggests that mycorrhizas were present and facilitated P acquisition in the control plants. Additional features for enhanced P acquisition that may have been present in the control plants are increased production and exudation of phosphatases and organic acids, modifications to root architecture to increase root surface area, and/or

enhanced expression of P transporters (Vance et al., 2003) Based on the paired comparisons in this study, the maintenance of leaf P concentration appears to be conserved across not only serpentine tolerant species, but also nonserpentine chaparral shrub species that frequently grow on nutrient-poor soils as well.

Effects of fertilization on both aboveground and belowground biomass production for each nonserpentine species tended to be greater than that of its serpentine congener, although initial biomass was similar between each pair at the experiment onset (Figure 1). The difference between serpentine and nonserpentine species with respect to biomass production may be due to differences in allocation plasticity. Fertilization resulted in a shift in biomass allocation with LWR increasing up to 2-fold compared to controls in all species. Species adapted to high stress environments tend to have slower growth rates and lower plasticity in response to nutrient addition (Aerts and Chapin, 2000). Species from less stressful environments, however, tend to have faster growth rates and respond more opportunistically to nutrient (especially N) addition through higher allocation plasticity and larger increases in LWR (Marschner, 2002). The results of this study are consistent with these patterns.

Although high root allocation has been well-documented in many species from nonserpentine, nutrient-poor environments, it has been poorly documented in serpentine environments. The serpentine species in this study appear to be very similar to their nonserpentine congeners in RWR under both control and fertilized conditions. *Quercus* had particularly high RWRs, consistent with its very slow growth (Figure 1).

In addition to effects on biomass allocation, fertilizer also influenced the formation of root nodules in both *C. cuneatus* and *C. jepsonii*, which are both symbiotic with nitrogen-fixing *Frankia* (an actinomycete) (Figure 2). Most of the *C. cuneatus* and *C. jepsonii* control plants bore one to several root nodule clusters while the fertilized plants of both species did not contain any root nodules. Past inspection of *C. cuneatus* plants growing on serpentine and nonserpentine soils in Oregon and California, USA revealed that nodulation of plants growing on nonserpentine soils was substantially higher (18–79%) than plants growing in serpentine soils (0–6%) (White,

1967). The author attributed the lack of nodulation in serpentine soils to either low soil NPK and/or low Mo concentration. The wide-spread deficiency of Mo (Walker, 2001) and NPK (Hunneke et al., 1990; Turitzin et al., 1982) in serpentine soils from the western USA has been verified, but not demonstrated to result in suppressed nodule formation for rhizobial or actinorhizal symbiotic species in that region. Although soil deficiency of NPK and Mo can greatly inhibit nodule formation, excess inorganic N, especially as NO_3^- can also suppress nodule formation through a shift from dependence upon symbiotic N nutrition to inorganic N nutrition (Marschner, 2002). In this study, an adequate NO_3^- supply is believed to have been responsible for the absence of nodules in the fertilized plants.

Regardless of whether N, P, or K is the most limiting nutrient for plant productivity, macronutrient deficiency appears to be a common feature of serpentine soils globally. Both serpentine and nonserpentine species responded physiologically and morphologically to macronutrient deficiency in nearly the same manner. Therefore, it is concluded that the *first hypothesis*, nonserpentine shrub species will exhibit significantly greater response to fertilization with NPKCa with respect to biomass production, biomass allocation, and tissue nutrient content than their congeneric serpentine counterparts, is *not supported*.

Ca and Mg plant nutrition

Of the nutrients examined, leaf Ca:Mg molar ratio accounted for the largest difference between serpentine and nonserpentine congeneric pairs (Figure 5). Each serpentine species achieved a leaf Ca:Mg molar ratio greater than that of its nonserpentine congener primarily through regulation of root to shoot translocation (Figure 6). Fine root Ca and Mg concentrations did not differ significantly between the nonserpentine and serpentine species of each congeneric pair in either the fertilized or control treatments ($P \geq 0.2$). Leaf Ca and Mg concentrations, however, were significantly different between the nonserpentine and serpentine *Arctostaphylos* and *Ceanothus* congeneric pairs ($P < 0.05$) and varied in the same direction for *Quercus*. Higher Ca and lower Mg translocation from roots to leaves in the control treatment resulted in a greater leaf Ca:Mg

molar ratio for *A. viscida* (1.0) than *A. manzanita* (0.5). Much greater Ca translocation and little change in Mg translocation from the roots to leaves resulted in a higher Ca:Mg molar ratio for *C. jepsonii* (1.0) than *C. cuneatus* (0.6). Lower Mg translocation and little change in Ca translocation resulted in a slightly higher Ca:Mg molar ratio for *Q. durata* (1.0) than *Q. berberidifolia* (0.8). These Ca and Mg translocation patterns are similar to the findings of Shewry and Peterson (1975) in which *Agrostis stolonifera* (creeping bentgrass; Poaceae) contained more Ca and less Mg in its shoots than roots. The authors attributed the difference in Ca and Mg between roots and shoots to selective transport of those elements from roots to the shoot. This supports the findings in this study that root-to-shoot Ca and Mg translocation patterns are more important than root level uptake and exclusion mechanisms in maintaining an elevated foliar Ca:Mg molar ratio. Based on these findings, it is concluded that the *second hypothesis*, serpentine shrub species will exhibit significantly greater tolerance of Ca deficiency, Mg toxicity, and have higher leaf Ca:Mg molar ratios than their congeneric non-serpentine counterparts, is *strongly supported*.

It is not uncommon for serpentine species to have leaf Ca:Mg molar ratios several times that of the soil they are growing in. The leaf Ca:Mg molar ratio for all three serpentine shrub species was greater than the control soil Ca:Mg molar ratio, while the leaf Ca:Mg molar ratio for the non-serpentine shrubs was less than or equal to that of the soil. Based on several studies, a total of 54/68 (79%) serpentine species examined globally have been measured to have leaf Ca:Mg molar ratios greater than that of their respective serpentine soil (Angelone et al., 1993; Koenigs et al., 1982; Kruckeberg, 1950; Lombini et al., 1998; Lyon et al., 1971; Nagy and Proctor, 1997; Proctor and Woodell, 1975; Shewry and Peterson, 1975). One study of California serpentine shrubs measured leaf Ca:Mg molar ratios for field-collected *A. viscida* and *Q. durata* (6.05 and 2.18, respectively) growing on serpentine soil compared to a soil Ca:Mg molar ratio of 0.32 (Wallace et al., 1982). A similar study found leaf Ca:Mg molar ratios for field-collected *A. viscida* and *Q. durata* as 1.29 and 0.69, respectively compared to a soil Ca:Mg molar ratio of 0.22 (Koenigs et al., 1982). Calculations of Ca and Mg accumulation

ratios (leaf [element]/soil [element]) for those plants revealed that selective Ca uptake and Mg exclusion were operating in both species, consistent with the finding in this study that selective root to shoot Ca translocation and/or Mg exclusion mechanisms exist in these two species.

The importance of selective Ca uptake and Mg exclusion in plants for survival and productivity in low Ca:Mg molar ratio media has been demonstrated in two studies comparing nonserpentine *H. annuus* (common sunflower; Asteraceae) and a serpentine endemic *H. bolanderi* (serpentine sunflower; Asteraceae) (Walker et al., 1955; Madhok and Walker, 1969). These two studies showed that *H. bolanderi* biomass production was maintained at the same level across a range of media Ca:Mg molar ratios from 4.2 to 0.1, while biomass production in *H. annuus* dropped to just 5% of the maximum over the same 4.2 to 0.1 range. The ability of *H. bolanderi* to maintain biomass production even at very low Ca:Mg molar ratios was attributed to its ability to maintain a significantly higher foliar Ca:Mg molar ratio (3.6–0.3) across the substrate Ca:Mg molar ratio range than *H. annuus* (2.0–0.1). *H. bolanderi* excluded significantly more Mg from leaves and accumulated slightly more Ca in leaves than *H. annuus* allowing it to maintain an elevated leaf Ca:Mg molar ratio. Whether root-to-shoot translocation differences or root exclusion/accumulation differences were responsible for the differences in leaf Ca:Mg molar ratios was not determined in these studies.

Leaf Ca and Mg concentrations appear to be phylogenetically constrained with most of the variation in this traits occurring at higher taxonomic levels (Broadley et al., 2003). The evolution of intra-generic differences for the species examined in this study, likely occurred subsequent to the evolution of angiosperms. It has been suggested by Kruckeberg (1950) that the divergence of a species into serpentine and nonserpentine edaphic ecotypes may be the first step in the evolution of serpentine endemic species from formerly nonserpentine species. The species *Gilia capitata* (blue field gilia; Polemoniaceae) is widespread in the western US and has populations that grow on both serpentine and nonserpentine soils. Kruckeberg (1950) demonstrated that a serpentine ecotype of *G. capitata* produced significantly more biomass and had a leaf Ca:Mg molar ratio (1.0) 4-fold that

of the nonserpentine ecotype (0.25) when grown on serpentine soil having a Ca:Mg molar ratio of 0.2. The serpentine ecotype of *G. capitata* excluded significantly more Mg from its leaves and accumulated slightly more Ca than its nonserpentine ecotype counterpart. Similarly, a comparison of *Achillea millefolium* (yarrow; Asteraceae) serpentine and nonserpentine ecotypes grown on serpentine soil (Ca:Mg molar ratio 0.2) demonstrated that the serpentine ecotype maintained a leaf Ca:Mg molar ratio (1.0) 3-fold that of the nonserpentine ecotype (0.3) (O'Dell and Claassen, 2005). Leaf and root Ca and Mg concentrations showed that the serpentine ecotype translocated significantly more Ca from roots to the shoot than the nonserpentine ecotype. The widespread occurrence of elevated leaf Ca:Mg molar ratios in serpentine plants and ecotypes (including the species examined in this study) relative to the low Ca:Mg molar ratio soils that support them suggests that selective Ca translocation to leaves and inhibited Mg translocation and sequestration by roots are important evolutionary adaptations in serpentine species which distinguish them from nonserpentine species.

Ni, Mn, and other heavy metals in plant nutrition

Comparison of control leaf and fine root Ni and Mn concentrations revealed two very different translocation patterns for these metals. Fine root Ni concentrations were up to 70-fold greater than leaf concentrations in all six species, indicating that in these dominant serpentine and nonserpentine chaparral shrubs translocation of this toxic metal to leaves is strongly suppressed in favor of sequestration in roots. The cumulative effect of root Ni sequestration is especially evident in the plant element pools (Figure 8). Analyses of the other heavy metals including Fe, Cu, Zn, Cr, Co, and Mo showed similar patterns of root sequestration.

The critical toxicity level for Ni in agricultural crops is 0.05–0.10 g kg⁻¹ in leaves (Marschner, 2002) and 0.01–0.10 g kg⁻¹ Ni is regarded as a normal leaf concentration range for western USA serpentine species (Reeves et al., 1983). All six shrub species displayed no leaf Ni toxicity symptoms during the study and had healthy, white roots at harvest which had thoroughly explored the serpentine potting media. The leaf

Ni concentration obtained for *A. viscida* (0.004 g kg⁻¹) and *Q. durata* (0.018 g kg⁻¹) in this study are similar to those measured by other researchers for field-collected samples (Koenigs et al., 1982; Wallace et al., 1982) and are well below the range considered to be toxic. No critical root tissue toxicity ranges for Ni have been suggested for serpentine species, although high levels of plant available Ni are known to inhibit root growth in some sensitive species (Gabbrielli et al., 1990). Elevated plant-available levels of Ca and occasionally Mg, are known to ameliorate the toxic effects of Ni and other heavy metals (Gabbrielli and Pandolfini, 1984; Lombini et al., 2003)

In contrast to Ni, root Mn concentrations were very similar to that of leaves, suggesting that Mn is readily translocated from roots to shoot in these shrub species. The degree of Mn translocation from roots to shoot varies considerably between species and can vary greatly even between ecotypes of the same species (El-Jaoual and Cox, 1998; Marschner, 2002). Most crops have a critical toxicity threshold for Mn between 0.2 and 5.3 g kg⁻¹ in shoots, depending on species (Marschner, 2002). Wildland plants tend to be more tolerant of such toxicity stresses and leaf Mn concentrations for the shrubs in this study were well within the lower end of the critical toxicity range for crops.

Although leaf and root Ni and Mn concentration were not significantly different between congeneric serpentine and nonserpentine species, fertilization significantly increased both leaf and root Ni and Mn concentration by 2- to 8-fold in all six species (Figure 7). Other heavy metals examined including Fe, Cu, Zn, Cr, Co, and Mo were not significantly affected by the fertilizer treatment and did not significantly differ between congeneric serpentine and nonserpentine species. Analyses of fertilized and control soils revealed that there was no significant effect of fertilization on bulk soil pH or the availability of Fe, Mn, Cu, Zn, Ni, Cr, Co, and Mo (Table 1).

A change in rhizosphere soil pH might be responsible for the increased plant Ni and Mn concentrations observed. Desorption of Ni and Mn from serpentine and metalliferous soils is strongly influenced by excess H⁺ and NH₄⁺ in soil solution (Gasser et al., 1995; Lorenz et al., 1994; Wiltshire, 1972). NH₄NO₃ is a source of

NH_4^+ and the nitrification of NH_4^+ to NO_3^- can generate excess H^+ (Tisdale et al., 1993; Wiltshire, 1972). In addition, root absorption of NH_4^+ results in the acidification of rhizosphere soil (Marschner, 2002). A study of serpentine and nonserpentine plant responses to fertilization with NH_4^+ on Ni–Cr rich serpentine soil and Cu–Pb minespoil revealed large differences with respect to plant accumulation and distribution of heavy metals (Wiltshire, 1974). Regardless of serpentine and nonserpentine habitat of origin, NH_4^+ fertilization of the Ni–Cr serpentine soil resulted in higher plant Ni concentrations than the control with greater Ni concentrations in roots compared to shoots. Likewise, NH_4^+ fertilization of the Cu–Pb minespoil resulted in higher shoot Mn concentration than the control. The author attributed the enhanced uptake of heavy metals to rhizosphere acidification of the soil by plants due to assimilation of NH_4^+ . Although fertilization with NH_4^+ containing fertilizer increased uptake of Ni and Mn in this study, the degree of heavy metal uptake did not differ significantly between the serpentine and nonserpentine shrub species examined. Thus, it is concluded that the *third hypothesis*, serpentine shrub species will exhibit significantly greater tolerance of heavy metal exclusion and toxicity tolerance than their congeneric nonserpentine counterparts, is *not supported*.

The inherent ability of these shrub species to sequester and tolerate heavy metals may have an evolutionary origin that predates the divergence of serpentine (metalliferous) taxa from nonser-

pentine (nonmetalliferous) taxa in the genera studied. Investigation of phylogenetic variation in heavy metal accumulation in hundreds of metalliferous and nonmetalliferous species spanning more than a dozen different plant orders found that significant variation in shoot metal content only occurred at the order classification level and above, suggesting an ancient evolution of metal accumulation and tolerance traits (Broadley et al., 2001).

Conclusions

Overall the three pairs of serpentine and nonserpentine congeneric shrubs examined in this study did not differ significantly in their response to fertilization with NPKCa fertilizer, nor did they differ in their accumulation of heavy metals from the metalliferous serpentine soil (see Table 2 for major results summary). The finding that the serpentine shrub species maintained significantly higher leaf Ca:Mg molar ratios than their nonserpentine counterparts, however, is of great interest since the low Ca:Mg molar ratio typical of serpentine soils is a unique feature that nonserpentine soils lack. *A. viscida* employed both selective Ca translocation from roots to leaves and Mg exclusion to roots to achieve a higher leaf Ca:Mg molar ratio than its nonserpentine counterpart *A. manzanita*. *C. jepsonii* depended mostly upon selective Ca translocation from roots to leaves and *Q. durata* relied primarily upon Mg exclusion to roots. Both serpentine and nonserpentine species had similar

Table 2. Summary of major results

Hypothesis	Results
(1) Nonserpentine species significantly more responsive to fertilization than serpentine species	(1) No difference between serpentine and nonserpentine species (2) N more limiting than P (3) N fertilization inhibited nodulation of <i>Ceanothus</i> species
(2) Serpentine species significantly more tolerant of low Ca/high Mg than nonserpentine species	(1) Significantly higher leaf Ca:Mg molar ratio in serpentine than nonserpentine species (2) Major differences in root-shoot transport of Ca and Mg
(3) Serpentine species exhibit significantly greater tolerance of heavy metals than nonserpentine species	(1) No difference between serpentine and nonserpentine species (2) Most heavy metals sequestered in roots of all species (3) N fertilization enhanced heavy metal uptake

root Ca and Mg concentrations, but leaf concentrations for the two elements differed. Thus, it was concluded that the translocation patterns for Ca and Mg from roots to leaves is more important than selective Ca uptake and Mg exclusion operating at the root level as proposed in previous studies on serpentine plant species. The widespread global prevalence of serpentine plant taxa that maintain leaf Ca:Mg molar ratios greater than that of the serpentine soil that supports them suggests that selective Ca transport and Mg retention mechanisms operating at the root-to-shoot transport level are important evolutionary adaptations for survival on serpentine and unique features that distinguish them from nonserpentine plant taxa.

Acknowledgements

This study was supported by the Packard Foundation (2000-01607) and the California Agricultural Experiment Station. The authors are grateful to R. E. Drenovsky, A. Dain-Owens, D. A. Enderlin, V. P. Claassen, and H. D. Safford for their comments and assistance. We thank the Donald and Sylvia McLaughlin UC Natural Reserve and the US Bureau of Land Management for permitting the collection of plant and soil materials used in this study. J. H. Richards also thanks the UC Valentine Eastern Sierra Reserve for support.

References

- Aerts R and Chapin F S III 2000 The mineral nutrition of wild plants revisited: A re-evaluation of processes and patterns. *Adv. Ecol. Res.* 30, 1–67.
- Angelone M, Vaselli O, Bini C and Coradossi N 1993 Pedogeochemical evolution and trace elements availability to plants in ophiolitic soils. *Sci. Total. Environ.* 129, 291–309.
- Baker A J M 1987 Metal tolerance. *New Phytol.* 106(Suppl.), 93–111.
- Broadley M R, Willey N J, Wilkins J C, Baker A J M, Mead A and White P J 2001 Phylogenetic variation in heavy metal accumulation in angiosperms. *New Phytol.* 152, 9–27.
- Broadley M R, Bowen H C, Cotterill H L, Hammond J P, Meacham M C, Mead A and White P J 2003 Variation in the shoot calcium content of angiosperms. *J. Exp. Bot.* 54, 1431–1446.
- Brooks R R 1987 *Serpentine and its Vegetation: A Multidisciplinary Approach*. Dioscorides Press, Portland, OR, USA 454 pp.
- Burt R, Fillmore M, Wilson M A, Gross E R, Langridge R W and Lammers D A 2001 Soil properties of selected pedons on ultramafic rocks in Klamath Mountains, Oregon. *Commun. Soil Sci. Plant Anal.* 32, 2145–2175.
- Carter S P, Proctor J and Slingsby D R 1988 The effects of fertilization on part of the Keen of Hamar serpentine, Shetland. *Trans. Bot. Soc. Edinburgh* 45, 97–105.
- Chiarucci A, Maccherini S, Bonini I and De Dominicis V 1999 Effects of nutrient addition on community productivity and structure of serpentine vegetation. *Plant Biol.* 1, 121–126.
- El-Jaoual T and Cox D A 1998 Manganese toxicity in plants. *J. Plant Nutr.* 21, 353–386.
- Freitas H and Mooney H 1996 Effects of water stress and soil texture on the performance of two *Bromus hordeaceus* ecotypes from sandstone and serpentine soils. *Acta Oecol.* 17, 307–317.
- Gabrielli R and Pandolfini T 1984 Effect of Mg^{2+} and Ca^{2+} on the response to nickel toxicity in a serpentine endemic and nickel-accumulating species. *Physiol. Plant* 62, 540–544.
- Gabrielli R, Pandolfini T, Vergnano O and Palandri M R 1990 Comparison of two serpentine species with different nickel tolerance strategies. *Plant and Soil* 122, 271–277.
- Gasser U G, Dahlgren R A, Ludwig C and Lauchli A E 1995 Release kinetics of surface-associated Mn and Ni in serpentinic soils: pH effects. *Soil Sci.* 160, 273–280.
- Huenneke L R, Hamburg S P, Koide R, Mooney H A and Vitousek P M 1990 Effects of soil resources on plant invasion and community structure in Californian serpentine grassland. *Ecology* 71, 478–491.
- Jeschke W, Kirkby E, Peuke A, Pate J and Hartung W 1997 Effects of P deficiency on assimilation and transport of nitrate and phosphate in intact plants of castor bean (*Ricinus communis* L.). *J. Exp. Bot.* 48, 75–91.
- Koenigs R L, Williams W A, Jones M B and Wallace A 1982 Factors affecting vegetation on a serpentine soil. II. Chemical composition of foliage and soil. *Hilgardia* 50, 15–26.
- Kruckeberg A R 1950 An experimental inquiry into the nature of endemism on serpentine soils. Ph.D. Thesis, University of California, Berkeley, California, USA, 154 p.
- Kruckeberg A R 1984 *California Serpentes: Flora, Vegetation, Geology, Soils and Management Problems*. University of California Press, Berkeley, California, USA 180 pp.
- Kruckeberg A R 2002 *Geology and Plant Life*. University of Washington Press, Seattle, Washington, USA 362 pp.
- Lombini A, Dinelli E, Ferrari C and Simoni A 1998 Plant–soil relationships in the serpentinite screes of Mt. Prinzera (Northern Apennines, Italy). *J. Geo. Expl.* 64, 19–33.
- Lombini A, Llugany M, Poschenrieder C, Dinelli E and Barcelo J 2003 Influence of the Ca/Mg ratio on Cu resistance in three *Silene armeria* ecotypes adapted to calcareous soil or to different, Ni- or Cu-enriched serpentine sites. *J. Plant Physiol.* 160, 1451–1456.
- Lorenz S E, Hamon R E, McGrath S P, Holm P E and Christensen T H 1994 Applications of fertilizer affect cadmium and zinc concentrations in soil solutions and uptake by plants. *Eur. J. Soil. Sci.* 45, 159–165.
- Lyon G L, Peterson P J, Brooks R R and Butler G W 1971 Calcium, magnesium, and trace elements in a New Zealand serpentine flora. *J. Ecol.* 59, 421–429.
- Madhok O P and Walker R B 1969 Magnesium nutrition of two species of sunflower. *Plant Physiol.* 44, 1016–1022.
- Marschner H 2002 *Mineral Nutrition of Higher Plants*. Academic Press, San Diego, California, USA 889 pp.

- Meyer G A and Keliher P N 1992 An overview of analysis by inductively coupled plasma-atomic emission spectrometry. *In* Inductively Coupled Plasmas in Analytical Atomic Spectrometry. Eds. A Montaser and D W Golightly. pp. 473–505. VCH Publishers Inc, New York, New York, USA.
- Nagy L and Proctor J 1997 Plant growth and reproduction on a toxic alpine ultramafic soil: Adaptation to nutrient limitation. *New Phytol.* 137, 267–274.
- Neter J, Wasserman W and Kutner M H 1990 Applied Linear Statistical Models: Regression, Analysis of Variance, and Experimental Design. 3 Irwin, Homewood, Illinois, USA 842 pp.
- O'Dell R E and Claassen V P 2005 Serpentine and non-serpentine *Achillea millefolium* accessions differ in serpentine substrate tolerance and response to organic and inorganic amendments. *Plant Soil*, DOI 10.1007/s11104-005-1360-2 (*in press*).
- Proctor J and Woodell S R J 1975 The ecology of serpentine soils. *Adv. Ecol. Res.* 9, 256–365.
- Proctor J 1999 Toxins, nutrient shortages and droughts: The serpentine challenge. *TREE* 14, 334–335.
- Reeves R D, MacFarlane R M and Brooks R R 1983 Accumulation of nickel and zinc by western North American genera containing serpentine-tolerant species. *Am. J. Bot.* 70, 1297–1303.
- Rice W R 1989 Analyzing tables of statistical tests. *Evolution* 43, 223–225.
- Roberts B A and Proctor J 1992 The Ecology of Areas with Serpentinized Rocks. A World View. Kluwer Academic Publishers, Boston, Massachusetts, USA 472 pp.
- Sah R N and Miller R O 1992 Spontaneous reaction for acid dissolution of biological tissues in closed vessels. *Anal. Chem.* 64, 230–233.
- SAS Institute 2001 SAS/STAT User's Guide Version 8. SAS Institute, Cary, North Carolina, USA.
- Sawyer J O and Keeler-Wolf T 1995 A Manual of California Vegetation. California Native Plant Society, Sacramento, California, USA 471 pp.
- Scheiner S M 1993 MANOVA: Multiple response variables and multispecies interactions. *In* Design and Analysis of Ecological Experiments. Eds. S M Scheiner and J Gurevitch. pp. 94–111. Chapman and Hall, New York, New York, USA.
- Shaw A J 1990 Heavy Metal Tolerance in Plants: Evolutionary Aspects. Ed. A J Shaw. CRC Press, Boca Raton, Florida, USA. 355 p.
- Shewry P R and Peterson P J 1975 Calcium and magnesium in plants and soil from a Shetland area on Unst, Shetland. *J. Appl. Ecol.* 12, 381–391.
- Smucker A J M, McBurney S L and Srivastava A K 1982 Quantitative separation of roots from compacted soil profiles by the hydropneumatic elutriation system. *Agron. J.* 74, 500–503.
- Tabachnick B G and Fidell L S 1996 Using Multivariate Statistics. Harper Collins College Publishers, New York, New York, USA 880 pp.
- Tisdale S L, Nelson W L, Beaton J D and Havlin J L 1993 Soil Fertility and Fertilizers. Macmillan Publishing Company, New York, New York, USA 634 pp.
- Turitiz S N 1982 Nutrient limitations to plant growth in a California serpentine grassland. *Am. Midl. Nat.* 107, 95–99.
- Vance C P, Uhde-Stone C and Allan D L 2003 Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. *New Phytol.* 157, 423–447.
- Walker R B, Walker H M and Ashworth P R 1955 Calcium-magnesium nutrition with special reference to serpentine soils. *Plant Phys.* 30, 214–221.
- Walker R B 2001 Low molybdenum status of serpentine soils of western North America. *S. Afr. J. Sci.* 97, 565–568.
- Wallace A, Jones M B and Alexander G V 1982 Mineral composition of native woody plants growing on a serpentine soil in California. *Soil Sci.* 134, 42–44.
- Westerbergh A 1994 Serpentine and non-serpentine *Silene dioica* plants do not differ in nickel tolerance. *Plant Soil* 167, 297–303.
- White C D 1967 Absence of nodule formation on *Ceanothus cuneatus* in serpentine soils. *Nature* 215, 875.
- Wiltshire G H 1972 Effect of nitrogen source on translocation of nickel in some crop plants and weeds. *Kirkia* 8, 103–123.
- Wiltshire G H 1974 Growth of plants on soils from two metalliferous sites in Rhodesia. *J. Ecol.* 62, 501–525.

Section editor: H. Schat