PLANT-MICROBE-ANIMAL INTERACTIONS - ORIGINAL RESEARCH

Edaphic factors and plant–insect interactions: direct and indirect effects of serpentine soil on florivores and pollinators

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Abstract Edaphic factors can lead to differences in plant morphology and tissue chemistry. However, whether these differences result in altered plant–insect interactions for soil-generalist plants is less understood. We present evidence that soil chemistry can alter plant–insect interactions both directly, through chemical composition of plant tissue, and indirectly, through plant morphology, for serpentine-tolerant *Mimulus guttatus* (Phrymaceae). First, we scored floral display (corolla width, number of open flowers per inflorescence, and inflorescence height), flower chemistry, pollinator visitation and florivory of *M. guttatus* growing on natural serpentine and non-serpentine soil over 2 years. Second, we conducted a common garden reciprocal soil transplant experiment to isolate the effect of serpentine soil on floral display traits and flower chemistry. And last, we observed arrays of field-collected inflorescences and potted plants to determine the effect of soil environment in the field on pollinator visitation and florivore damage, respectively. For both natural and experimental plants, serpentine soil caused reductions in floral display and directly altered flower tissue chemistry. Plants in natural serpentine populations received fewer pollinator visits and less damage by florivores relative to non-serpentine plants. In experimental arrays, soil environment did not influence pollinator visitation (though larger flowers were visited more frequently), but did alter florivore damage, with serpentine-grown plants receiving less damage. Our results demonstrate that the soil environment can directly and indirectly affect plant–mutualist and plant–antagonist interactions of serpentine-tolerant plants by altering flower chemistry and floral display.

Keywords Serpentine · Soil chemistry · Pollination · Florivory

Communicated by Christina Caruso.

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Introduction

Biotic interactions can be influenced by abiotic factors, thus identical communities found in disparate environments (i.e., with different resource availability) may differ in both the strength (Breitburg et al. 1997; Alonso 1999; Chalcraft and Andrews 1999) and direction (Pugnaire and Luque 2001) of interactions. Abiotic conditions have been documented to alter biotic interactions across a broad range of organisms, including effects of temperature and moisture on insect (Park 1954) and bivalve competition (Connell 1961). Plants, in particular, are heavily dependent upon their abiotic environment for inorganic nutrient acquisition, and as a consequence may be particularly susceptible to abiotic-mediated variation (Klanderud and Totland 2005) in morphology and plant tissue chemistry, which in turn may affect how they interact with animals. Chemical and physical aspects of soils are extremely variable and this variation can alter both plant morphology and tissue chemistry (e.g., Cunningham et al. 1999; Murren et al. 2006; Burnett et al. 2008). For plants that occur in a variety of soil types, it is unclear whether interactions with mutualists (e.g., pollinators) or

antagonists (e.g., herbivores) are affected by soil context, and whether soil could modify these interactions via direct effects on plant chemistry or indirect effects on morphology. However, such modification of biotic interactions could be instrumental in varying patterns of coevolution (i.e., the geographic mosaic of coevolution; Thompson 1999).

The soil environment can influence plant reproductive morphology, which can in turn affect both plant–florivore and plant–pollinator interactions. Macronutrients in the soil, such as N, P, K, Ca, and Mg, have been shown to influence flower size and number (e.g., Nagy and Proctor 1997; Murren et al. 2006; Burnett et al. 2008). In addition, toxic elements, such as heavy metals, often result in stunting of growth when present in high concentrations in the soil (Antonovics et al. 1971) and are also known to influence flower size (Hladun et al. 2011) and flower number (Saikkonen et al. 1998). Soil-induced changes in floral morphology can have consequences for plant reproduction, as both pollinators (Mitchell et al. 2004; Ivey and Carr 2005) and herbivores (Juenger et al. 2005; Ashman and Penet 2007) generally favor plants with large floral displays. As a result, soil chemistry may mediate the quantity and quality of plant interactions with florivores and pollinators. Many studies have documented the effects of environment on plant reproductive morphology, yet few (e.g., Galen 2000; Lau et al. 2008) have determined whether these morphological changes lead to altered plant–animal interactions across different environments.

Soil chemistry can also have effects on plant tissue chemistry (Cunningham et al. 1999), although the consequences for plant–animal interactions are less well understood. While plant–herbivore interactions are often studied in the context of plant secondary compounds (reviewed in Mithöfer and Boland 2012), recent studies suggest that primary metabolites (e.g., N, P, K) may also greatly influence both herbivore preference (Alonso and Herrera 2003) and fitness (Beanland et al. 2003; Perkins et al. 2004). In addition, soils that contain toxic elements can alter plant–herbivore interactions. For instance, *Streptanthus polygaloides*, a serpentine soil endemic, hyperaccumulates Ni (i.e., tissues >1,000 ppm Ni; Baker and Brooks 1989), which results in less leaf damage by herbivores (Boyd and Moar 1999) and pathogens (Boyd et al. 1994). The effect of metal accumulation on plant–pollinator interactions, however, is unclear. For example, interactions with pollinators may also be affected if metals are translocated to floral tissues and pollinator rewards (e.g., nectar and pollen). A recent study of a non-metal (Se) hyperaccumulator has shown that flower constituents, including nectar, can accumulate non-essential elements in high concentrations (Hladun et al. 2011), though metal accumulators from serpentines have not been similarly studied, and the implications of floral metal and metalloid accumulation on plant–pollinator

interactions have only begun to be explored (Quinn et al. 2011). Moreover, serpentine soils may generally influence plant–animal interactions (i.e., for non-hyperaccumulating plants) through changes in tissue chemistry, as concentrations of metals in plant tissues far below hyperaccumulator thresholds have been shown to be toxic to herbivores (Coleman et al. 2005). In addition, while studies of folivory are important, an understanding of how flower chemistry alters florivory is needed, as many insects supplement their diets with nutrient-rich flower tissue (Held and Potter 2004). In fact, some studies suggest that florivory can be just as common as leaf herbivory in natural populations (e.g., Zangerl and Rutledge 1996; Wolfe 2002), with potentially negative implications for plant reproductive success (Mothershead and Marquis 2000). Florivores can affect male and female fitness both directly, through consumption of gamete-housing structures, and indirectly by altering floral traits important for biotic interactions (e.g., pollinator attraction; reviewed in McCall and Irwin 2006). Therefore, understanding how the soil environment alters plant–florivore interactions may be vital towards explaining plant adaptation to unique soils.

Serpentine soil is distinct from adjacent soils by having low Ca:Mg ratios, mineral nutrient deficiencies (e.g., P, K), and relatively high concentrations of several metals (Co, Cr, Ni, Fe, Mg, and Zn; Brady et al. 2005; Safford et al. 2005; Table S1). These soils provide an ideal model system to test whether the soil environment alters plant–florivore and plant–pollinator interactions as they are (1) globally distributed, (2) host many species of tolerant plants, and (3) are chemically distinct from adjacent soil types (Brady et al. 2005; Harrison and Rajakaruna 2011). We address whether biotic interactions are soil-dependent by answering the following questions with respect to a serpentine-tolerant species, *Mimulus guttatus*: (1) Does growth on serpentine soil alter traits that mediate plant–animal interactions, i.e., flower size, flower number, and inflorescence height? (2) Does serpentine soil directly influence floral chemistry, specifically for minerals known to be enriched or deficient in serpentine and/or known to influence plant–animal interactions (macronutrients: Ca, Mg, P, K; micronutrients: Fe, Ni, Zn, B; or other beneficial nutrients: Al, Na; Marschner 1986)? And (3) is (a) pollinator visitation rate, (b) pollinator diversity, and/or (c) florivore damage lower for plants growing in serpentine versus non-serpentine soils?

Materials and methods

Study system

Mimulus guttatus (Phrymaceae) is a widespread herbaceous plant native to western North America that inhabits

creeks or seepage areas (Vickery 1978). It can grow on serpentine and non-serpentine soil (Vickery 1978), and thus is regarded as a serpentine-tolerant species (Gardner and Macnair 2000). It is self-compatible and predominantly pollinated by bees, including honeybees (*Apis mellifera*) and bumblebees (*Bombus* spp.), although it is also visited by beetles, flies, and butterflies (Gardner and Macnair 2000; Meindl, Arceo-Gomez, and Ashman, unpublished data). Its flowers are damaged by insect florivores including grasshoppers (Orthoptera) and beetles (e.g., Buprestidae and Melyridae; G. A. Meindl, personal observation).

Study sites

This study was conducted in serpentine (S) and non-serpentine (NS) seeps at the Donald and Sylvia McLaughlin Natural Reserve in Napa and Lake counties of California. We studied *M. guttatus* in two serpentine (S1: 38°51′N, 122°25′W and S2: 38°51′N, 122°27′W) and two non-serpentine seeps (NS1: 38°51′N, 122°22′W and NS2: 38°52′N, 122°26′W), separated by 1–5 km, in May–August of 2010 and 2011. Soils at the two types of seeps are chemically distinct in most major macro- and micronutrients (Table S1; http://nrs.ucdavis.edu/McL/natural/geology/index.html). Specifically, serpentine soil at the study sites was higher in Fe, Mg, Ni, and Zn (56, 326, 422, and 69 % respectively), lower in Al, Ca, K and P (27, 44, 39, and 49 %, respectively) than non-serpentine soil, but similar in B and Na (Table S1). While bioavailable fractions of Ni in serpentine soils at McLaughlin are lower compared to other serpentine sites (e.g., Oze et al. 2008), concentrations of Ni are higher in serpentine soils relative to non-serpentine soils on the reserve (Wright et al. 2006; Table S1).

Each seep supported several hundred *M. guttatus* along with *Vicia villosa*, *Melilotus alba* (Fabaceae), *Torilis arvensis* (Apiaceae), and *Stachys ajugoides* (Lamiaceae) at non-serpentine seeps and *Castilleja rubicundula* (Scrophulariaceae), *Triteleia peduncularis* (Liliaceae), *Lotus micranthus* (Fabaceae), and *Plagiobothrys stipitatus* (Boraginaceae) at serpentine seeps. *Zigadenus venenosus* (Liliaceae) and *Trifolium obtusiflorum* (Fabaceae) were present at both seep types.

Abiotic and biotic interactions in natural populations

Floral display/flower chemistry

We assessed whether plants on serpentine and non-serpentine soil differed in aspects of floral display. We established four to six 1 × 2 m plots at 5-m intervals along each of seven transects/seep over the course of two seasons. At mid-bloom, we measured corolla width (widest distance across lower lip of corolla to the nearest 0.1 mm; Robertson

et al. 1994) and inflorescence height (mm) with digital calipers, number of open flowers per inflorescence, and the percent of open flowers with visible florivore damage (i.e., corolla tissue missing) on three plants in a standard position in each plot. Corolla width was always measured on the second most recently opened flower on an inflorescence. Trait averages for corolla width, inflorescence height, and the number of open flowers per inflorescence were calculated for each of 164 plots (42 at NS1 and S2; 40 at NS2 and S1).

To determine whether flower tissue chemistry differed between serpentine and non-serpentine plants, in 2010 we bulk-collected entire, freshly opened flowers during peak flowering from 10 plots/site. These were rinsed with diH$_2$O and dried at 60 °C for 48 h. A 0.1 g sample (4–5 flowers) was microwave-digested in 4 mL of trace metal grade HNO$_3$ and brought to a final volume of 15 mL with MilliQ (Millipore, Bedford, MA, USA) H$_2$O (Esslemont et al. 2000). We analyzed element composition using inductively coupled plasma mass spectrometry (ICP-MS; Perkin/Elmer NEXION 300X), and present data on ten elements known to differ between serpentine and non-serpentine soils and/or known to influence plant–animal interactions (macronutrients: Ca, Mg, P, K; micronutrients: Fe, Ni, Zn, B; other beneficial: Al, Na; e.g., Alonso and Herrera 2003; Beanland et al. 2003; Wang and Mopper 2008; Pilon-Smits et al. 2009).

We analyzed corolla width, inflorescence height, number of open flowers per inflorescence, and floral tissue chemistry with mixed linear models (PROC MIXED; SAS 2010) with soil type (serpentine vs. non-serpentine) and year (2010, 2011) as factors. Soil type and year (for display traits only) were fixed effects, while site identity (nested within soil type) was a random effect. To control for potential Type I errors due to multiple comparisons, we used Bonferroni corrections to adjust alpha levels. The data for elements B, Na, Al, Fe, and Zn were log transformed to improve normality.

Pollinators

To determine whether pollinator visitation rate differed between serpentine and non-serpentine *M. guttatus*, 10–10.5 h of observation/site was conducted across three (2010) to four (2011) days/site in June/July. Observations were conducted for 15 min/plot between 1000 and 1600 hours on sunny days. For each week of observation, the order that serpentine and non-serpentine sites were observed was reversed, and sites were visited alternately in the morning and afternoon on successive days of observation. For each plot, we recorded the number of *M. guttatus* flowers and two 'context' characters: the number of heterospecific flowers and percent bare ground (a measure

of plant density), for use as covariates as these might also influence visitation (e.g., Bernhardt et al. 2008; Duffy and Stout 2011). Visitors to *M. guttatus* flowers were recorded as small bees, medium bees, large bees (including honey-bees and bumblebees), and beetles. Unknown visitors were collected and later identified to species or family. We calculated visitation rate as the number of visits/flower/h, pooled across all visitors. To distinguish flower visitors foraging for nectar and pollen rewards from florivores, we refer to them as 'pollinators', but acknowledge that some of these flower-visiting insects may not be effective pollinators.

The effect of soil type on visitation rate to *M. guttatus* was determined using mixed linear models (PROC MIXED; SAS 2010), with site soil type (serpentine vs. non-serpentine) and year (2010, 2011) as fixed factors, and site identity (nested within site soil type) as a random effect. Average *M. guttatus* floral display (i.e., corolla width, number of open flowers per inflorescence, and inflorescence height) per plot, time of day of observations, and context characters (i.e., the number of heterospecific flowers and percent bare ground) were included as covariates. Visitation rate was transformed [log(visitation rate + 1)] to meet the assumption of normality. Composition of the pollinator assembly, pooled across all observations/year, to *M. guttatus* growing on serpentine and non-serpentine was compared using two-way Chi-square analysis (PROC FREQ; SAS 2010) with visitor type and soil type as factors.

Florivores

To assess whether *M. guttatus* on serpentine and non-serpentine soil differed in terms of florivore damage, we measured the percent of open flowers with corolla tissue missing on three plants/plot. Average florivore damage/plot was analyzed using a generalized linear mixed model (PROC GLIMMIX; SAS 2010) with site soil type and year as factors and display traits (corolla width, number of open flowers per inflorescence, and inflorescence height) as covariates. Site soil type and year were fixed effects, while site identity (nested within site soil type) was a random effect.

Abiotic and biotic interactions for experimental plants

Floral display/flower chemistry

To isolate the specific effects of soil on reproductive morphology and/or chemistry, we conducted a common garden reciprocal soil transplant experiment using field-collected seedlings and soil. Soil from the two serpentine seeps was mixed together in equal proportions to create a generic serpentine soil, and soil from the two non-serpentine sites was similarly treated to create the non-serpentine soil. All soils were augmented with 15 % vermiculite (Perlite Vermiculite

Packaging Industries, OH, USA) to increase water-holding capacity in 27 cm³ 'rocket' pots (Deepots; Stuewe). Fifty *M. guttatus* seedlings, in the two-cotyledon stage, were collected from each seep and assigned randomly to one of the soil treatments. These were arranged in 25 blocks of eight plants (one each per site-treatment combination; total $n = 200$) on an outside bench and bottom watered as needed. Field-collected seedlings were used in these experiments so they would be phenologically synchronized with the natural populations. We measured corolla width (mm) for the first three flowers produced by each plant, and inflorescence height (mm) and number of open flowers per plant 2 weeks after the first flower opened. The first 15 freshly opened flowers per plant were bulk-collected for elemental analysis as above.

To determine whether soil type affected corolla width, number of open flowers per inflorescence, or inflorescence height, we used mixed linear models (PROC MIXED; SAS 2010) with experimental soil type, source population soil type, and their interaction as factors. Experimental soil type and source population soil type were treated as fixed effects, while source population (nested within source population soil type) and block were treated as random effects. We used separate mixed linear models (PROC MIXED; SAS 2010) for each element in floral tissue as above. To control for potential Type I errors due to multiple comparisons, we used Bonferroni corrections to adjust alpha levels. The data for elements B, Al, Fe, Ni, and Zn were log-transformed to improve normality.

Pollinators

To determine whether pollinator preference depends on soil type, we created arrays of inflorescences. Inflorescences were collected from both serpentine and non-serpentine sites, corolla width and flower number recorded, and placed in 225-mL centrifuge tubes filled with water and topped with florist's foam. Each array consisted of two inflorescences from each site evenly spaced in a circle with a circumference of 52 cm. On each day of observation, two arrays were placed within the four sites and observed for 615-min intervals (18 h of total; $n = 6$ arrays per site; total $n = 24$ arrays). Following each observation interval, the position of the arrays was switched. Visitation to each inflorescence was recorded as visits/flower/h.

The effect of site soil type on pollinator visitation rate to inflorescences of *M. guttatus* within arrays was determined using a mixed linear model (PROC MIXED; SAS 2010). The model included site soil type and source population soil type as factors and corolla width and time of day as covariates. Site soil type and source population soil type were treated as fixed effects, while site identity (nested within site soil type) and source population (nested within source

Table 1 Results from mixed models ANOVA on the effects of serpentine versus non-serpentine soil (site soil) and year (2010, 2011) on inflorescence height, corolla width, and number of open flowers per inflorescence of *M. guttatus* plants in natural populations

Source of variation	*df* (num., den.)	Display trait		
		Inflorescence height	Corolla width	Number of open flowers per inflorescence
		F	*F*	*F*
Site soil type	1, 2	80.96[†]	175.59*	187.09*
Year	1, 159	1.13	29.18*	4.90[†]

[†] Significance of fixed effects at $P \leq 0.05$

* Significance of fixed effects at $P \leq 0.01$

population soil type) were treated as random effects. Visitation rate to inflorescences was transformed [log(visitation rate + 1)] to meet the assumption of normality.

Florivores

We placed arrays of 16–20 potted plants, half grown in serpentine and half grown in non-serpentine soil, within each site for 72 h. After exposure, florivore damage was estimated as percent of corolla removed on a 0–5 scale (where 0 = no damage, 1 up to 20 %, 2 = 20–40 %, 3 = 40–60 %, 4 = 60–80 %, and 5 = 80–100 %). One array/site was set out for three consecutive weeks (208 total plants).

The proportion of plants placed within serpentine versus non-serpentine sites that received damage from florivores was compared using a two-way Chi-square analysis (PROC FREQ; SAS 2010) with site soil type and florivore damage (present or absent) as factors. For those *M. guttatus* plants that received damage by florivores, florivore damage score was analyzed using a generalized linear mixed model (PROC GLIMMIX; SAS 2010). The model included site soil type and source population soil type as factors and corolla width and inflorescence height as covariates. Site soil type and source population soil type were treated as fixed effects, while site identity (nested within site soil type) and source population (nested within source population soil type) were treated as random effects.

Results

Abiotic and biotic interactions in natural populations

Floral display/flower chemistry

Serpentine soil influenced floral display in *M. guttatus*. Plants growing on serpentine soils produced 60 % shorter inflorescences, 12 % smaller corollas and 52 % fewer open flowers per inflorescence (Table 1; Fig. 1) than those growing on non-serpentine. Differences between years were also evident for mean corolla width (2010: 22.08 ± 0.2 mm;
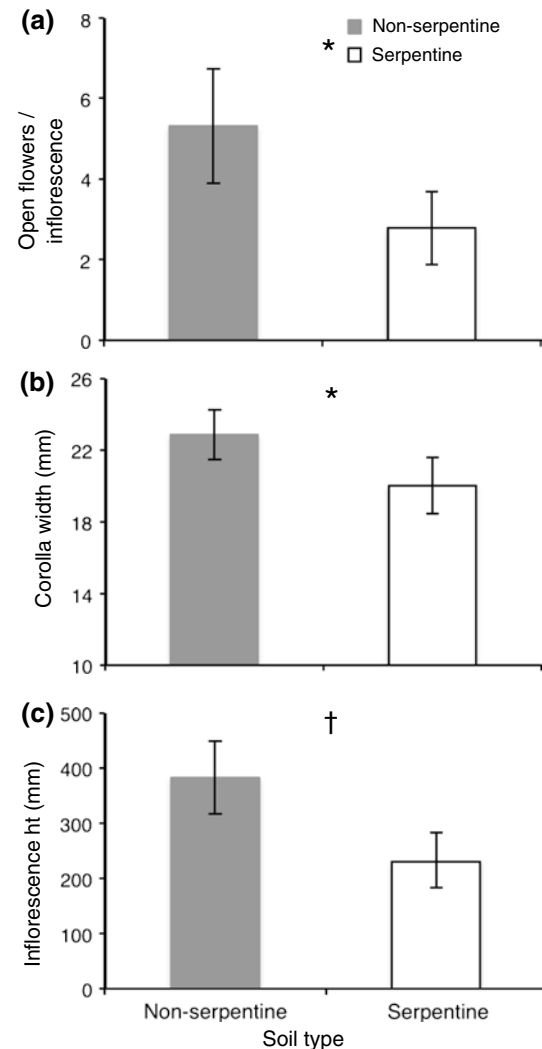

Fig. 1 Comparison of **a** number of open flowers per inflorescence, **b** corolla width, and **c** inflorescence height of *M. guttatus* plants growing in natural non-serpentine and serpentine sites. *Bars* means (±SE; n = 82 per soil type); [†]$P \leq 0.05$, *$P \leq 0.01$ indicating significant soil type effects

2011: 20.92 ± 0.23 mm) and mean number of open flowers per inflorescence (2010: 4.28 ± 0.2; 2011: 3.87 ± 0.18; Table 1).

Fig. 2 Differences in flower concentration of **a**, **b** (%) macronutrients and **c**, **d** (ppm) micronutrients and beneficial elements of *M. guttatus* plants growing in (**a**, **c**) natural populations and (**b**, **d**) experimental soils. *Bars* means (±SE; $n = 82$ per natural soil type; $n = 83–88$ per experimental soil type); *$P \leq 0.005$ indicating a significant soil type (serpentine vs. non-serpentine) effect
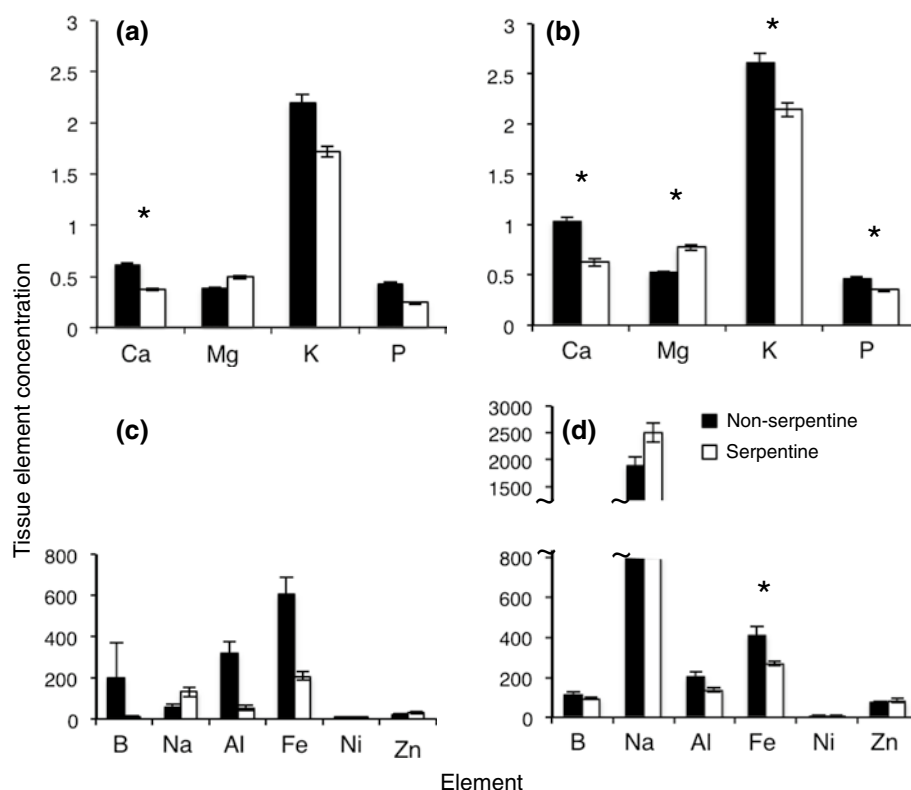
Flowers of *M. guttatus* on serpentine soil differed in chemical content from those on non-serpentine soil. Floral tissue was more concentrated in Mg (29 %), but less concentrated in Ca, P, and K (39, 43, and 22 % respectively) than those of non-serpentine plants (Fig. 2; Table S2). Flowers on serpentine plants were more concentrated in Zn (42 %) and Na (97 %), but less concentrated in Fe, Ni, Al, and B (16, 15, 33, and 42 % respectively) than those produced by non-serpentine plants (Fig. 2; Table S2).

Pollinators

Visitation rates differed between serpentine and non-serpentine *M. guttatus*. Plants growing on non-serpentine received three times more pollinator visits per flower per hour by all insects pooled relative to plants growing in serpentine populations (Fig. 3; Table 2), and this difference exists even after corolla width was accounted for (Table 2). There was a difference in visit rate between years (2010 vs. 2011: 0.63 ± 0.08 vs. 0.73 ± 0.07; Table 2).

Pollinator assemblage differed between serpentine and non-serpentine seeps in both 2010 ($\chi^2 = 51.47$, $df = 3$, $P < 0.0001$) and 2011 ($\chi^2 = 29.68$, $df = 3$, $P < 0.0001$). Across both years, large bees and beetles made up a larger percentage of all pollinators observed on *M. guttatus* at non-serpentine seeps than at serpentine seeps (large bees: 26 vs. 10 %; beetles: 18 vs. 3 %; Tables S3, S4).

Florivores

Flowers of serpentine plants received 60 % less damage than non-serpentine plants, though this difference was only marginally statistically significant ($F_{1,2} = 6.32$; $P = 0.064$; Fig. 3; Table 3). Similar to pollinator visitation, floral display also influenced florivore damage in natural populations, as flower number and inflorescence height explained a significant amount of the variation in damage amount (Table 3).

Abiotic and biotic interactions for experimental plants

Floral display/flower chemistry

Similar to the natural populations, serpentine soil affected floral display traits of *M. guttatus* in the transplant experiment, and this was true regardless of their soil-type origin (experimental soil type × source population soil type interaction: all $P > 0.1$). Plants grown in serpentine soil produced 12 % shorter inflorescences and 22 % smaller corollas relative to plants grown in non-serpentine soil, but there was no difference in open flowers per inflorescence (Fig. 4; Table 4).

In addition, flowers from *M. guttatus* growing in serpentine soil were found to be chemically distinct from those on non-serpentine soil, regardless of the soil type in which
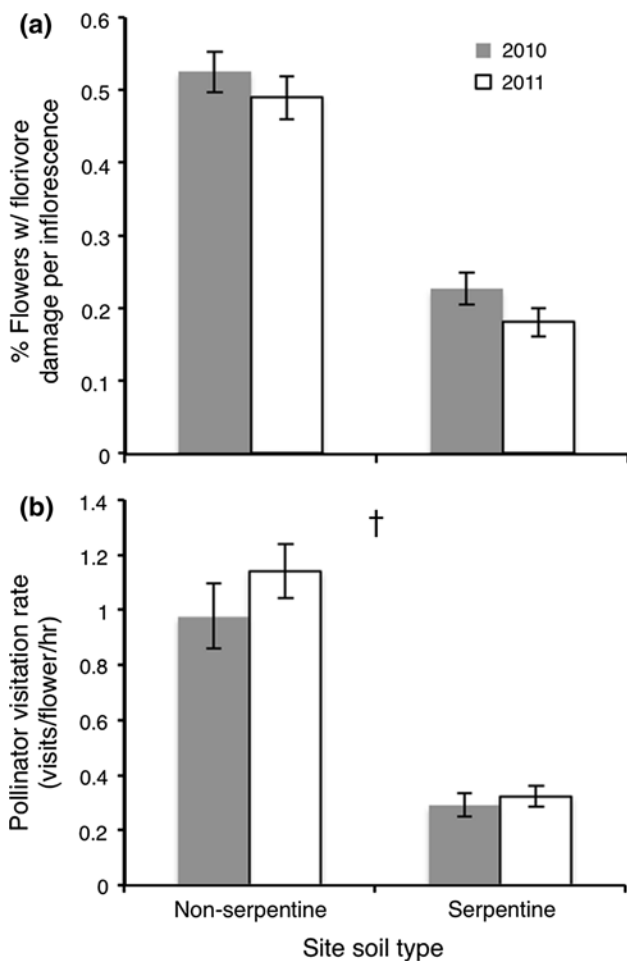
Fig. 3 Pollinator visitation rates (visits/flower/h) and florivore damage (% of flowers damaged per inflorescence) at non-serpentine and serpentine populations of *M. guttatus* in 2010 and 2011. *Bars* are means (±SE; $n = 82$ per soil type); $^{†}P \leq 0.05$ indicating a significant site soil type effect

Table 2 Mixed model ANCOVA of pollinator visitation rate (visits/flower/h) to *M. guttatus* plants growing at serpentine vs. non-serpentine sites (*Site soil type*) in 2 years (2010, 2011)

Source of variation	*df* (num., den.)	*F*
Site soil type	1, 2	17.35†
Year	1, 154	8.29*
Corolla width	1, 154	9.23*
Open flowers per inflorescence	1, 154	0.32
Inflorescence height	1, 154	0.06
Time of day	1, 154	3.76
Number of heterospecific flowers	1, 154	1.19
% Bare soil	1, 154	0.10

Time of day of observation, the number of heterospecific flowers within plots, percent bare soil within plots, and plot-level means of corolla width, open flowers per inflorescence, and inflorescence height were included as covariates

† Significance of fixed effects at $P \leq 0.05$

* Significance of fixed effects at $P \leq 0.01$

Table 3 Results from mixed model ANOVA of florivore damage (percentage of flowers with florivore damage per inflorescence) to *M. guttatus* plants from natural serpentine versus non-serpentine populations (site soil) in 2 years (2010, 2011)

Source of variation	*df* (num., den.)	*F*
Site soil type	1, 2	6.32
Year	1, 146	0.73
Flower number	1, 146	5.46†
Corolla width	1, 146	1.51
Inflorescence height	1, 146	15.57*

Plot-level means of corolla width, open flowers per inflorescence, and inflorescence height were included as covariates

† Significance of fixed effects at $P \leq 0.05$

* Significance of fixed effects at $P \leq 0.01$

they originated. Floral tissues of plants on serpentine were more concentrated in Mg (34 %), but less concentrated in Ca, P, and K (39, 24, and 18 %, respectively) compared to those grown on non-serpentine soil (Fig. 2; Table S5). For micronutrients and other beneficial elements, floral tissues of plants on serpentine were more concentrated in Zn (8 %) and Na (24 %), but less concentrated in Fe, Al, Ni, and B (36, 33, 19, and 19 %, respectively) compared to those on non-serpentine soil (Fig. 2; Table S5). In one case, there was a significant experimental soil type by source population soil type interaction, where plants from non-serpentine populations accumulated more Fe into flowers when grown on non-serpentine soil compared to all other plants (Table S5).

Pollinators

Regardless of where inflorescences were collected from, insects visited arrays placed at non-serpentine sites three times more often than those placed at serpentine sites (Table 5). Source population soil type did not influence pollinator visitation rates to flowers in arrays (Table 5). Pollinator visitation increased with flower size regardless of source population soil type (Table 5).

Florivores

There was a strong effect of site soil type on the frequency of florivore damage: 19 % of plants placed at serpentine sites received florivore damage, compared to 40 % at non-serpentine sites ($\chi^2 = 8.83$, $df = 1$, $P < 0.01$). Moreover, flowers of potted plants growing in serpentine soil received 34 % less damage compared to plants growing in non-serpentine soil, a marginally significant difference ($F_{1,2} = 16.59$; $P = 0.055$; Fig. 5; Table 6). Neither flower size nor inflorescence height,

Table 4 Results from the mixed models ANOVA on floral display traits (inflorescence height, corolla width, and number of open flowers per inflorescence) of *M. guttatus* plants in reciprocal transplant experiment

Source of variation	df (num., den.)	Display Trait		
		Inflorescence height	Corolla width	Number of open flowers per inflorescence
		F	F	F
Experimental soil type	1, 160	13.35*	207.97*	2.83
Source population soil type	1, 2	0.03	0.48	0.89
Experimental soil type × Source population soil type	1, 160	0.17	1.78	0.15

Experimental and source population soils are serpentine and non-serpentine

* $P \leq 0.01$ indicating significant fixed effects

Table 5 Results from mixed model ANCOVA of pollinator visitation rate (visits/flower/h) to experimental arrays of *M. guttatus* inflorescences collected from serpentine vs. non-serpentine sites (source population soil) and presented to pollinators at serpentine or non-serpentine sites (site soil)

Source of variation	df (num., den.)	F
Time of day	1, 182	3.18
Site soil type	1, 2	20.35[†]
Source population soil type	1, 2	1.81
Corolla width	1, 182	67.48*

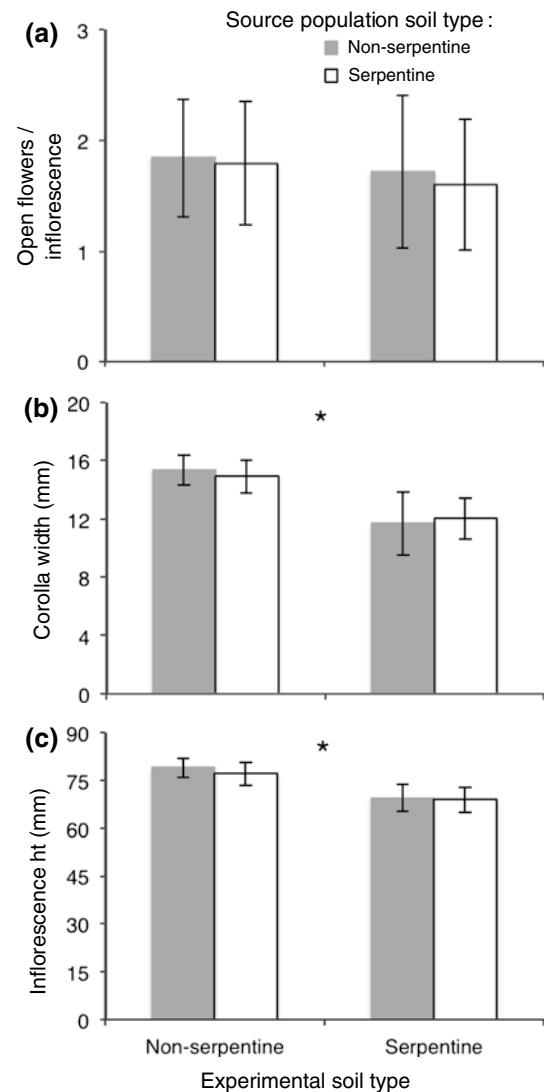Corolla width of experimental plants was included as a covariate

[†] Significance of fixed effects at $P \leq 0.05$

* Significance of fixed effects at $P \leq 0.01$

however, influenced the amount of damage by florivores (Table 6).

Discussion

Our study simultaneously shows that serpentine soil alters plant–insect interactions both directly, through plant tissue chemistry, and indirectly, through floral display, and thus it adds a new dimension to the growing body of work on the effect of serpentine on plant morphology and chemistry (Harrison and Rajakaruna 2011). While plant–mycorrhizae interactions across serpentine and non-serpentine plant populations are beginning to receive attention (Schechter and Bruns 2008; Davoodian et al. 2012), plant–pollinator and plant–florivore interactions across populations of serpentine-tolerant plant species have rarely been characterized (but see Westerbergh and Saura 1994; Lau et al. 2008). Not only were florivore damage and pollinator visitation rates altered by soil habitat but pollinator assemblage was also more diverse for *M. guttatus* in non-serpentine soils (Tables S3, S4), indicating that serpentine soil influences the quantity and perhaps the quality of plant–animal interactions.

Fig. 4 Effect of experimental serpentine soil on **a** number of open flowers per inflorescence, **b** corolla width, and **c** inflorescence height of *M. guttatus* plants in reciprocal soil transplant experiment. *Bars* means (±SE, $n = 83$–88 per experimental soil type); *$P \leq 0.01$ indicating a significant experimental soil type (serpentine vs. non-serpentine) effect
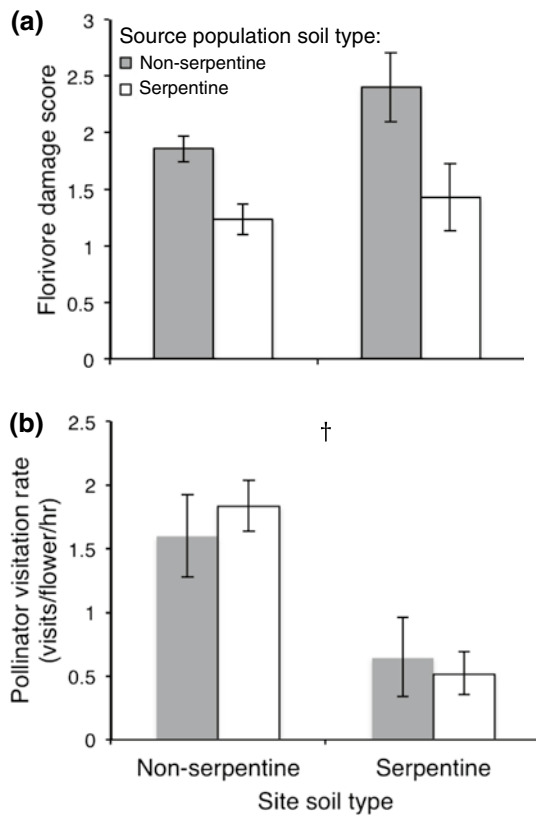
Fig. 5 Effects of site soil type and source population on **a** florivore damage score and **b** pollinator visitation rates (visits/flower/h) to potted *M. guttatus* in experimental arrays. *Bars* means (±SE, $n = 96$–104 plants per site soil type); †$P \leq 0.05$ indicating a significant site soil type effect

Table 6 Results of the generalized mixed model of florivore damage score to *M. guttatus* plants collected from serpentine versus non-serpentine sites (source population soil) and presented to florivores at serpentine or non-serpentine sites (site soil)

Source of variation	df (num., den.)	F
Site soil type	1, 2	3.14
Source population soil type	1, 2	16.59[+]
Inflorescence height	1, 42	0.02
Corolla width	1, 42	1.69
Week	2, 42	0.43

Over the course of 3 weeks, one array ($n = 16$–20 plants) was monitored weekly for florivore damage at each site. Inflorescence height and corolla width were included in the model as covariates

[+] $P \leq 0.06$

Mimulus guttatus on serpentine had reduced floral display (i.e., smaller flowers, fewer flowers per inflorescence, and shorter inflorescences), and our common garden reciprocal soil transplant experiment confirmed the direct effect of serpentine soil on flower size and inflorescence height

(Fig. 4) in response to nutrient limitation. Interestingly, plants responded similarly to soil treatments in terms of morphology and tissue chemistry, regardless of the soil type they originated. In addition to similar morphological and chemical responses, all experimental plants survived equally well on both soil types, regardless of whether they were collected from serpentine or non-serpentine populations ($\chi^2 = 0.14$, $df = 1$, $P = 0.71$). This suggests a lack of adaptation to soil chemistry for these serpentine/non-serpentine populations of *M. guttatus*, which may be explained by high levels of gene flow between populations growing in different soil environments (Sambatti and Rice 2006). And if our survival data are indicative of total fitness, then our findings are in contrast to others that have found evidence of local adaptation and ecotypic differentiation for other serpentine-tolerant plant species (e.g., *Collinsia sparsiflora*; Wright et al. 2006). However, other studies have documented the importance of phenotypic plasticity for serpentine-tolerant *M. guttatus* (Murren et al. 2006), and it is clear from our data that soil-induced changes in plant morphology have consequences for plant–pollinator and plant–florivore interactions in this species. While flower size varied across serpentine and non-serpentine populations, pollinator visitation was greater in plots with larger flowers within both serpentine and non-serpentine sites (Table 2), and pollinators responded in terms of increased visitation to larger flowers within experimental arrays (Table 5). Additionally, the number of open flowers per inflorescence and inflorescence height altered plant–florivore interactions, as plants with more flowers and taller inflorescences experienced greater levels of florivore damage in natural populations (Table 3). Therefore, indirect effects of soil environment on pollinator visitation and florivore damage may affect plant–animal dynamics in plant species expressing phenotypic plasticity across multiple environments. Phenotypic plasticity due to environmental heterogeneity is likely an important, yet understudied, mechanism altering the evolution of plant–animal interactions (Fordyce 2006).

Traditionally, studies of plant–herbivore interactions have focused on the role of plant secondary compounds in influencing levels of herbivore damage (reviewed in Mithöfer and Boland 2012). However, primary metabolites may be equally important in affecting herbivore damage (e.g., Alonso and Herrera 2003). Studies of herbivory of plants growing on serpentines have often focused on toxic elements in the soil, such as the heavy metal Ni in hyperaccumulating species (Boyd et al. 1994; Martens and Boyd 1994; Boyd and Moar 1999). Our work with *M. guttatus*, a serpentine-tolerant species, did not reveal significant differences in Ni concentrations in floral tissues of plants growing in serpentine versus non-serpentine soils (Fig. 2), yet there were tendencies for higher florivore damage on non-serpentine grown plants in both natural and experimental

settings (Figs. 3, 5). Because plants growing in serpentine versus non-serpentine soils had distinct chemical profiles (Fig. 2), these findings suggest that other metals (e.g., Mg) or primary metabolites, such as Ca, P, and K, may be just as likely to alter herbivore feeding as the toxic metal Ni present in serpentines. While the effects of primary metabolites on the growth of insect herbivores is well studied, the influence of primary metabolites on herbivore choice is less understood (Joern et al. 2012). Furthermore, previous work has suggested that herbivores may respond to ratios of elements, rather than single element concentrations, which may be the case in serpentine plants as the chemical profiles of serpentine versus non-serpentine tissues differed in multiple elements. For example, Beanland et al. (2003) manipulated the ratios of B, Zn, and Fe present in diets fed to herbivores, and found that herbivore development could not be described as a linear response to any one element but instead depended upon ratios of these elements. As several elements varied in the floral tissues of *M. guttatus* in this study, it is reasonable to suspect that *M. guttatus* florivores are also responding to multiple element variation. Our work shows that soil generalist, non-hyperaccumulating plants may display variation in the magnitude of plant–florivore interactions across multiple environments, and that this variation is largely explained by the direct effect of soil chemistry on floral tissue chemistry.

We did not find evidence of a direct effect of flower chemistry on pollinator visitation, which may be due to one of several factors. For example, our tissue analysis was based on whole flowers, therefore we do not know if the soil environment alters pollen or nectar chemistry for *M. guttatus*, or rather strictly perianth tissues. Furthermore, *M. guttatus* is not known to produce large volumes of nectar (Robertson et al. 1999), thus flower-visiting insects may not have been exposed to chemically variable resources when visiting serpentine versus non-serpentine *M. guttatus*, particularly if pollen chemistry is unaffected by soil environment. It is also possible that pollinating insects are less affected by changes in tissue chemistry relative to herbivorous insects, as visitation by bees has been shown to be unaffected by high concentrations of trace elements in floral rewards (e.g., Se: Hladun et al. 2013). Other studies, however, have shown that the presence of Ni in nectar can decrease visitation by bumblebees (Meindl and Ashman 2013), suggesting soil chemistry may alter biotic interactions more so than previously thought.

It is important to consider whether differences observed in plant–pollinator and plant–florivore interactions across serpentine and non-serpentine habitats translate into differences in individual fitness of plants in natural populations. Provided that plants are pollen-limited, which is common for many flowering plants (reviewed in Knight et al. 2005), pollinator visitation is generally considered a good proxy of fitness, as higher visitation rates often translate into higher seed and fruit production (e.g., Ghazoul 2005). However, in nutrient-limited environments, like serpentine, limited resource availability may preclude any added benefit of increased pollinator visitation towards individual fitness (Asikainen and Mutikainen 2005). Additionally, while corolla damage by florivores can decrease pollinator visitation (e.g., Botto-Mahan et al. 2011), florivore damage to structures that house the gametes, such as anthers and pistils, may have greater consequences for plant fitness (McCall and Irwin 2006; Hargreaves et al. 2009), especially if plant tolerance to herbivory is low (Strauss and Agrawal 1999). To fully appreciate the evolutionary consequences of abiotic-mediated changes in plant–animal interactions, studies are needed that document differential fitness of soil-generalist plants across different environments and that tie this directly to altered plant–animal interactions.

Our study shows that soils have both direct and indirect effects on how plants interact with animal mutualists and antagonists. We demonstrate that soils can affect these plant–animal interactions more generally than previously thought, i.e., in addition to affecting plant–insect interactions of metal hyperaccumulators, even those of non-accumulating soil generalist species are affected. As such, plant species that occur in a variety of substrates may differ in both the quality (e.g., visits by effective pollinators) and quantity (e.g., number of pollinator visits) of plant–animal interactions across soil types. Soil chemistry may therefore be an important geographic variant that contributes to altered interactions, leading to small-scale spatial mosaics with the potential to influence evolutionary dynamics between plants and animals (Thompson 1999).

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