Experimental dispersal reveals characteristic scales of biodiversity in a natural landscape

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Ecological theory posits that dispersal among habitat patches links local communities and is a key "regional" process that maintains biological diversity. However, manipulations required to experimentally test regional processes are infeasible for most systems, and thus more work is needed to detect the scales at which regional processes manifest and their overall effect on diversity. In a Californian grassland, a hotspot for global biodiversity, we used a seed vacuum to increase dispersal at spatial scales varying from 1 m to 10 km while maintaining a realistic spatial structure of species pools and environmental conditions. We found that dispersal limitation has a profound influence on diversity; species richness increased with the spatial scale of seed mixing, doubling in plots that received seed from large (≥5 km) compared with small (≤5 m) scales. This increase in diversity corresponded to an increase in how well species distributions were explained by environmental conditions, from modest at small scales ($R^2 = 0.34$) to strong at large scales ($R^2 = 0.52$). Responses to the spatial scale of seed mixing were nonlinear, with no differences below 5 m or above 5 km. Nonlinearities were explained by homogeneity of environmental conditions below 5 m and by a lack of additional variation in the species pool above 5 km. Our approach of manipulating natural communities at different spatial scales reveals (*i*) nonlinear transitions in the importance of environmental sorting and dispersal, and (*ii*) the negative effects of dispersal limitation on local diversity, consistent with previous research suggesting that large numbers of species are headed toward regional extinction.

dispersal limitation | McLaughlin Natural Reserve | metacommunity | seed addition | spatial scale

> The problem of pattern and scale is the central problem in ecology, unifying population biology and ecosystem science, and marrying basic and applied ecology.
>
> S. A. Levin (1992)

The processes that structure ecological populations, biodiversity, and ecosystem properties transition in importance across spatial scales (1, 2). As the spatial scale of observation increases, the range of environments sampled (1, 3) and the geographic distance separating localities (4) become increasingly important in shaping species distributions. As a consequence, the relative spatial scaling of different ecological processes is thought to underlie some of the most important patterns in ecology, such as species–area (5) and biodiversity–ecosystem function relationships (6). Because identifying the important scales is challenging, ecologists often compare ecological patterns among local and regional scales to simplify theoretical (7–9) and empirical research (10, 11). However, how closely local and regional delineations match up with the actual scaling of ecological processes is rarely known (2, 12). Quantifying the spatial scaling of these processes promises to enrich our understanding of the mechanisms that maintain diversity, yet remains elusive even in biodiversity hotspots that require this information for conservation decisions (13).

A major challenge to testing how ecological processes transition among spatial scales in natural communities is that regional processes, unlike local processes, are not often amenable to experimental manipulation in the field due to the inability to move most communities of organisms. Manipulative tests of local and regional diversity are typically performed in mesocosm experiments on communities constructed using simplified environments, species pools, or dispersal patterns (14). Because it is often unclear how such simplifications affect experimental outcomes (15), mesocosm experiments allow essential tests of the range of potential outcomes under different sets of experimental conditions, but cannot capture the importance of processes that occur in nature. Finding new ways to combine the power of manipulative field experiments with the biological realism of natural landscape structure is necessary to address fundamental questions, such as: how widespread is dispersal limitation in a community and at what spatial scales does it manifest? How strongly do species sort along environmental gradients in the absence of dispersal limitation? And, even more basically, at what spatial scales are local and regional communities most appropriately defined?

The above questions can be tested by experimentally removing dispersal limitation through the homogenization of species pools, that is, by redistributing species equally across habitat patches while maintaining their abundance distributions. Theory makes distinct predictions for how such homogenization would affect species diversity, and whether it would increase or decrease the probability that species are found in environmentally suitable habitat patches (9, 16, 17). This latter concept, referred to here as "species–environment associations," is commonly measured by the amount of variation in species distributions that is explained by environmental covariates (18). If dispersal chronically limits species movement and the landscape is patchy and heterogeneous, then homogenization of species pools would increase both species richness (19, 20) and the strength of species–environment associations (16) (*SI Appendix*, Fig. S1A). These changes occur as species access suitable localities that were previously inaccessible (9), causing the average number of species in a site to increase and the

Significance

Biological communities differ dramatically in numbers and identities of species, a pattern that could be explained by many mechanisms that each vary with spatial scale. Testing how ecological mechanisms transition among scales is key to understanding the maintenance of diversity but is infeasible in most systems. In a natural plant community, we experimentally enhanced seed dispersal over scales ranging from 1 m to 10 km using a seed vacuum. Our results indicate that pervasive dispersal limitation constrains local communities at and above 100 m, causing communities to contain half as many species as the environment is capable of supporting. Our results suggest that many species at risk for extirpation in this global biodiversity hotspot could be restored through managed dispersal.

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variation in species distributions explained by the environment to similarly increase.

Alternatively, if species are not dispersal-limited and already well-matched to environmental conditions (*SI Appendix*, Fig. S1B), the strength of species–environment associations should decrease even as local richness increases due to mass effects (16, 17), meaning that species are increasingly found in environments to which they are not suited. When species–environment associations are neutral with respect to species identity, species–environment associations should not change whether species have limited dispersal (*SI Appendix*, Fig. S1C; increased species richness) or are already dispersing evenly among communities (*SI Appendix*, Fig. S1D; no change in species richness) (8, 16). In the metacommunity ecology literature, these four potential outcomes of increasing dispersal would correspond to species sorting, mass effects, and neutral dynamics (with or without dispersal limitation), respectively (9, 16). The approach of homogenizing species pools and tracking diversity responses to local conditions is similar to that taken by many experiments that use mesocosm (e.g., ref. 11) or field (e.g., refs. 19–21) manipulations with artificial species pools (14, 15, 22), but has yet to be implemented in the field with natural spatial structure in species pools, abundance distributions, and environments.

We tested the spatial scales at which dispersal and environmental heterogeneity impose constraints on species distributions and diversity using a "hay transfer" technique (23) in serpentine annual plant communities in California. This technique involved vacuuming the seed bank and other loose material from field plots, pooling the material among plots, and then redistributing it. Our "species-pooling" treatments captured five spatial scales (~1 m, 5 m, 100 m, 5 km, and 10 km; *SI Appendix*, Fig. S2), with the 100-m treatment receiving a mix of seeds from all plots within 100 m, for example. In the annual plant communities studied, pooling the seed bank redistributes all plants during their dormant stage. In the following growing season, we collected data on species occupancy and environmental conditions to answer four questions: (*i*) How common is dispersal limitation in a metacommunity? (*ii*) How strongly do species sort along environmental gradients once potential dispersal limitation is experimentally removed? (*iii*) At what spatial scales do signals of dispersal limitation and environmental sorting manifest? (*iv*) Do these signals correspond to the natural structure of species pools and environmental conditions? Our surveys were conducted at peak flowering, after the filtering effects of competition and environment, which can be strongest at the seedling stage (24, 25), had time to take effect.

Our serpentine study system is ideal for testing questions of the spatial scaling of diversity for two reasons. First, the high occurrence of annual species and patchy distribution of serpentine habitat among a nonserpentine matrix is well-suited to testing spatial questions (26) with the hay transfer method (23). Second, our study took place in the California Floristic Province, a global biodiversity hotspot that is threatened by the pervasive invasion of European grasses (26, 27). Serpentine soils are hypothesized to act as spatial refugia for native species to escape the direct competitive effects of invasive grasses (28), but less often considered are the indirect negative effects of invaders on diversity through the isolation of habitat patches (13, 29).

Given that invasive grasses and other human impacts [road building, fire suppression (30)] have increased fragmentation of native plant communities in the past 200 y, we hypothesized that diversity is constrained by limited dispersal and that, if this dispersal constraint were removed, species would sort deterministically according to their environmental niche requirements (i.e., increased species–environment associations; *SI Appendix*, Fig. S1A). Environmental conditions were described by two composite variables—principal components analysis (PCA) axes—that summarized soil chemistry, fertility, moisture, and site topography. The strength of species–environment associations was the pseudo-R^2 of logistic regressions that predicted the occurrences of all species with environmental conditions (PCA axes) used as predictors (see *Data analysis* in Materials and Methods and *SI Appendix*, Fig. S4 A–C for an example of data that underlie pseudo-R^2 results). The degree of dispersal limitation, and the scales at which its effects manifest, are important to understanding biodiversity in this global hotspot and to projecting community stability over longer timescales (13).

Results

Increasing the spatial scale of species pooling caused a net increase of 12 species in plots ($F_{4,112} = 69.4$, $P < 0.001$; Fig. 1*A*). This increase was sigmoidal rather than linear, with no significant differences in species richness between the two smallest (1 m and 5 m; $P = 0.72$) or the two largest (5 km and 10 km; $P = 0.34$) spatial scales, but highly significant (all $P < 0.001$) differences among small (≤5 m), intermediate (100 m), and large (≥5 km) spatial scales. As a result, species richness in our plots doubled ($F_{4,112} = 69.9$, $P < 0.001$) from 10 species in the 1-m treatment to 21 species in the 10-km treatment (*SI Appendix*, Fig. S3A; all measures are for 0.75- × 0.75-m plots). Species richness postmanipulation was composed of species initially present at sites (not dispersal limited) plus those gained by species pooling (dispersal limited; *SI Appendix*, Fig. S3B).

To explore whether this increase in species richness corresponded with an increase or decrease in species–environment associations, we tested whether environmental conditions predicted the occurrences of all 73 annual species observed in our study. Environmental conditions were highly correlated and thus were summarized by the first and second axis scores of a principal components analysis (Materials and Methods). Axis 1 primarily summarized soil chemistry (i.e., Ca/Mg, Olsen-P, X-K, NO_3-N, and organic matter), whereas axis 2 summarized elevation and soil moisture (*SI Appendix*, Fig. S5B). We found the proportion of variance explained (pseudo-R^2) by our models increased from 0.34 to 0.52 with increasing spatial scale of species pooling (Fig. 1*B*); the sigmoidal response of species richness to the spatial scale of species pooling was closely mirrored by the sigmoidal increase in variation explained by the environment (Fig. 1). Supplementary analyses of species–environment associations with percentage of cover data, rather than presence/absence data, produced broadly similar increases with increasing spatial scale of species pooling (*SI Appendix*, Fig. S9 and discussion in *SI Appendix*, *Supplementary Methods, Results, and Discussion*).

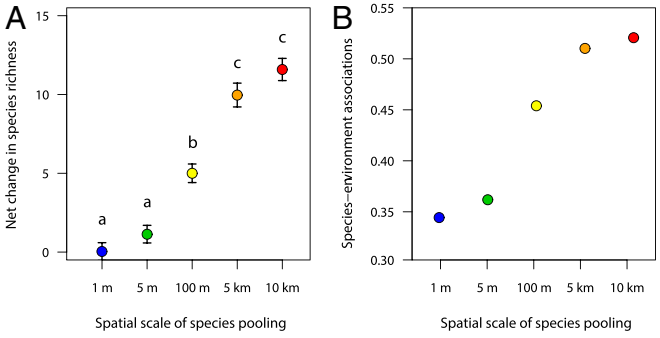

Fig. 1. Effect of spatial scale of species pooling on (*A*) net change in species richness (mean ± SE), relative to an unmanipulated control plot (C3 in *SI Appendix*, Fig. S2B) and (*B*) the strength of species–environment associations across a community of species (pseudo-R^2 values); observed patterns correspond to prediction in *SI Appendix*, Fig. S1A. Points with the same letter were not significantly different in a multiple comparisons test. Net changes in species richness are driven by new species gained, which translates into a doubling in species richness when species are pooled at the 1-m versus at the 10-km scale (*SI Appendix*, Fig. S3).

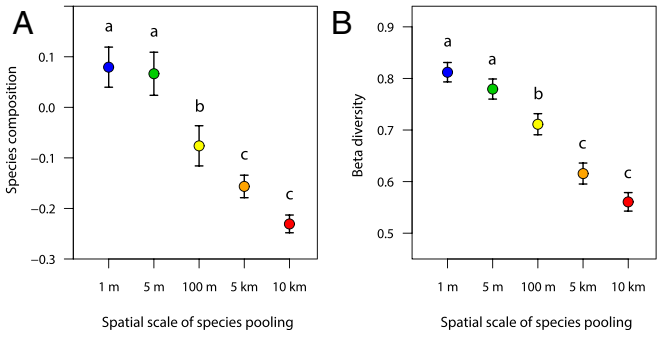

Fig. 2. Effect of spatial scale of species pooling on (*A*) species composition and (*B*) the compositional dissimilarity of plots (beta diversity). Species composition shows the mean and SE of the first axis scores from a PCoA with Jaccard's dissimilarity index (see biplot in *SI Appendix*, Fig. S5*A*); results with second-axis scores are qualitatively similar. Points with the same letter were not significantly different in a multiple comparisons test. Beta diversity patterns mirror changes in species richness, as increasing local (alpha) diversity with no change in regional (gamma) diversity reduces among-plot compositional dissimilarity.

In addition to plot-level species richness (alpha diversity), we tested the effects of our species-pooling treatments on other components of diversity. As with species richness, the composition of species in plots responded sigmoidally to the spatial scale of species pooling (Fig. 2*A*), as estimated using the axis 1 and 2 scores of a principal coordinates analysis (PCoA) (Materials and Methods). These axes were significantly associated with 42 plant species ($P < 0.05$), including many endemic serpentine-associated species (e.g., *Clarkia gracilis*, *Collinsia sparsiflora*, and *Navarretia jepsonii*). Beta diversity (i.e., among-plot turnover in species composition, measured with Jaccard's dissimilarity index) decreased with the scale of species pooling from 0.81 to 0.56 between the 1-m to 10-km spatial scaling treatments (Fig. 2*B*). The size of the regional species pool (gamma diversity), estimated as the total number of unique species that emerged across plots, was not affected by the species-pooling treatments, with a mean pool size of 58.0 ± 0.5 SE across treatments (*x*-intercept; *SI Appendix*, Fig. S6). At a common gamma diversity, alpha and beta diversity are inversely related, as compositional dissimilarity among sites decreases as a consequence of species being found in more sites (31). Site occupancy (the number of sites occupied by each species) also increased with the spatial scale of species pooling (*SI Appendix*, Fig. S6), as did community evenness due to the increasing occurrences of regionally rare species (shallower slope in *SI Appendix*, Fig. S6). Not all 73 species were observed in all treatments; on average, each treatment had 1.2 species (±0.58 SE) that were not observed in any other treatment.

Differences in the naturally occurring spatial structure of environmental conditions and species pools provide further evidence of how regional processes structure local patterns of diversity. We first tested for differences in environmental conditions among sites at spatial scales corresponding to our experimental setup (*SI Appendix*, Fig. S2) to characterize the spatial scaling of the environment. Environmental conditions differed significantly among groups of sites within halves (100-km scale; $F = 14.05$, $P = 0.001$), but differences between halves of the reserve (5-km scale; $F = 247.68$, $P = 0.001$) were larger, with limited overlap in environmental conditions (Fig. 3*A*). Differences among reserve halves were driven by axis 2 (% soil moisture, elevation) but not axis 1 (soil chemistry, fertility). For those environmental variables that were quantified with multiple within-site measurements (5-m scale), there were no statistically significant differences among plots that occurred at the same site (all $P > 0.3$; *SI Appendix*, Table S2). Despite these average changes in environmental conditions with distance, even distant sites (≥10 km apart) frequently shared similar environmental conditions (Fig. 3*A* and *SI Appendix*, Figs. S7 and S8), supporting the finding that dispersal limitation at these scales can have important consequences.

In contrast to environmental conditions, the composition of species significantly differed at the 100-m scale ($F = 1.96$, $P = 0.001$) but not between halves of the reserve (5-km scale; $F = 1.26$, $P = 0.168$). Despite the lack of a significant difference in species composition, the two halves of the reserve had only 62% (37/60) of species in common, with 20% (12/60) of species unique to the northern half and 18% (11/60) unique to the southern half; these numbers were calculated from the 1-m treatment and sum to less than 73 because not all species were observed in all treatments. The species unique to one-half of the reserve were significantly less associated with the axis 1 and 2 PCoA scores than species that were common to both halves ($P = 0.023$), which potentially reconciles the high compositional similarity among halves of the reserve despite the presence of unique species.

Discussion

Understanding the scale dependency of ecological processes is central to protecting biodiversity and a core interest of ecology research (1, 2, 32-34), yet experimental demonstrations have remained elusive for natural communities (2). By experimentally manipulating the spatial scale of seed dispersal in a biodiversity hotspot, our study resulted in two key findings: (*i*) compared with natural patterns, species richness and the strength of environmental matching doubled when seeds were distributed at large spatial scales, and (*ii*) nonlinear diversity responses to the spatial scale of species pooling suggests that there are distinct spatial transitions in the forces that structure diversity. These findings provide insight into how metacommunities and the distributions of their constituent species are structured, with implications for conservation.

In the absence of species pooling, species in our study showed modest associations with environmental conditions (blue point in Fig. 1*B*), consistent with the findings of several observational studies (e.g., refs. 34, 35). However, this type of observational evidence alone cannot identify the mechanisms underlying the pervasive lack of environmental associations, such as dispersal limitation, neutrality, and mass effects (*SI Appendix*, Fig. S1). The experimental enhancement of dispersal via seed pool manipulations allowed us to discriminate among potential mechanisms and supports a model in which dispersal limitation precludes the colonization of otherwise suitable sites (*SI Appendix*, Fig. S1*A*). Consistent with these results, a subsequent analysis showed that species with more effective

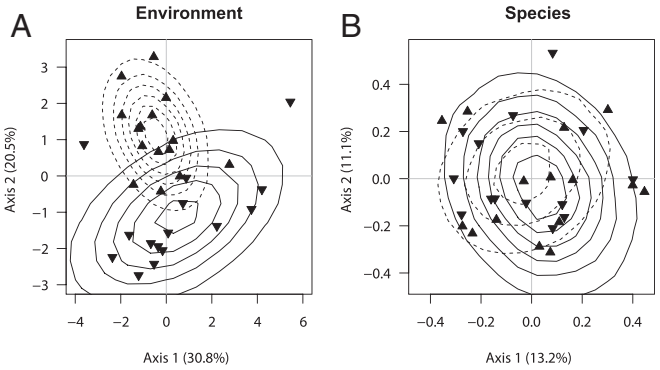

Fig. 3. Natural differences among halves of the reserve in (*A*) environmental conditions (from a PCA) and (*B*) species composition (from a PCoA). The contours outline the bivariate normal distributions of each half of the reserve; dashed contours/upward triangles are sites that occur in the northern half, and solid contours/downward triangles are sites that occur in the southern half. Environmental conditions ($P = 0.001$) but not species ($P = 0.175$) significantly differed among halves (Results).

dispersal modes (wind and vertebrate dispersed) tended to occupy more sites in the absence of species pooling (*SI Appendix*, Fig. S10 and *Supplementary Methods, Results, and Discussion*).

Similar evidence of the role of dispersal limitation has been obtained for individual species, such as the canary violet (4); we extend this perspective beyond individual species to an entire plant community and show a near doubling in the strength of species–environment associations that explains over half of the variation in how communities of species are distributed (Fig. 1*B*). An additional feature of our community-level seed additions is that they allowed natural spatial structure in both species composition and relative abundances, rather than fixed amounts of seed added for all sites and all species (19). Doing so most closely reflects natural variation in propagule pressure, an important determinant of dispersal limitation and community structure (20, 36). Although we cannot refute the possibility that the observed responses are transient, evidence from related seed addition research has shown that initial diversity effects are persistent, even in competitive environments (37), and increases in species–environment associations would not be expected in a transient scenario. Additionally, our results were insensitive to the exclusion of species with small populations, which are those most likely to be "sink" populations (*SI Appendix*, Table S3); we discuss these points at length in *SI Appendix*, *Supplementary Methods, Results, and Discussion*.

To what extent differences in species composition among local communities can be explained by distance or environment has been a major area of ecological research (38, 39), stimulated by the development of statistical methods to partition their relative influences from observational data (18, 40). However, variance-partitioning methods can produce biased estimates of spatial and environmental components of variation, as well as inflated model fits (41), and should be interpreted as rough estimates. Moreover, partitioning approaches cannot isolate the interactive effects of dispersal limitation and environmental components (18), yet it is these interactive effects that determine the persistence of species within landscapes (13, 29). In our study, we find that environmental conditions explain 32% of variation in species occupancy patterns in the absence of species pooling and an additional 20% when distance effects are experimentally removed (Fig. 1*B*). This increase when distance effects are removed quantifies the interactive effect of distance and environmental conditions, or the degree to which dispersal limitation prevents species from accessing suitable habitats.

Nearly all components of diversity that were examined showed consistent, nonlinear responses to the spatial scale of species pooling, including species richness (alpha diversity), species–environment associations, and compositional turnover (beta diversity). At small spatial scales (<5 m), conditions are homogenous, and most species are able to disperse these distances in one to few generations (42); our species-pooling manipulations had negligible effects at this scale (blue and green points, Figs. 1 and 2 and *SI Appendix*, Fig. S3). At intermediate (100 m) and larger (≥5 km) scales, our manipulations allowed seed to cross the nonserpentine grassland matrix to reach distant serpentine sites, resulting in increased species richness and species–environment associations consistent with dispersal limitation. Although environmental conditions also tended to be more different as distance increased, many sites were similar even when separated by ∼10 km (Fig. 3 and *SI Appendix*, Figs. S7 and S8), making dispersal across these distances important for accessing distant sites with similar environments.

Together, our evidence reveals three "characteristic scales" [*sensu* Levin (2)] or "domains of scale" [*sensu* Wiens (1)] among which strengths of ecological processes appear to differ. When seed pooling occurs at local scales ≤5 m, communities experience relatively homogenous environmental conditions, but the make-up of communities is severely limited by dispersal, such that species–environment associations frequently fail to emerge. As the scale of species pooling increases to 100 m and beyond, the increased environmental difference in new sites appears to be outweighed by the large numbers of similar sites (*SI Appendix*, Figs. S7 and S8) and the release from dispersal limitation. The net result is that species are better able to exploit the increased range of environmental heterogeneity, increasing species–environment associations. However, as the scale of species pooling increases beyond 5 km, dispersal limitation no longer alters community responses to continually increasing environmental heterogeneity.

The lack of differences among 5-km and 10-km scales was surprising given their differences in spatial extent and environmental conditions, particularly soil moisture and elevation (as summarized by PCA axis 2, Fig. 3*A*); there are two potential, nonmutually exclusive explanations for this trend. First, the high similarity in the composition of species pool among halves of the reserve (the 5-km scale, Fig. 3*B*) indicates that mixing seed among halves (10-km scale) would introduce little variation to the species pool, consistent with the lack of responses that we observed. Although we did observe a number of species that were unique to each half, these species were so rare that they did not contribute strongly to compositional variation among sites or the strengthening of species–environment associations in response to seed mixing. Second, it is possible that most species in the reserve are more specialized to soil fertility and biochemistry than to differences in elevation and soil moisture (Fig. 3, axis 1 and 2, respectively). Because the reserve halves did not significantly differ in axis 1 scores, specialization along this axis did not generate among-half differences in species composition.

Our finding that local communities are half as speciose as the environment is capable of supporting sheds light on the past and potential future of this global biodiversity hotspot. Californian landscapes no longer resemble those of 200 y ago; European invaders now form a matrix of unsuitable habitat (28) and have restricted native species to harsh refuge environments, such as serpentine (13, 26). Our findings of low species richness and high regional rarity (*SI Appendix*, Fig. S6) are consistent with those predicted for areas experiencing regional "extinction debts"—the delayed extinction of species from a region through chronic reductions in colonization rates (13). The prognosis for native diversity under this scenario is bleak because old invaders are adapting to tolerate harsh serpentine conditions (43) and new invaders that thrive in these harsh environments are spreading rapidly [e.g., barbed goatgrass (*Aegilops triuncialis*)]. The net effect is a shrinking and loss of native plant refuge patches that threaten the regional stability of native plant diversity.

In this scenario and others like it, a more regionally focused approach to conservation is needed to preserve many types of local communities and their constituent species, understanding that local persistence relies on colonization from adjacent patches (44). Management at the wrong scale may miss important covariation in species pools and environments, generating mismatches between species and local conditions (1, 4). In our system, management at a spatial extent of 5 km is likely to be most effective, particularly through assisted dispersal or creation of habitat patches that act as stepping stones between otherwise isolated patches. Although the exact scale of spatial delineations will differ among study systems depending on landscape structure, habitat matrix permeability, and dispersal distances of focal organisms, we have offered a clear example of how these critical scales can be identified. In other systems, approaches such as genetic analyses may be required to better understand characteristic scales of dispersal before assessing scales of environmental turnover.

The forces that dictate how species are distributed across landscapes have long fascinated ecologists. In the serpentine system, we have identified scale-specific processes that structure plant communities and how this spatial scaling is explained by the accumulation of species and environments across space. These results provide insights that are specific to an area that is considered a model system for biodiversity research (45) and more generally provide an experimental test of predictions from a well-developed

body of theory (e.g., ref. 9). Broadly applying such approaches can better match conservation actions with ecological processes and promises to advance our understanding of one of the longest-standing challenges in ecology.

Materials and Methods

Study System. The field experiment was conducted at the 2,800-ha McLaughlin Natural Reserve (nrs.ucdavis.edu/McL/) in northern California (38.8739° N, 122.4317° W). The region has a Mediterranean climate, featuring cool, wet winters (November–March) and hot, dry summers (April–October) with ∼750 mm of annual rainfall. The landscape is largely composed of chaparral, oak woodland, and grassland meadow habitat; common herbivores include mule deer, jack rabbits, and pocket gophers. The reserve lies on the San Andreas Fault and has unique soil chemistry owing to the emergence and erosion of the Earth's mantle into the serpentine ultramafic soils that characterize the region. Serpentine soils have Ca/Mg ratios <1, as well as low levels of essential nutrients, high heavy metal content, and poor soil moisture retention.

We focused specifically on serpentine meadow habitat and observed 113 species in our plots from a potential pool of the 310 species that occur in all habitat types at the reserve; 73 of the 113 species were annual plants (*SI Appendix*, Table S1). The four most common species observed in unmanipulated plots were *Vulpia microstachys* (89% of sites), *Hemizonia congesta* (79%), *Plantago erecta* (69%), and *Lasthenia californica* (52%). Sixteen of the 113 total species could not be identified or classified as having an annual or perennial life history and were thus excluded from all analyses; however, these individuals occurred only at single sites and are thus unlikely to have large effects on diversity patterns.

Experimental Setup. In May 2013, we surveyed the reserve for 30 serpentine meadow sites. Site locations were chosen such that they could be hierarchically grouped at five spatial scales, which resulted in three groups of five sites each at the northwestern and southeastern ends of the reserve (*SI Appendix*, Fig. S2). Within each site, we flagged and GPS-located eight 0.75- × 0.75-m plots arranged in a 2 × 4 block of plots, with plots separated by 1 m of bare ground. Each plot in a block was randomly assigned to receive a different experimental treatment (discussed below). In total, there were 240 plots (eight plots × 30 sites) and five spatial scales for comparison [at the level of the plot (1 m), a block of plots at a site (5 m), a group of sites (100 m), a reserve half (5 km), and a whole reserve (10 km)] (*SI Appendix*, Fig. S2). In late July 2013, after all winter annual species had senesced and the majority of summer annual species had set seed, we harvested all seed and standing vegetation from seven of the eight plots at each site using garden shears and a powerful gas-powered leaf vacuum (Stihl BG86); the eighth plot (C2; *SI Appendix*, Fig. S1B) was left unaltered to evaluate any unintended effects of the vacuuming procedure on plant diversity. All collected materials were stored outside in paper bags to allow natural heat stratification until they could be processed (<6 wk).

All collected materials were reintroduced to the seven vacuumed plots per site in two control and five species-pooling treatments (*SI Appendix*, Fig. S2B). The control treatments were as follows: a "vacuum without replacement" treatment (C1) to identify individuals that were left behind following vacuuming, and a "vacuum without movement" treatment (C3), where the collected material was homogenized at the plot level and redistributed back onto the source plot. The five spatial scaling treatments involved the pooling, homogenization, and redistribution of material collected from a single plot (1 m; blue plot in *SI Appendix*, Fig. S2B), multiple plots at a site (5 m; green plot), 5 sites of a single group (100 m; yellow plot), 15 sites from the same half of the reserve (5 km; orange plot), and all 30 sites across the entire reserve (10 km; red plot). Because treatments were nested within sites, and plots within sites are highly similar in environments and species composition, all treatments received a similar regional pool in terms of species richness and relative abundances. The redistributed material was secured to each recipient plot with twine.

We surveyed the plot-level community structure and corresponding environmental parameters during peak biomass in the following growing season. Plots were surveyed April 20 to May 2 in 2014, using percentage of cover estimates of each species because small-statured annuals can occur at densities up to 5,500 individuals per square meter (46); additional surveys were conducted later in the season to confirm the identities of late-flowering species. We surveyed the innermost 0.5×0.5 m^2 of each plot to account for any edge effects in our analyses. We measured plot-level percentage of soil moisture content, understory photosynthetically active radiation (PAR) in full sun, and slope inclination, as well as site-level elevation, slope aspect, hillside slope steepness, and soil depth. We also performed site-level soil fertility analyses (NO_3-N, Olsen-P, X-K, X-Na, X-Ca, X-Mg, pH, cation exchange capacity, organic matter; University of California at Davis Analytical Lab) on soil samples collected and pooled between four plots per site; X-Ca and X-Mg were converted to a ratio of Ca/Mg. Although site-level estimates of environmental conditions preclude finer-scale estimates, those variables for which we do have plot-level estimates showed no difference among plots that occur at the same site (all $P > 0.35$; *SI Appendix*, Table S2). This means that, although site-level estimates preclude estimates of within-site (among-plot) error in most of our environmental variables, all treatments and controls are subject to the same error because each is represented within all sites, and plots within sites do not significantly differ in environmental conditions (or species composition, Fig. 3).

Data Analysis. A presence/absence matrix was created from percentage of cover estimates of all 73 annual species in 29 of the 30 sites that were sampled; data from one site were lost due to a corrupt data file. We used presence/absence data because it is most appropriate for tracking gains and losses of species in response to manipulations of species pools and is most comparable to other studies (14, 29).

We tested the responses of five components of diversity to our species pool manipulations: net changes in species richness, species richness, regional site occupancy, species composition (PCoA axis 1 and 2 scores), and beta diversity. The first four components were tested using separate linear mixed-effects models in the "lmerTest" R package, each with spatial species-pooling treatment as a fixed factor and plot nested within site as a random factor. The nested random effects account for the nonindependence of our nested experimental design in terms of both error structure and degrees of freedom (47). Because species-pooling treatment emerged as a significant predictor in all analyses, we used post hoc Tukey tests using the "multcomp" package to identify treatment levels that differed significantly from each other. Net changes in species richness were estimated by subtracting the control plot C3 (*SI Appendix*, Fig. S2B) presence/absence matrix from each species-pooling treatment matrix; doing so allowed us to decompose net changes into gains and losses of individual species (*SI Appendix*, Fig. S3B). Species composition was the first two axis scores of a PCoA using a Jaccard dissimilarity matrix (R package "vegan"; *SI Appendix*, Fig. S5A).

The last component, beta diversity, was calculated as a matrix of Jaccard dissimilarity coefficients among all pairwise site combinations and separately for each species-pooling treatment. We then performed a permutational multivariate analyses of variance ("adonis" function in R package vegan) to test differences in Jaccard dissimilarity among treatments, constrained to account for treatments being nested within sites. This analysis is analogous to a univariate analysis of variance, except expanded to handle multivariate response variables. Because the overall model was significant, we ran adonis with a Bonferroni correction on all pairwise treatment combinations to identify treatments that significantly differed.

Before testing the responses of species–environment associations to our species-pooling treatments, we used complementary multivariate methods in the R package vegan to reduce the dimensionality of the environmental variables that were measured. First, we used variance inflation factors (VIFs) to confirm that multicollinearity was low (VIFs < 10) and performed a constrained correspondence analysis (CCA), forward-selecting environmental variables using the R function "ordistep" to identify the subset of environmental variables that significantly influenced species occurrences (all but soil depth). Second, we ran a PCA on the subset of environmental variables identified as meaningful in the CCA, with the axis 1 and 2 scores of the PCA summarizing 51.4% of the variation in the among-site environmental dataset (*SI Appendix*, Fig. S5B).

We used the first (PCA1) and second (PCA2) PCA axis scores as composite environmental variables to examine how the species-pooling treatments affected the strength of species–environment associations. We performed a generalized linear model, separately for each species-pooling treatment, with species occurrences as the binomially distributed response variable and poly(PCA1,2)*species + poly(PCA2,2)*species as predictor variables. The poly(*x*,2) function in the R "stats" package calculates orthogonal first- and second-order polynomials of PCA1 and PCA2 to detect linear and quadratic relationships; species present in sites of intermediate environmental conditions but absent at the extremes would be best characterized by quadratic relationships. "Species" was a fixed factor in these analyses to facilitate model convergence and because we were directly interested in the amount of variation explained (pseudo-R^2 [1-{residual deviance/null deviance}]) by species-specific differences in responses to environmental conditions (47). We then compared the amount of variation explained by composite environmental conditions among species-pooling treatments (18), with the prediction that pseudo-R^2 values should increase with the spatial scale of species pooling. To further examine species-level responses (i.e., how strongly the occurrences of each species were associated with environmental conditions), we performed separate generalized linear models for each species and species-pooling treatment, testing the additive effects of poly(PCA1,2) and poly(PCA2,2). We visualized how the distribution of

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the pseudo-R^2 values of the 73 species shifted across species-pooling treatments. We then visualized the distribution of pseudo-R^2 values across species for each of the species-pooling treatments.

To quantify similarity among sites in environments and species that occurred naturally at distinct spatial scales, we performed permutational multivariate analyses of variance (adonis function in R package vegan). The response variables were matrices of Euclidian (for environment) and Jaccard (for species) distances among sites, and each matrix was tested for differences among groups of five sites (100-m scale) nested within each half of the reserve (5-km scale). The species distance matrix was created from plots that received the 1-m treatment only, as this treatment most closely reflected natural unmanipulated species distributions.

The control plots were used to assess the presence of any unintended effects of the hay transfer manipulation that were unrelated to the species-pooling treatment. We found no difference in species richness or composition between the unmanipulated (C2 in *SI Appendix*, Fig. S2) plots and our 1-m treatment plots (all $P > 0.998$), indicating that the hay transfer manipulation did not affect local diversity. Similarly, there was no difference among plots that received their own hay back (C3) and the 1-m treatment plots that received hay from a different adjacent plot (all $P > 0.807$); thus, the removal and replacement of hay did not affect plot diversity. As a result, we report only the results from the 1-m treatment plots. The removal without replacement control plot (C1) had significantly lower species richness than the unmanipulated control plot (C2) and all treatment plots (all $P < 0.002$), indicating that our seed vacuum was effective.

We incorporated percentage-of-cover data to assess whether our species-pooling manipulations caused small transient "sink" populations to establish, leading to biased diversity estimates. To do this, we revisited the original percentage-of-cover matrix from which the presence/absence matrix was generated and converted any percentage-of-cover entries less than or equal to four cutoff values (0.05, 0.10, 0.25, and 0.5%) to zero. For small-statured annual plants, 0.5% cover roughly translates into 10 individual plants; values greater than 0.5% risks removing small but stable populations. We then converted those four percentage-of-cover matrices to presence/absences and reanalyzed our species richness and species–environment association results (*SI Appendix*, Table S3).

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Supporting Information

Table S1. Information for 73 annual species observed in the unmanipulated control plots (C2 in SI Appendix Fig. S2).

Scientific name	Family	Status	Site occupancy	Dispersal mode†
Achyrachaena mollis	Asteraceae	Native	3	V
Acmispon americanus	Fabaceae	Native	1	U
Acmispon brachycarpus	Fabaceae	Native	5	U
Acmispon wrangelianus	Fabaceae	Native	10	U
Agoseris heterophylla	Asteraceae	Native	9	W
Amsinckia menziesii	Boraginaceae	Native	2	V
Anagallis arvensis	Primulaceae	Naturalized	3	V
Ancistrocarphus filagineus	Asteraceae	Native	14	V*
Astragalus gambelianus	Fabaceae	Native	2	W
Athysanus pusillus	Brassicaceae	Native	8	V*
Avena spp.*	Poaceae	Invasive	7	V
Bromus diandrus	Poaceae	Invasive	2	V
Bromus hordeaceus	Poaceae	Invasive	14	V
Bromus madritensis	Poaceae	Naturalized	11	V
Calandrinia ciliata	Portulacaceae	Native	5	U
Calycadenia pauciflora	Asteraceae	Endemic	8	W
Camissonia graciliflora	Onagraceae	Native	2	U
Cardamine oligosperma	Brassicaceae	Native	1	U
Castilleja attenuata	Orobanchaceae	Native	0	W
Castilleja rubicunda	Orobanchaceae	Endemic	1	W
Centaurea solstitialis	Asteraceae	Invasive	2	W
Clarkia gracilis	Onagraceae	Native	6	U
Clarkia purpurea	Onagraceae	Native	1	U
Collinsia sparsiflora	Plantaginaceae	Native	9	U
Croton setigerus	Euphorbiaceae	Native	16	W
Cuscuta californica	Convolvulaceae	Native	6	W
Daucus pusillus	Apiaceae	Native	1	V
Epilobium brachycarpum	Onagraceae	Native	7	W
Eriogonum covilleanum	Polygonaceae	Native	3	V
Eriogonum vimineum	Polygonaceae	Native	0	V
Erodium cicutarium	Geraniaceae	Invasive	2	V
Euphorbia crenulata	Euphorbiaceae	Native	6	U
Galium aparine	Gentianales	Native	1	V
Gilia tricolor	Polemonaceae	Endemic	2	U

Githopsis specularioides	Campanulaceae	Endemic	7	U
Hemizonia congesta	Asteraceae	Native	24	U
Hesperolinon spp.*	Linaceae	Endemic	9	U*
Holocarpha virgata	Asteraceae	Endemic	3	W
Hypochaeris glabra	Asteraceae	Invasive	2	W
Juncus bufonius	Juncaceae	Native	1	U*
Lactuca spp.*	Asteraceae	Naturalized	10	W
Lagophylla minor	Asteraceae	Endemic	3	U
Lasthenia californica	Asteraceae	Native	16	V
Lepidium nitidum	Brassicaceae	Native	4	U
Lessingia ramulosa	Asteraceae	Endemic	7	W
Linanthus bicolor	Polemoniaceae	Native	3	U*
Linanthus dichotomus	Polemoniaceae	Native	1	U*
Lolium multiflorum	Poaceae	Naturalized	11	V
Lupinus bicolor	Fabaceae	Native	2	U*
Lupinus succulentus	Fabaceae	Native	1	U*
Micropus californicus	Asteraceae	Native	10	U*
Microseris douglasii	Asteraceae	Native	12	W
Mimulus douglasii	Phrymaceae	Native	5	U
Mimulus guttatus	Phrymaceae	Native	1	W
Minuartia douglasii	Caryophyllaceae	Native	1	U
Navarretia jepsonii	Polemoniaceae	Rare/Endemic	4	V
Navarretia pubescens	Polemoniaceae	Native	1	V
Nemophila heterophylla	Boraginaceae	Native	2	U
Nemophila pedunculata	Boraginaceae	Native	1	U*
Phlox gracilis	Polemoniaceae	Native	3	W
Plantago erecta	Plantaginaceae	Native	24	V
Riggiopappus leptocladus	Asteraceae	Native	6	W
Sidalcea diploscypha	Malvaceae	Endemic	5	V
Stellaria nitens	Caryophyllaceae	Native	3	U
Taeniatherum caput-medusae	Poaceae	Invasive	8	V
Torilis arvensis	Apiaceae	Invasive	1	V
Trifolium albopurpureum	Fabaceae	Native	5	U*
Trifolium bifidum	Fabaceae	Native	5	U*
Trifolium depauperatum	Fabaceae	Native	1	U*
Trifolium fucatum	Fabaceae	Native	2	U*
Trifolium gracilentum	Fabaceae	Native	4	U*
Velezia rigida	Caryphyllaceae	Naturalized	1	U*
Vulpia microstachys	Poaceae	Native	26	V

Notes: Species' statuses were cross-checked with CalFlora Plant Database (www.calflora.org); species that occurred in 0 sites were observed in one of the other treatment or control plots.

**Avena fatua/A. barbata* and *Lactuca saligna/L. serriola* were indistinguishable, and *Hesperolinon* could not be identified to species. †Dispersal modes are those reported in the literature (1); in species with multiple dispersal modes, we report the mode associated with the greatest mean dispersal distance: unassisted/ant (U) < wind (W) < vertebrate (V). *Species with unreported dispersal modes or with seed morphologies suggestive of dispersal modes that differ from those reported in the literature (1) were assigned based on seed morphology as per convention (2).

Table S2. Fine-scale estimates of soil moisture, light availability, and slope inclination among plots at the same site, among groups of sites occurring within 100 m, and among sites occurring within the same half of the reserve.

Nested plot design	**spatial extent**	**soil moisture (%)**		**light availability (PAR)**		**slope inclination (º)**	
		F-value	*P*	*F*-value	*P*	*F*-value	*P*
halves w/i reserve	10 km	159.5	<**0.001**	22.3	<**0.001**	17.3	<**0.001**
groups w/i halves	5 km	3.7	0.055	151.1	<**0.001**	68.2	<**0.001**
sites w/i groups	100 m	6.5	**0.011**	122.4	<**0.001**	0.04	0.851
plots w/i sites	5 m	0.47	0.493	0.3	0.579	0.75	0.388
residual *df*		711		474		475	

Notes: Analyses are nested analysis of variance using 'aov' R function; data are from two (light availability, slope inclination) or three (soil moisture) subsamples per plot for all eight plots per site. The statistical annotation of the independent factors is ~halves/groups/sites/plots.

Table S3. Sensitivity of spatial scale of species pooling effects on species richness and species-environment associations to the removal of species with small population sizes, as estimated through percent cover.

Cut-off	**>0% - all data**	**>0.05%**	**>0.10%**	**>0.25%**	**>0.5%**
~ # individuals	0	1	2	5	10
Species richness					
1 m	9.9 (0.7)	8.7 (0.6)	7.7 (0.6)	6.6 (0.6)	5.2 (0.5)
5 m	10.9 (0.8)	9.8 (0.7)	8.7 (0.7)	7.4 (0.6)	5.7 (0.5)
100 m	14.6 (0.7)	13.0 (0.7)	11.4 (0.7)	9.9 (0.7)	7.7 (0.6)
5 km	19.6 (1.0)	17.1 (1.0)	14.5 (1.0)	12.3 (0.9)	9.6 (0.8)
10 km	21.2 (1.0)	18.4 (1.1)	15.8 (1.1)	13.1 (0.9)	10.2 (0.8)
Species-environment associations					
1 m	0.34	0.33	0.29	0.31	0.26
5 m	0.36	0.36	0.34	0.34	0.29
100 m	0.45	0.43	0.41	0.41	0.35
5 km	0.51	0.48	0.45	0.45	0.40
10 km	0.52	0.49	0.45	0.47	0.39
Mean number of observations at or below cut-off per plot					
1 m	0	1	2	3	5
5 m	0	1	2	4	5
100 m	0	2	3	5	7
5 km	0	3	5	7	10
10 km	0	3	6	8	11

Note: Small populations are not necessarily transient sink populations, were present even when species were only pooled locally (1 m and 5 m), and did not qualitatively affect the spatial scaling patterns presented in the manuscript (>0% cut-off). Cut-offs above 0.5% risk removing species with small but stable populations.

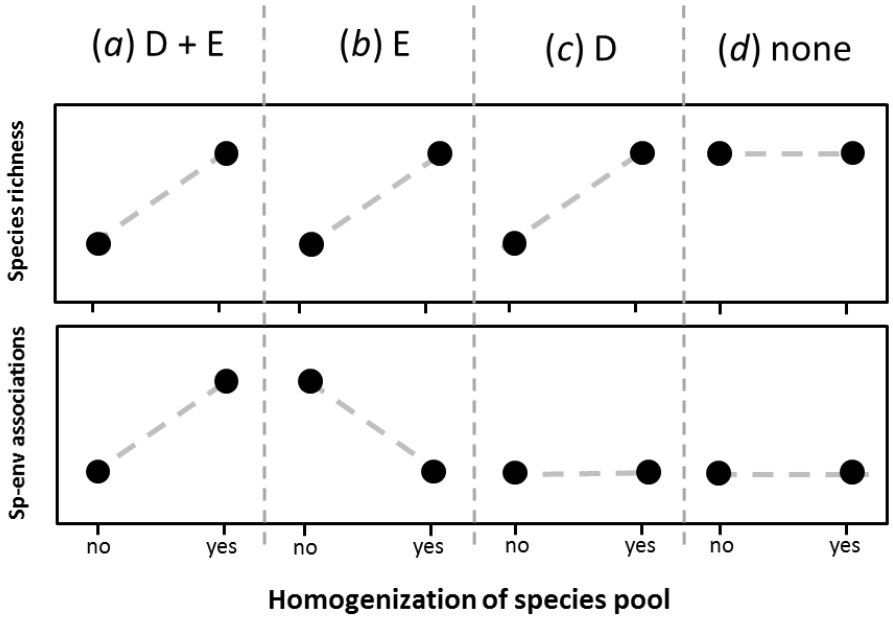


Fig. S1. Predicted responses of species richness (top panel) and species-environment associations (bottom panel) to the homogenization of the species pool, depending on whether distance (D) or environment (E) influence species distributions. We do not include the possibility that dispersal decreases both diversity and species-environment associations, which is predicted to occur when dispersal is already so common as to cause mass effects prior to species pooling (3); we considered this possibility highly unlikely because we only increase dispersal at a single point in time and because of the documented dispersal limitation of annual plants discussed in the main article.

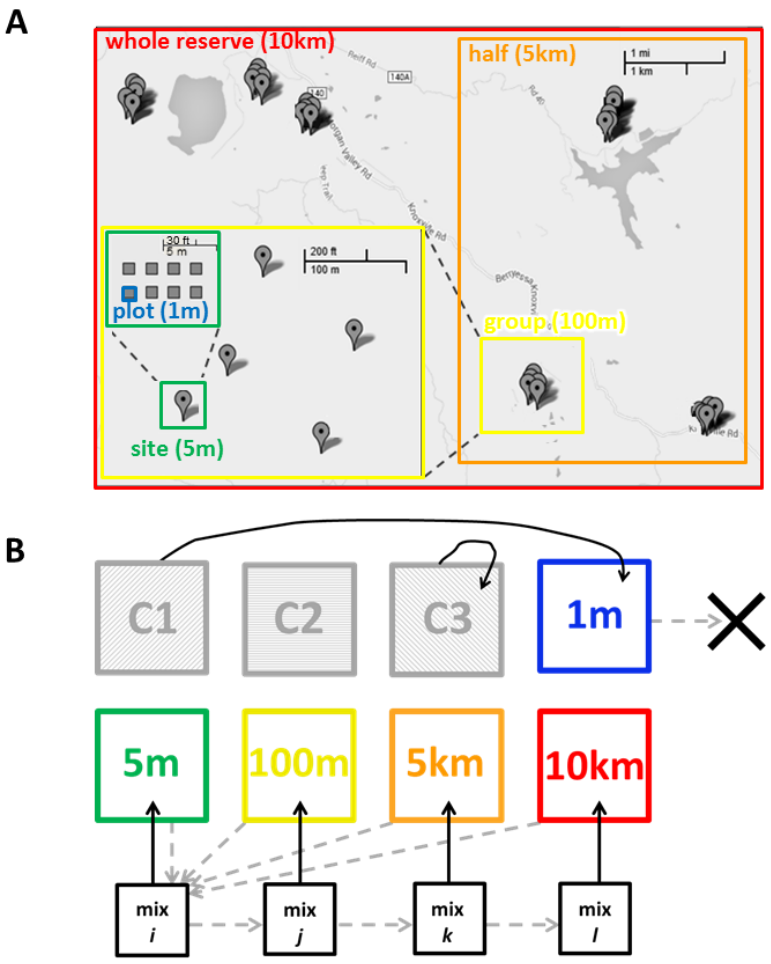
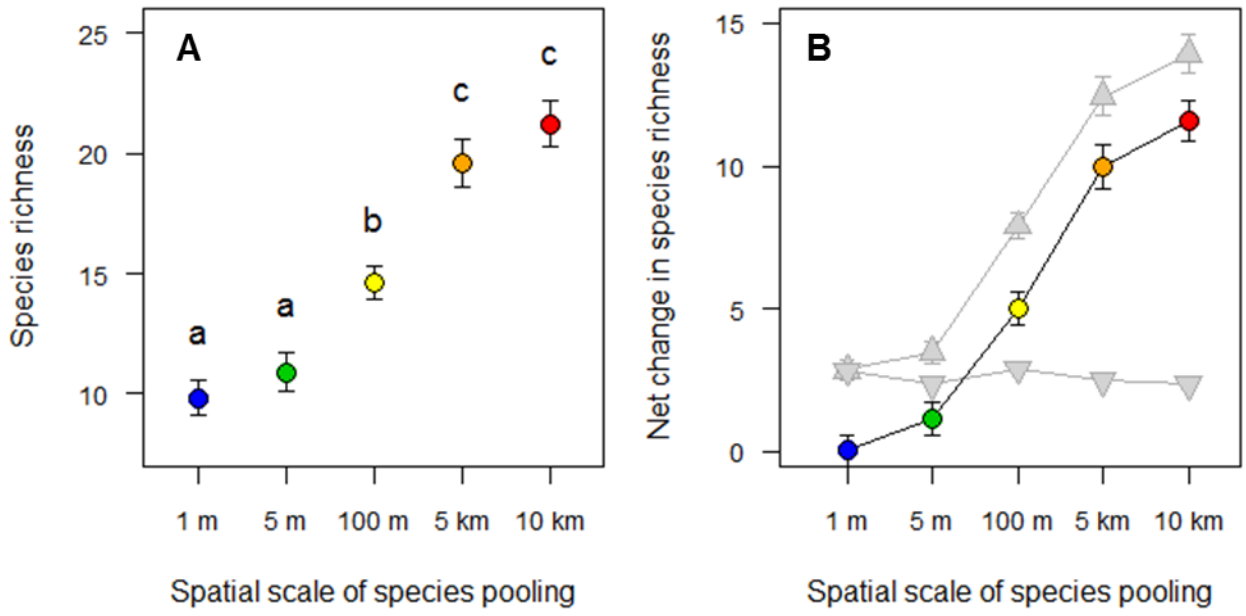


Fig. S2. (A) Map of McLaughlin reserve with the six groups of five sites and (B) the species pooling treatment design for a block of plots at a single site. Dashed grey arrows indicate source plots of seed material, and solid black arrows indicate recipient plots. Plots C1 to C3 are control plots: material removed without replacement (C1), no manipulation (C2), and material collected and transferred to the same plot (C3). Colored plots are the treatment plots, receiving material mixed from plots at increasing spatial scales: a single plot from the same site (1 m), a mix of plots within a site (5 m, mix *i*), a mix of plots from the same group of five sites (100 m, mix *j*), a mix of plots within the same half of the reserve (east vs. west side; 5 km, mix *k*), and a mix of plots across the entire reserve (10 km, mix *l*). Source material from the recipient plot of the 1 m treatment was discarded.



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78 **Fig. S3.** The effect of spatial scale of species pooling on (A) species richness (mean ± se) and
79 (B) net changes in species richness. Net changes in species richness (circles) are species gains
80 (upwards triangles) minus species losses (downwards triangles) – spatial scaling trends were
81 driven solely by species gains. Points with the same letter were not significantly different in a
82 multiple comparisons test.

83

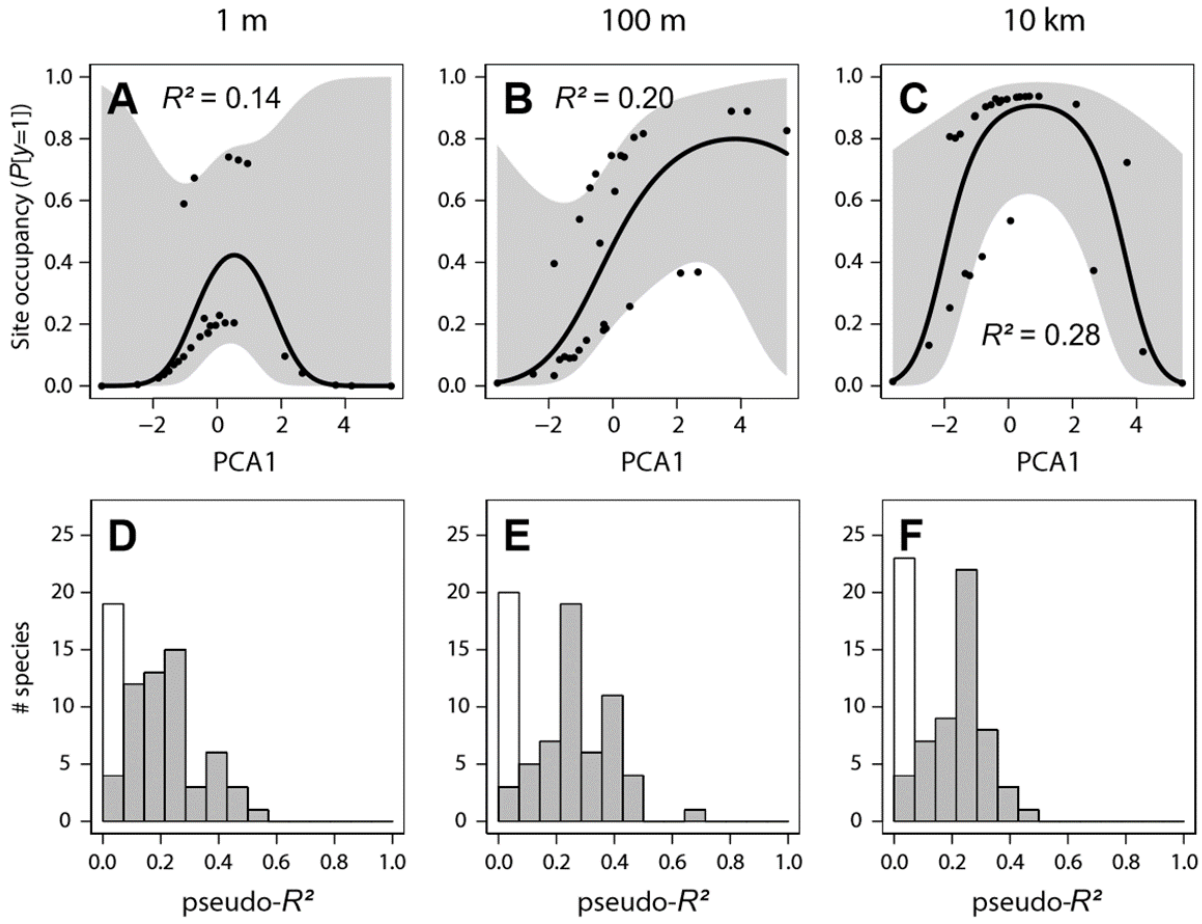


Fig. S4. Effect of spatial scale of species pooling on relationships between site occupancy and environmental conditions broken down by species. Fitted relationships of an example species, *Clarkia gracilis* (A-C), showing the raw data points, fitted relationships, 95% confidence bands, and model fits [pseudo-R^2]. The distribution of pseudo-R^2 values (species-environment associations) determined individually for the 73 species are shown (D-F); the white bar is species present in 0% or 100% of sites. Analyses were logistic regressions of species presences/absences as a function of environment, and are presented as probabilities of being present at a site. All analyses were performed with PCA1 and PCA2 as linear and quadratic predictors; PCA1 is shown for simplicity.

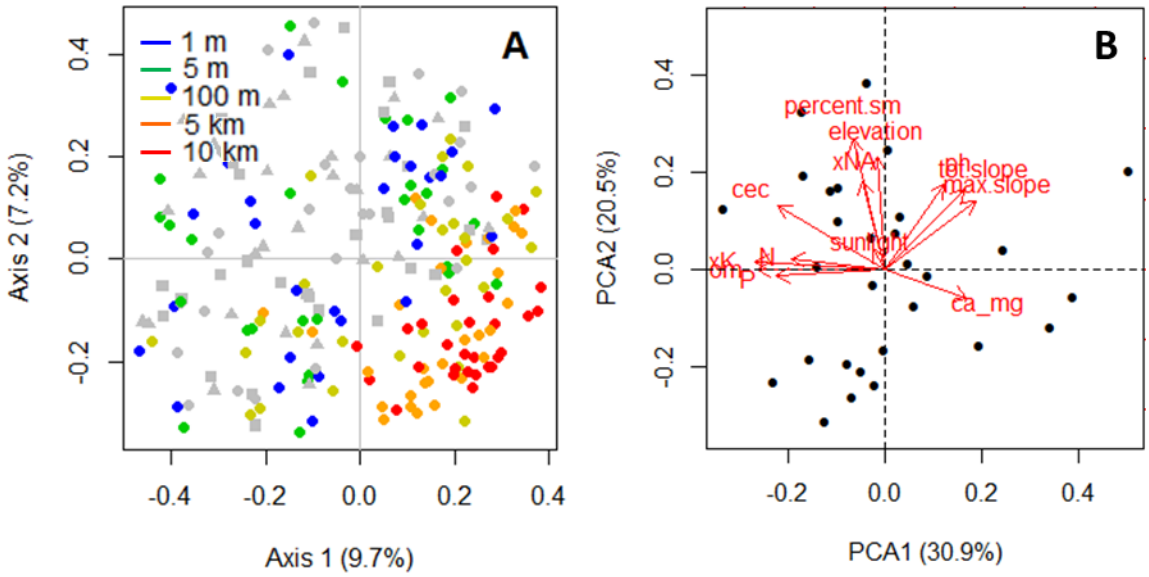
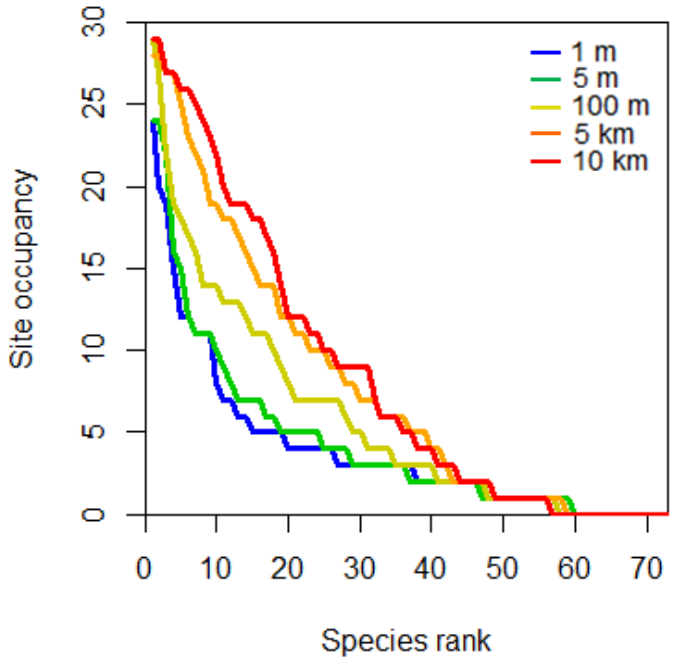


Fig. S5. Biplots of (A) community composition and (B) environmental conditions. The community composition biplot (A) is from a principle coordinates analysis on a Jaccard's dissimilarity matrix; points in grey are control treatments (SI Appendix Fig. S2): vacuumed without replacement (C1; triangles), unmanipulated plots (C2; circles), and vacuumed with replacement but not pooled (C3; squares). Species composition becomes more distinct and less variable with increasing spatial scale. The biplot of environmental conditions (B) is from a principle components analysis of 13 environmental variables. Black points are sites, and red arrows are how strongly each environmental variable loads on each axis. N = nitrogen, P = phosphorus, xK = potassium, xNa = sodium, ph = pH, cec = cation exchange capacity, om = organic matter, percent.sm = percent soil moisture, ca_mg = calcium/magnesium ratio, max.slope = slope of hillside, tot.slope = average slope of plots per site.



106

107 **Fig. S6.** Site occupancy (based on number of sites each species occurs in) increases with spatial

108 scale of species pooling (also note upwards shift in *y*-intercept) and becomes more even

109 (decrease in regional rarity).

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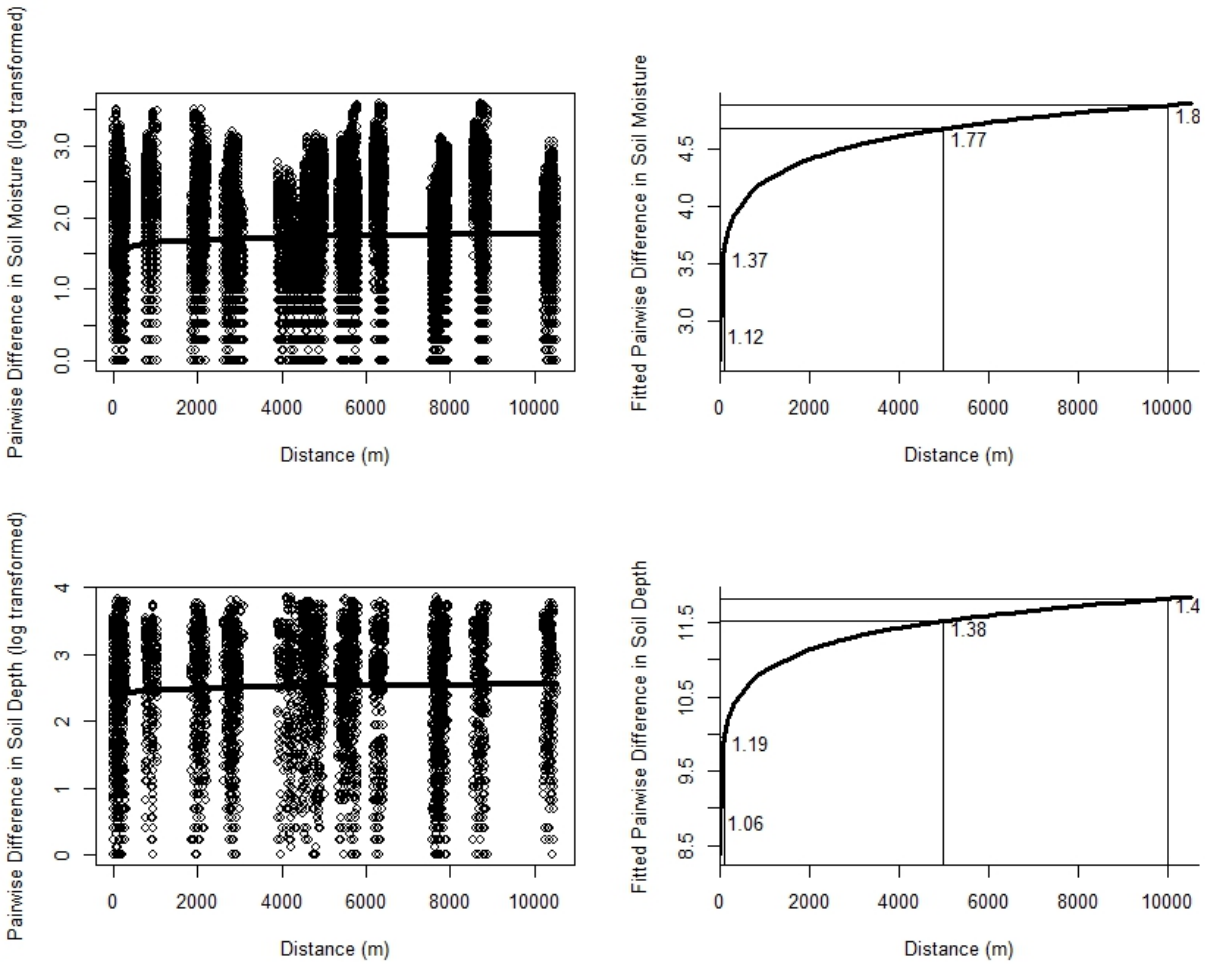

Fig. S7. The spatial structure of the environment from small (5 m) to large (10 km) scales. Pairwise difference in soil conditions (soil moisture – top; soil depth – bottom). Panels on left show raw data with lines fitted using log-log relationships as tested by Mantel tests (both P = 0.001). Panels on the right show the fitted relationships, with vertical lines marking the scales of our experimental mixing of species pools (5 m, 100 m, 5 km, 10 km) and numbers showing the ratio of mean among-site differences at that scale relative to the reference scale (1 m). These numbers highlight the large changes from 5 to 100 m and 100 m to 5 km relative to the changes at smaller (1 to 5 m) and larger (5 to 10 km) scales. Note that the *y*-axis is of log-transformed values in panels on the left and back-transformed to original values in panels on the right.

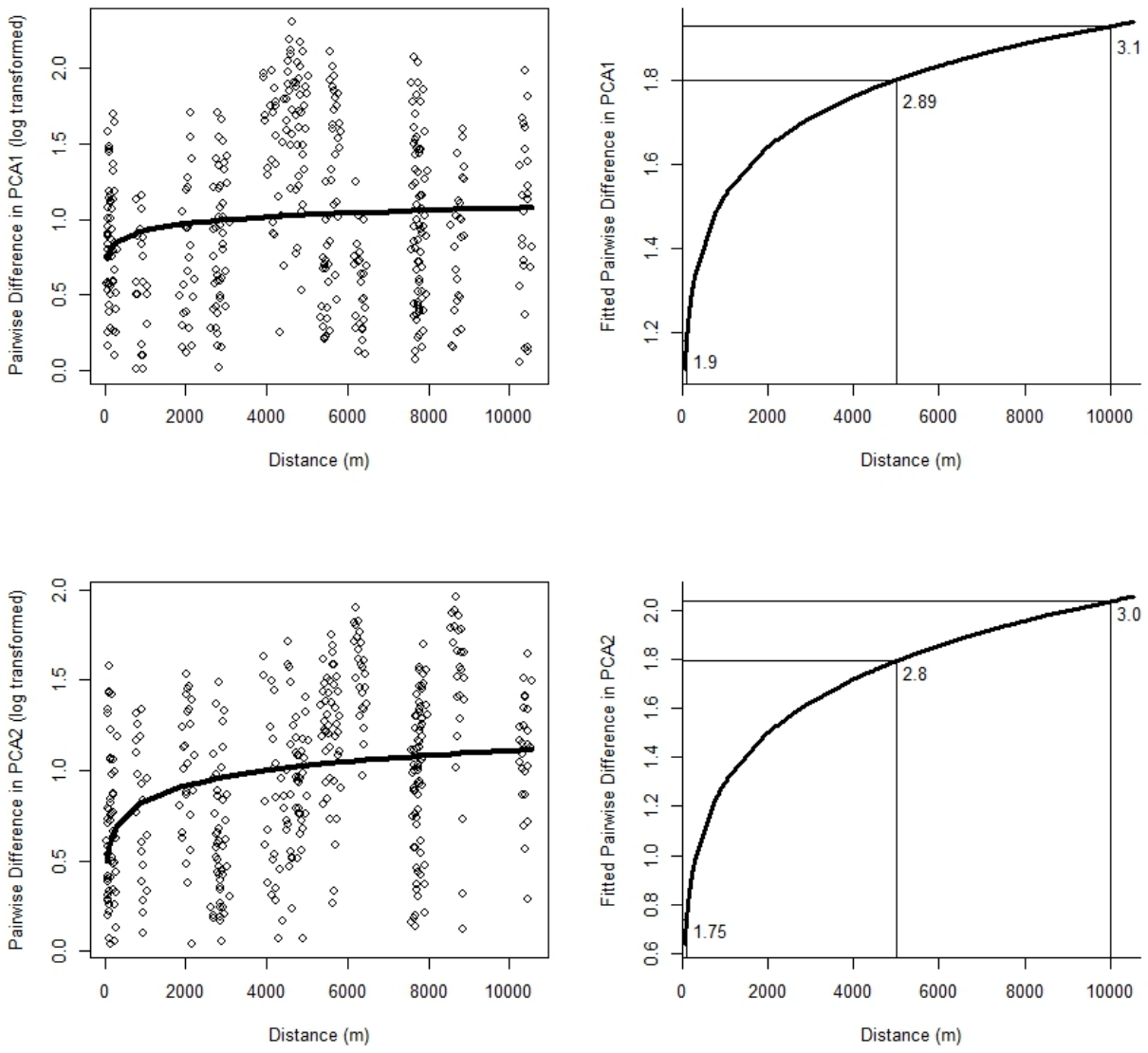

Fig. S8. The spatial structure of the environment from moderate (100 m) to large (10 km) scales. Pairwise difference in environmental conditions (PCA1 – top; PCA2 – bottom). Panels on left show raw data with lines fitted using log-log relationships as tested by Mantel tests (both $P <$ 0.004). Panels on the right show the fitted relationships, with vertical lines marking the scales of our experimental mixing of species pools (100 m, 5 km, 10 km) and numbers showing the ratio of mean among-site differences at that scale relative to the reference scale (1 m). These numbers highlight the large changes to 100 m and from 100 m to 5 km relative to the changes at larger (5 to 10 km) scales. The relationship for PCA2 (bottom panel) was stronger than for PCA1 (top

panel; Mantel *r* of 0.35 versus 0.16 respectively), as shown in Fig. 3*A*. Among-site differences at the 5 m scale are not given because measurements for the PCA variables were taken at the site level. Note that the *y*-axis is of log-transformed values in panels on the left and back-transformed to original values in panels on the right.

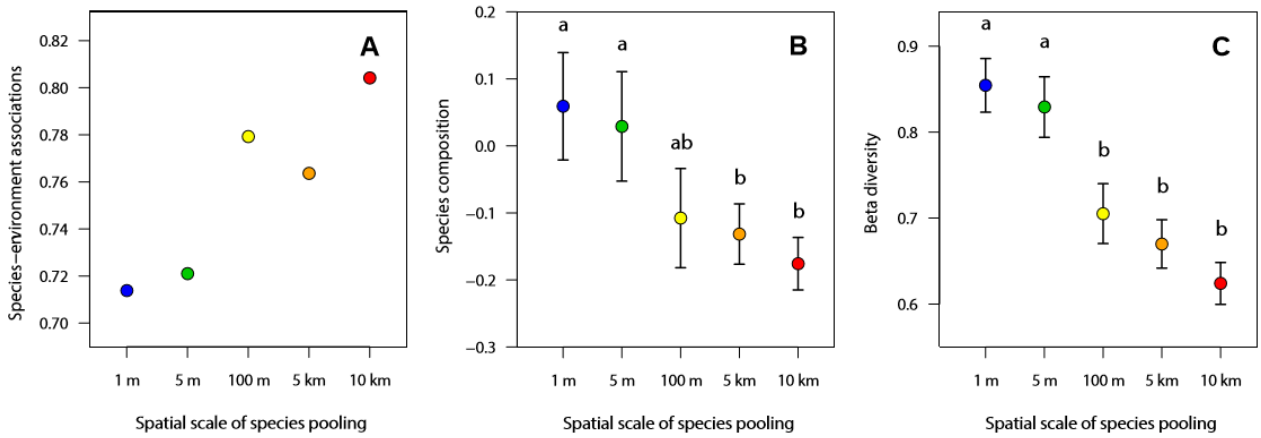

Fig. S9. The effect of spatial scale of species pooling on (A) species-environment associations, (B) species composition, and (C) beta diversity, estimated on percent cover data. Points with the same letter were not significantly different in a multiple comparisons test.

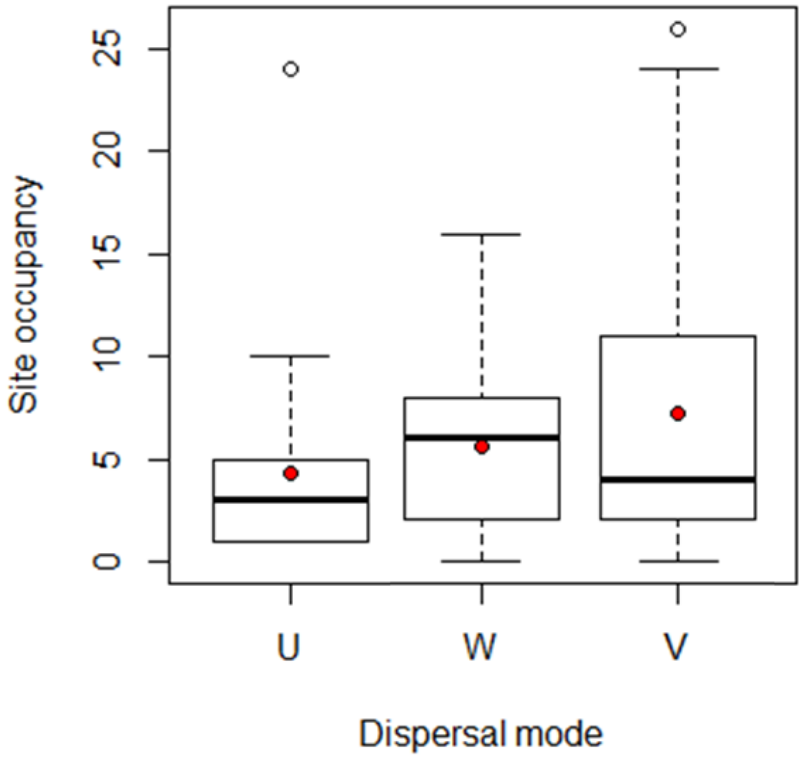

Fig. S10. The distribution of site occupancies among species that differ in dispersal mode (unassisted, wind, and vertebrate dispersal); site occupancy data is from the 1 m treatment, which best reflects natural occupancy patterns. The thick black bars and red circles are median and mean site occupancies, respectively. Species dispersal modes are reported in SI Appendix Table S1; the outlier in the 'unassisted' category is *Plantago erecta*, which may be animal-dispersed via a mucilaginous seed coat (R. M. Germain *pers. obs.*) though more research is needed.

Supplementary methods, results, and discussion

The persistence of diversity responses to species pooling manipulations

The rapid and dramatic increase in local diversity and species-environment associations that we observed as the scale of the species pool increased raises questions about the transient and longer-term effects of altering scales of dispersal. Although we cannot refute the possibility that the observed responses are transient in nature, both our results and those of related studies suggest that they likely reflect long-term responses for six reasons. First, theory suggests that increasing dispersal will increase diversity but dampen species-environment associations in source-sink communities (SI Appendix Fig. S1*B* (4)), but increase both community metrics when species are favoured by distinct environmental conditions and are dispersal limited (5), as observed here. Second, a number of community ecology studies have added seeds from many species to communities, and found that short-term gains in diversity from these seed additions correspond to long-term increases in diversity (*e.g.*, (6)). Third, California annual plant species are considered chronically dispersal limited. These small-stature plants are frequently less than 20 cm tall, and at those heights plants have been shown to disperse very locally regardless of whether they have specific adaptations for dispersal (7). This low dispersal, combined with relatively low densities of plants on serpentine soils and recent habitat fragmentation, causes very small numbers of seeds to reach distant serpentine patches (8). Fourth, in plant communities, the filtering effects of competition and environment tend to manifest most strongly at the seedling stage (9, 10). Because our surveys were conducted at peak flowering, long after the seedling stage, our diversity responses account for some of these influences. Fifth, the results that we have reported are consistent with hypotheses about metapopulation declines that have been proposed for heavily invaded serpentine grasslands like those used in this study (8).

Although previous research has been unable to characterize the change in diversity and species-environment associations that we have shown here, these patterns are nonetheless predicted from that research. Finally, non-linear increases in components of diversity were insensitive to the exclusion of species with local percent cover values ≤0.5 (~10 individuals), potential sink populations (SI Appendix Table S3). In sum, the patterns from our study are consistent with theory and previous studies that highlight the persistent nature of seed addition effects on plant diversity.

The effect of environmental resolution on diversity and composition

Environmental conditions were increasingly different as the distance between sites increased (all $P < 0.004$; SI Appendix Figs. S7, S8), with the largest differences occurring at those scales that showed the greatest change in diversity (Figs. 1; SI Appendix Figs. S7, S8), and the smallest changes occurring between the two smallest and two largest scales (SI Appendix Figs. S7, S8), corresponding to non-significant changes in diversity (Figs. 1, 2). These results support our nested analysis of environmental structure, using geographical distance rather than scales that correspond to our species pool manipulations.

The experiment was designed so that the resolution of the environmental data would affect all treatments equally. At each site, we pooled soil samples from four plots to get an average soil, and analysed this soil for typical soil nutrients (nitrate, phosphorus, calcium, etc.), pH, organic matter, and cation exchange capacity (details in *Materials and Methods*). We similarly used site-averaged soil moisture, slope, and so on. Although these average values are not entirely correct for any plot within a site, they are equally representative of all our treatments within sites; species-environment associations for all levels of pooling are subject to the same

error that arises from this averaging. Our analyses of fine-scale environmental measurements (for soil moisture, PAR and slope) indicate that this averaging error should be small, as there was very little variation among plots within a site. In other words, spatial resolution of environmental variables likely created a negative bias in all our estimates of species-environment associations, but this bias should be equal among treatments.

Spatial scale of species pooling on abundance-based metrics

We explored the effects of spatial scale of species pooling on species composition, beta diversity, and species-environment associations, generated from percent cover values and not presences/absences (SI Appendix Fig. S9). We used the percent cover matrix to perform the exact analyses described in *Data analysis* on the presence/absence data, except for two differences. First, species composition and beta diversity were calculated using Bray-Curtis dissimilarity instead of Jaccard dissimilarity, since the former is the abundance-based analog of the latter. Second, the analyses used to estimate species-environment associations were performed with quasiPoisson error distributions instead of binomial distributions, a more appropriate distribution for non-Gaussian overdispersed abundance-based data.

Our abundance-based results were similar to those generated using presence/absence data, with a general tendency for increased species-environment associations, decreased beta diversity, and altered species composition when species are pooled at small vs. large spatial scales; however, the exact shapes of these relationships differed. In the presence/absence data, all response variables varied sigmoidally with the spatial scale of species pooling, whereas beta diversity and species composition appeared to respond more-or-less linearly. Species-

environment associations also appeared linear overall, but spiked at 100 m spatial scales of species pooling.

We present these results because they would likely be of interest to readers, but are cautious not to overinterpret their biological meaning. The main challenge with the abundance data, as a consequence of our seed manipulations, is that the total amount of seed added per species per plot sets an upper limit on plant abundance the year after species pooling. Specifically, since seed is homogenized among plots at different scales, homogenization creates an averaging effect, where species pooling caused plots that initially contained variable numbers of seeds of each species to receive numbers of seeds averaged among plots at the spatial scale of species pooling. As a result, abundance distributions would partly reflect site suitability, and partly reflect the total amount of seed added, but the relative contributions of each explanations cannot be parsed out from our data. Species' presences/absences would not be affected by this problem, which is supported by species present initially at a site making it back post-species pooling (SI Appendix Fig. S3*B* upwards triangle [gains]), but would bias analyses based on abundances.

The effect of dispersal mode on site occupancy patterns

A future goal for this work is to identify differences among species that contribute to the severity of dispersal limitation, given that our results point towards dispersal limitation as an important driver of species distributions and diversity patterns. Although in-depth analyses are beyond the scope of this paper, we performed a preliminary analysis exploring whether species occupancy patterns depended on the vector of seed dispersal, or 'dispersal mode' (2). To do this, we categorized the dispersal modes of each species as having no apparent dispersal vector

(unassisted; U), wind dispersed (W), or vertebrate dispersed (V) based on existing literature (1) and seed morphology (2). We then used a glm with a negative-binomial distribution to test if the average number of sites occupied by each species varied by dispersal mode. We predicted that site occupancy would be lowest among species with no apparent dispersal mechanism (unassisted), intermediate for wind-dispersed species, and greatest among vertebrate-dispersed species; common vertebrates at our study site are mule deer, jackrabbits, and coyotes, all of which have broad home ranges.

Dispersal mode emerged as a marginally-significant predictor of site occupancy patterns ($X^2 = 4.97$, $P = 0.083$), with shifts in site occupancy (SI Appendix Fig. S10) that are consistent with our predictions. That site occupancy was greater on average among species with more dispersive seed morphologies ('V' in SI Appendix Fig. S10) provides an additional line of evidence that dispersal limitation is important to the distributions of species in this system. However, our categorizations of dispersal modes serve only as coarse proxies of dispersal ability. We aim to seek further resolution by incorporating specific traits that are known to affect dispersal ability within and among dispersal modes, such as plant height (7) and seed morphology (*e.g.*, seed mass, pappus), as well as tracking differences in responses to our species pooling manipulations depending on dispersal strategies.

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