Photos taken in the beginning of May show the diversity of plant communities and environmental conditions among 3 sites.
Fine-scale trait variation of five native forbs along environmental gradients

Sasha Victor, Kyle Doherty, Mary Ellyn DuPre, Philip W Ramsey, and Ylva Lekberg

ABSTRACT

Seed-transfer zones attempt to match seed source and habitat, but the extent of environmental variation within climate-based zones can be substantial and potentially relevant to seed-transfer guidelines. We surveyed abiotic and biotic soil properties across elevation and insolation (amount of solar radiation) gradients within a 5 km² (1.9 mi²) area in Montana and assessed relationships with plant traits in 5 native species. We detected substantial variation in soil physical, chemical, and biological properties along gradients. Differences in insolation due to topographical features predicted variation in soil moisture and maximum temperature, organic matter content, and magnesium, whereas elevation predicted minimum soil temperature and relative abundance of arbuscular mycorrhizal fungi. Elevation and insolation combined predicted plant productivity, soil potassium, calcium, sulfate, and iron concentrations. Yet, neither elevation nor insolation consistently correlated with variation in plant traits across species, potentially because of insufficient selection pressure or widespread seed dispersal throughout the area. Nonetheless, specific leaf area and seed weight, both heritable traits, correlated with changes in elevation and insolation in some species. Whether this variation is attributable to local adaptation or plasticity requires reciprocal transplant experiments and longer-term monitoring of the survival and fitness of plants. We show that elevation and insolation gradients can be effective predictors of some aspects of the soil environment. Systematically collecting seeds along elevation and insolation gradients can provide a breadth of plant traits to test trait-environment interactions in reciprocal transplant studies and reveal if matching heritable traits to environments within a climatic seed zone is beneficial.


KEY WORDS
plant functional traits, specific leaf area, seed mass, seed-transfer zones, elevation, insolation, fungal communities

NOMENCLATURE
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The need for native seeds in ecological restoration is continuously increasing and far exceeds the availability (Pedrini and Dixon 2020; Pedrini and others 2020). Given the cost of production and high demand, it is important to maximize the chance of restoration success, which historically has been quite low from seeding (Larson and others 2015; Nolan and others 2021; Shackelford and others 2021) at least partly because of environmental mismatch between the seed source and restoration site (Kettenring and others 2014; Buchanova and others 2019). To change this, restoration ecologists developed seed-transfer zones to guide suitable plant movement (Bower and others 2014; Erickson and Halford 2020), a concept first developed in forestry decades ago (discussed by Johnson and others 2004). While seed-transfer zones represent best guesses for matches between seed source and habitat, they tend to rely on coarse-scale environmental variation such as climate (Bower and others 2014; but see Havens and others 2015). Less is known about the importance of small-scale environmental difference in mediating restoration success, and whether more targeted guidance could improve outcomes (Bischoff and others 2006; Havens and others 2015; Balazs and others 2020; Davidson and Germino 2020). As such, those of us who work in this field need to better understand the extent of environmental variation within seed-transfer zones and to assess if it coincides with differences in plant form and function.

Plants respond to their environment by adjusting their morphology, physiology, and phenology (Shipley and others 2006; Gratani 2014; Tang and others 2016). The response is influenced by both abiotic factors such as light and moisture, as well as biotic factors such as plant–plant competition and interactions with soil biota. For example, plants in shady, moist areas tend to have taller structures with larger, thinner leaves and shallower roots than those in more arid sites with high solar inputs (Wright and others 2004; Poorter and others 2009), and competition shapes the relative allocation of resources to shoot versus roots and affects phenology (Goldberg and Landa 1991; Liancourt and others 2005; Tang and others 2016; Durham and others 2017). Likewise, resource availability and the level of stress plants perceive influence interactions with soil biota. Plants tend to increase allocation to beneficial arbuscular mycorrhizal (AM) fungi to aid with nutrient acquisition and drought tolerance when nutrients and water are limiting (Delavaux and others 2017; Revillini and others 2016), whereas pathogens and disease may be more abundant where resources are plentiful (Reynolds and others 2003). Thus, environmental variation can affect plants directly and indirectly (Rudgers and others 2020), but the extent of this variation on fine spatial scale is little known.

Responses by plants can be quantified and compared within and among plant species by measuring functional traits (Pérez-Harguindeguy and others 2013; Adler and others 2014). Functional traits can be plastic and heritable, conferring fitness advantages that lead to local adaptation (Geber and Griffen 2003; McKay and others 2005; Roybal and Butterfield 2018). Commonly measured traits include plant size; specific leaf area (SLA), which is indicative of cumulative responses to light and water availability (Violle and others 2009); relative growth rate; as well as seed number and size (Diaz and others 2004; Pérez-Harguindeguy and others 2013; Leger and others 2021). Trait variation within and among species along environmental gradients is not well known but is relevant for restoration, because functional traits and environmental conditions shape restored plant community development and functions (Zirbel and others 2017).

Sampling a multitude of environmental variables and plant traits is inefficient without a spatially explicit strategy. In the adjacent field of conservation planning, elevation range and variation in topography are used as proxies for niche diversity and species turnover and are employed to develop reserve systems that maximize biodiversity per unit area (Stein and others 2014; Lawler and others 2015). Elevation-derived guidance may also be a suitable strategy for sampling diversity of plant traits and micro-environments. Elevation is now mapped globally at a fine scale (< 98 ft), and insolation (the solar inputs in an area as governed by terrain) is readily computed from it (Corripio 2019). These data are freely available and have been shown to influence soil moisture and temperature on a relatively small spatial scale with relevance to patterns of biodiversity (Reid 1973; Sundqvist and others 2013; Kunkel and others 2016). For example, insolation predicted the biological soil crust abundance and richness on a property in western Montana (Durham and others 2018), and fine-scale topographic diversity was strongly predictive of biodiversity and rarity in ground-nesting bees (Doherty and others 2021). Also, aspect affected soil moisture and temperature, and this corresponded with differences in soil organic matter, total nitrogen, plant biomass allocation to shoots versus roots, and microbial community composition in a study on the Tibetan plateau (Xue and others 2018). Thus, both elevation and insolation may be good proxies for abiotic and biotic soil properties that determine the environment wherein plants germinate, establish, grow, and reproduce (Rustad and others 2001; Ren and others 2018).

In this study, we characterized a suite of abiotic and biotic soil properties and measured traits of 5 intermountain grassland species occurring along insolation and elevation gradients within a 5 km² (1.9 mi²) area. Specifically, we asked the following questions:

1) What is the variation in soil abiotic (soil temperature, moisture, and nutrient availabilities) and biotic (productivity, relative abundance of fungal mutualists and pathogens) properties along gradients in elevation and insolation?
2) In a topographically complex site, do plant functional traits vary along elevation and insolation gradients at a fine spatial scale, and is the variation trait and species-specific?

**METHODS**

**Site Selection and Plant Species**

Our survey occurred on MPG Ranch (46.68000 N, 114.02778 W), which is a 6000 ha (14,826 ac) conservation property in western Montana. The landscape habitat types include riparian corridors, former and current agricultural areas, sagebrush steppe and intermountain grasslands, and montane mixed-conifer forests (Montana Natural Heritage Program 2017). This area of Montana is characterized by short, dry summers, cold winters, and cool, wet springs. Mean annual precipitation ranges from 300 to 350 mm (11.8–13.8 in; valley to mountain summits) and mean annual temperature of 7.6 °C (45.7 °F), ranging from –4.7 °C (23.5 °F, January) to 19.4 °C (66.9 °F, July; 30-y trend from a weather station in nearby Missoula, Montana; https://ncdc.noaa.gov). All survey sites occurred in the grassland habitat, which had never been cultivated and was dominated by cool-season bunchgrasses including bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) Á. Löve [Poaceae]), Sandberg bluegrass (*Poa secunda* J. Presl [Poaceae]), Idaho fescue (*Festuca idahoensis* Elmer [Poaceae]), and the perennial forbs silky lupine (*Lupinus sericeus* Pursh [Fabaceae]) and arrowleaf balsamroot (*Balsamorhiza sagittata* (Pursh) Nutt. [Asteraceae]) (Mueggler and Stewart 1980).

We selected 5 native forbs based on their widespread occurrence and their relevance for restoration; blue-eyed Mary (annual; *Collinsia parviflora* Lindl. [Scrophulariaceae]), thread-leaf phacelia (annual; *Phacelia linearis* (Pursh) Holz. [Hydrophyllaceae]), cutleaf daisy (perennial; *Erigeron compositus* Pursh [Asteraceae]), blanketflower (perennial; *Gaillardia aristata* Pursh [Asteraceae]), and prairie smoke (perennial; *Geum triflorum* Pursh [Rosaceae]). We used elevation and insolation data to divide the area into 11 strata using a k-means clustering algorithm (Hartigan and Wong 1979; R Core Team 2020, stats package). Insolation was calculated as the megajoules of solar radiation each site received on the 2016 summer solstice (Doherty and others 2021); 2021 values showed minimum deviation relative to 2016 and were strongly correlated, \( r = 0.99 \). This technique for modeling insolation considers both direct and diffuse irradiance for solar zenith angles throughout the day, as well as shadows cast by surrounding terrain and site slope (Corripio 2019).

Within this selected area, elevation ranged from 1130 to 1670 m (3707–5479 ft), and insolation ranged from 27.43 to 31.86 MJ m\(^{-2}\) (2.55 MJ ft\(^{-2}\)–2.96 MJ ft\(^{-2}\)). Within each stratum we surveyed the focal forb species starting in mid-March 2021. Two 50 m\(^2\) (538 ft\(^2\)) sites were designated within each stratum where the greatest number of focal species were found, except in one stratum where only 1 suitable site was located (Figure 1). Sites within a specific stratum were typically located on separate ridgelines at least 300 m (984 ft) apart to increase the likelihood of independence. Sites in different strata along the same ridgeline were at least 145 m (475 ft) from other strata. All 5 plant species occurred along the elevation gradient, but thread-leaf phacelia and cutleaf daisy were not found in the lowest insolation sites. Our study sites spanned 2 Generalized Provisional Seed Zones (Bower and others 2014): 1) 2 sites in minimum temperature class –9.4 to –6.7 °C (15–20 °F), and annual heat moisture index 2 to 3, and 2) 19 sites in minimum temperature class –9.4 to –6.7 °C (15–20 °F), and annual heat moisture index 3 to 6.

**Measurements of Abiotic and Biotic Site Conditions**

At each site, we measured soil temperature, soil moisture and nutrients, and site productivity. Soil temperature was monitored every 90 min from May (5th, 6th, 11th, or 17th depending on site) to 20 or 22 July with iButton temperature loggers (Thermocron, Sydney, Australia) placed 5 cm into the soil. Minimum, maximum, and mean monthly temperatures were calculated for each site. On 6 to 7 June, we collected 5 soil cores (0–10 cm [0–0.39 in]) within each site, which were pooled, sieved through a 2 mm (0.08 in) sieve, and placed in resealable plastic bags in a cooler to bring to the laboratory for further processing. We subsampled 100 g (0.03 oz) that was air-dried and then sent for analyses of soil pH, percent organic matter, available nitrogen (NO\(_3\)), phosphorus (Merlich), potassium,
calcium, sulfur, magnesium, manganese, iron, sodium, zinc, and copper (Ward Laboratories, Kearney, Nebraska, USA). Ten g was dried at 105 °C (221 °F) for 48 h to determine gravimetric water content, and 10 g (0.35 oz) was freeze-dried for molecular analyses of fungal communities (see below). Gravimetric water content was also measured 12 to 13 July following the same protocols. During the July sampling, we also collected 2 samples of aboveground biomass using a 0.5 × 0.5 m (3.3 × 3.3 ft) frame as a measure of site productivity. Biomass was clipped at the soil and dried at 65 °C (149 °F) for 48 h before being weighed.

Plant Traits Measurements

Every week we visited sites to assess phenology. We recorded the time of flowering and seed ripening based on the prominent growth stage of the entire site (not individual plants). At each site, we randomly selected 2 individuals of each species to measure plant traits. We counted the number of flowers and then cut the shoots for measurements of specific leaf area (SLA) and aboveground biomass. While this involved destructive sampling that precluded seed collections on the same individuals, it was necessary to capture peak biomass before shoots started to senesce. Shoots were wrapped in wet paper towels, placed in a cooler, stored at 4 °C (39.2 °F), and processed within 2 d. Two leaves were removed per plant at a consistent stem location for SLA analysis to reduce the variation of leaf morphology within a plant (Shipley 1995). Leaves were flattened onto a sheet of white printer paper and scanned at 200 dpi using a scanner (Dell H825c/w Printer/Scanner) according to Pérez-Harguindeguy and others (2013). Leaf area was calculated in R using k-means algorithm (Hartigan and Wong 1979) and the HDBSCAN algorithm (McInnes and others 2017) for image processing. Specifically, we assigned each pixel to a white background group or leaf foreground group with k-means. We removed the group corresponding to white background pixels from further analysis. To isolate each of the 2 leaves in the scanned images, we applied HDBSCAN to the remaining leaf pixels. This method resulted in 2 clusters, one corresponding to each leaf. We counted the pixels in each leaf cluster and multiplied the counts by the area of a pixel (1.6e-8 meters-squared in a 200 DPI scanner) to derive area for each leaf. SLA was then calculated by dividing leaf area by leaf biomass after drying leaves at 65 °C (149 °F) for 48 h. The remainder of the shoot was also dried at 65 °C (149 °F) for 48 h. Values from the 2 plants per species were averaged within sites. SLA was not measured on threadleaf phacelia because plants began to dry down due to a heat wave before collection and we could not get accurate leaf area scans.

We collected ripe seeds from each species to measure seed mass. We collected from a minimum of 10 individuals at each site to capture seed weight diversity and to ensure that we had enough seeds for future reciprocal transplant studies. At a few sites, blue-eyed Mary seeds were collected farther than 10 m (32.8 ft; but not more than 30 m [98.4 ft]) away from the site center given the low seed numbers per plant. Seeds were stored in paper envelopes at room temperature to after-ripen, and we measured seed mass in September 2021 (Sartorius scale, precision = 0.1 mg [3.5 e-8 oz]). For larger-seeded species (blue-eyed Mary, blanket flower, and prairie smoke), we weighed 5 seeds individually from 5 plants for a total of 25 seeds per species per site. Smaller-seeded species (cutleaf daisy and threadleaf phacelia) had seeds that were too small to be weighed individually,
so 5 seeds were weighed together and the average individual seed mass was calculated. In total, 5 replicates of 5 seeds from 5 plants were weighed for 25 replicates (125 seeds) per species per site.

**Amplification of Fungal Communities and Assessment of Fungal Guilds**

We chose to focus on fungi because they constitute the most important group of soil mutualists and pathogens (Smith and Read 2010; Raaijmakers and others 2009). Briefly, we extracted DNA from 250 mg (0.009 oz) soil per site using the DNeasy PowerSoil Pro DNA isolation kit (Qiagen, Redwood City, California) as well as a blank to monitor potential background or cross-contamination among samples. Amplification, sequencing, and bioinformatics followed Bollington and others (2020). Briefly, we amplified the internal transcribed spacer 2 (ITS2) region using the fungal-specific forward primer pairs fITS7 and ITS7o and the general eukaryotic reverse primer ITS4 and attached barcodes in a second PCR. Amplicons were pooled based on band intensity and sequenced using 2 × 300 paired-end run on an Illumina MiSeq sequencing platform (Illumina Inc, San Diego, California, USA). Raw sequence data were processed using Quantitative Insights into Molecular Microbial Ecology 2 (QIIME2 version 2018.4; https://qiime2.org/), and forward and reverse reads were trimmed to where median quality score fell below 30. We used the q2-dada2 plugin to produce sequence clusters with 100% similarity. We then clustered the sequences at 97% similarity using the q2-vsearch plugin to produce operational taxonomic units (OTUs), and taxonomy was assigned using the database UNITE (Nilsson and others 2019). Taxa were placed into fungal guilds based on potential function (plant pathogens and plant mutualists) with the database FUNGuild (Nguyen and others 2016). To represent plant pathogen communities, we used OTUs identified solely as “plant pathogens” within “highly probable” and “probable” confidence rankings, whereas mutualists were identified as any sequence of Glomeromycota origin. Samples were rarified to 3500 sequences before statistical analyses, in which plant pathogens made up 17% and mutualists 4% of total fungal sequences. Sequences have been submitted to the National Center for Biotechnology Information’s Sequence Read Archive under accession number PRJNA983821.

**Statistics**

To determine if abiotic and biotic site characteristics or plant traits varied along elevation and insolation gradients and if a significant interaction occurred between elevation and insolation, we conducted two-way ANOVAs (type II) on multiple regression models with insolation and elevation as predictors. We anticipated opposing effects of elevation and insolation on drought conditions, with lower elevation and higher insolation corresponding to the strongest drought conditions. For this reason, we specified linear models with the inverse of insolation to test for multiplicative interactions among drivers of drought. However, we discuss results using the original insolation definition wherein increased insolation equals increased solar radiation and decreased topographical shade. Flower count and phenology were also analyzed using a two-way ANOVAs (type II) on multiple linear regression models but required a generalized linear model with a Poisson distribution. Normality for all site and plant trait data was determined using Shapiro-Wilk tests and visually using QQ-plots. Variables were log-transformed when necessary. Statistics for each species were run independently. Given strong pairwise correlations (>0.70, assessed using Pearson’s correlation), we removed gravimetric water content for July (correlated with gravimetric water content for June), and soil temperatures for May and July (correlated with June temperatures, which had a more complete data set for the entire month). There were no significant differences in phenology within species among sites, potentially attributable to measurements being too coarse (only once per week), and these data are therefore not presented. To assess if certain species exhibited greater trait variation and if certain traits were more variable than others, we relativized absolute trait values around the mean for each species and performed one-way Levene’s tests. We conducted Tukey adjusted post-hoc comparisons to reveal the nature of group differences. Statistical analyses were carried out using R 4.0.2 (R Core Team 2020), the car (v3.0.8; Fox and others 2012), and the hmisc (v4.4.0; Harrell 2020) packages.

**RESULTS AND DISCUSSION**

What Is the Variation in Soil Properties along Gradients in Elevation and Insolation?

Soil moisture and temperature, both known to affect plant phenology and growth (Reid 1973; Sundqvist and others 2013; Kunkel and others 2016), varied by orders of magnitude across our site. For example, June soil moisture ranged tenfold, minimum soil temperature ranged nearly threefold, and maximum temperature nearly twofold (Table 1). This corresponded with variation in other soil variables, including a sixfold difference in soil organic matter, a tenfold difference in available N, a threefold difference in S, Z, Mn, Ca, and a twofold difference in K, Fe, Mg, Na, and P (Table 1). Finally, site productivity differed eightfold among sites, and we documented more than a sixfold difference in the relative abundance of putative fungal mutualists and pathogens (Table 1).

We then determined if and how this substantial variation changed predictably along elevation and insolation gradients and found that both were suitable proxies for at least some of the abiotic and biotic properties measured. Soil moisture decreased with increased insolation in accordance with previous...
findings (Reid 1973; Sundqvist and others 2013) but did not change along the elevational gradient (Table 1). The availability of several nutrients (Ca, K, Mg, Fe) also decreased with increasing insolation, although the effect of insolation depended on elevation for three of them (Ca, K, Fe) (Table 1). We also observed a significant interaction between insolation and elevation for productivity, most likely because the effect of insolation was greater at lower elevation sites. Soil moisture has been shown to drive differences in soil fertility and productivity (Daly and Porporato 2005; Meisner and others 2013), which may explain the responses to insolation we observed. Elevation and insolation also affected soil temperature in predictable ways; minimum June temperature decreased with increased elevation (P = 0.002), and maximum temperature increased with insolation (P = 0.004). At one high insolation site with extensive rock cover and low productivity, soil temperature reached 66 °C (150 °F). Even with these extreme conditions, we were able to locate 4 of 5 target species at this site. Similarly, high soil temperatures have been recorded in eroded patches in the Alps with plants exhibiting heat resistance exceeding 60 °C (140 °F) (Larcher and others 2010), suggesting that high-elevation plants may be adapted to large temperature ranges. High-elevation plants may also associate less with AM fungi at our site, because the relative sequence abundance belonging to Glomeromycota decreased with increasing elevation (P = 0.008, although this was primarily driven by high

### Table 1

<table>
<thead>
<tr>
<th>Site / Soil property</th>
<th>Range, mean, and variation</th>
<th>Elevation</th>
<th>Insolation</th>
<th>Elevation * Insolation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site productivity (g / 0.25m²)</td>
<td>8.71–68.9 28.1 16.5</td>
<td>3.37 0.084</td>
<td>23.89 &lt; 0.001</td>
<td>- 7.46 0.014</td>
</tr>
<tr>
<td>pH</td>
<td>6.00–6.80 6.45 0.211</td>
<td>3.71 0.071</td>
<td>0.960 0.341</td>
<td>11.72 0.003</td>
</tr>
<tr>
<td>OM (log; %)</td>
<td>3.00–19.7 0.069 0.037</td>
<td>0.477 0.499</td>
<td>7.55 0.014</td>
<td>- 0.002 0.970</td>
</tr>
<tr>
<td>Nitrate (log; ppm)</td>
<td>0.500–4.90 1.95 1.27</td>
<td>0.726 0.406</td>
<td>0.994 0.763</td>
<td>3.04 0.099</td>
</tr>
<tr>
<td>Potassium (ppm)</td>
<td>163–431 257 70.3</td>
<td>1.26 0.278</td>
<td>11.11 0.004</td>
<td>- 16.17 &lt; 0.001</td>
</tr>
<tr>
<td>Sulfate (ppm)</td>
<td>7.00–24.7 13.1 4.34</td>
<td>0.270 0.610</td>
<td>3.16 0.093</td>
<td>9.77 0.006</td>
</tr>
<tr>
<td>Zinc (log; ppm)</td>
<td>1.37–5.35 2.88 1.21</td>
<td>0.505 0.487</td>
<td>0.251 0.623</td>
<td>0.253 0.621</td>
</tr>
<tr>
<td>Iron (ppm)</td>
<td>21.2–61.0 0.5 9.18</td>
<td>3.25 0.089</td>
<td>10.38 0.005</td>
<td>- 8.50 0.010</td>
</tr>
<tr>
<td>Manganese (log; ppm)</td>
<td>12.7–45.0 22.0 8.1</td>
<td>0.053 0.820</td>
<td>2.18 0.158</td>
<td>0.587 0.454</td>
</tr>
<tr>
<td>Copper (ppm)</td>
<td>0.460–93.8 6.48 21.3</td>
<td>1.39 0.256</td>
<td>2.62 0.124</td>
<td>1.19 0.290</td>
</tr>
<tr>
<td>Calcium (ppm)</td>
<td>982–2995 1853 472</td>
<td>0.008 0.931</td>
<td>0.251 0.623</td>
<td>0.253 0.621</td>
</tr>
<tr>
<td>Magnesium (ppm)</td>
<td>151–330 217 44.8</td>
<td>0.050 0.826</td>
<td>4.80 0.043</td>
<td>- 1.11 0.308</td>
</tr>
<tr>
<td>Sodium (ppm)</td>
<td>8.00–22.0 10.7 2.91</td>
<td>0.267 0.612</td>
<td>1.95 0.181</td>
<td>0.440 0.516</td>
</tr>
<tr>
<td>Phosphorus (ppm)</td>
<td>45.0–102 68.0 15.8</td>
<td>1.87 0.189</td>
<td>0.358 0.558</td>
<td>2.19 0.158</td>
</tr>
<tr>
<td>June gravimetric water content (log; g/g)</td>
<td>0.030–0.260 0.093 0.064</td>
<td>0.703 0.413</td>
<td>35.32 &lt; 0.001</td>
<td>- 0.673 0.424</td>
</tr>
<tr>
<td>June soil temperature (°C)</td>
<td>Maximum 35.0–66.0 49.5 8.46</td>
<td>0.021 0.886</td>
<td>11.07 0.004</td>
<td>+ 0.106 0.749</td>
</tr>
<tr>
<td></td>
<td>Minimum 3.00–8.50 5.14 1.31</td>
<td>13.42 0.002</td>
<td>- 0.026 0.873</td>
<td>0.448 0.513</td>
</tr>
<tr>
<td>Relative abundance (log)</td>
<td>Mutualistic Fungi 0.500–13.1 4.00 4.00</td>
<td>9.00 0.008</td>
<td>- 2.55 0.128</td>
<td>0.042 0.839</td>
</tr>
<tr>
<td></td>
<td>Pathogenic Fungi 4.10–42.9 16.0 10.0</td>
<td>0.087 0.772</td>
<td>0.368 0.552</td>
<td>0.204 0.657</td>
</tr>
</tbody>
</table>

Notes: We used the inverse of insolation due to an opposing effect of elevation and insolation found during preliminary data exploration. Data transformation noted. +/- denote the direction of relationship (positive or negative). Boldface indicates variables with significant results.
abundances at the 2 lowest elevation sites (Table 1). Previous work has observed greater proportions of non-mycorrhizal plants in the Arctic, possibly because host benefits from AM fungi decrease in colder soils (Kytöviita 2005). Putative soil pathogens, by comparison, did not change predictably along elevation or insolation gradients, even though their abundance varied greatly within this area (Table 1).

Overall, elevation and insolation were joint—possibly multiplicative—drivers of the soil environment in our grassland study area. However, where only main effects were significant...
predictors, insolation effects were more abundant, notably for water content. At this time, these results are limited to this system and additional studies would be necessary to determine if this is a local, regional, or more widespread pattern. Furthermore, we did not test for nonlinear relationships among soil properties and environmental gradients, though we encourage investigating this possibility with a larger data set capable of resolving these complex patterns. For future work studying the influence of soil properties on ecological phenomena, such as trait-by-environment interactions, we advise including both insolation and elevation in the sampling strategy and analysis.

**Do Plant Traits Vary along Fine-Scale Elevation and Insolation Gradients and Is Variation Trait Species-Specific?**

Despite the large variation in environmental conditions outlined above, we found few consistent patterns in trait differentiation. For example, no measured traits in threadleaf phacelia or prairie smoke varied along insolation and elevation gradients, although for threadleaf phacelia this could be caused by its more restricted distribution to only half of the sites surveyed. Absence of trait relationships with gradients may be attributable to extensive seed dispersal across the landscape.

### Table 2: Statistical results for linear regression of functional traits with elevation and insolation.

<table>
<thead>
<tr>
<th>Species</th>
<th>Trait</th>
<th>Range, mean, and variation</th>
<th>Elevation</th>
<th>Insolation</th>
<th>Elevation * Insolation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>P</td>
<td>+ / –</td>
</tr>
<tr>
<td>Blue-eyed Mary</td>
<td>SLA (log; cm²/g)</td>
<td>15.4–61.0</td>
<td>28.0</td>
<td>10.6</td>
<td>2.08</td>
</tr>
<tr>
<td></td>
<td>Seed Weight (mg)</td>
<td>0.300–1.03</td>
<td>0.629</td>
<td>0.163</td>
<td>0.911</td>
</tr>
<tr>
<td></td>
<td>Aboveground Biomass (g)</td>
<td>0.013–0.122</td>
<td>0.054</td>
<td>0.031</td>
<td>4.53</td>
</tr>
<tr>
<td></td>
<td>Flower Count</td>
<td></td>
<td>2–30</td>
<td>12.3</td>
<td>9.90</td>
</tr>
<tr>
<td>Cutleaf daisy</td>
<td>SLA (cm²/g)</td>
<td>8.84–13.1</td>
<td>10.5</td>
<td>1.15</td>
<td>0.650</td>
</tr>
<tr>
<td></td>
<td>Seed Weight (mg)</td>
<td>0.072–0.303</td>
<td>0.207</td>
<td>0.070</td>
<td>45.4</td>
</tr>
<tr>
<td></td>
<td>Aboveground Biomass (g)</td>
<td>0.616–1.47</td>
<td>0.910</td>
<td>0.231</td>
<td>0.079</td>
</tr>
<tr>
<td></td>
<td>Flower Count</td>
<td></td>
<td>2–6</td>
<td>3.43</td>
<td>1.40</td>
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<tr>
<td>Blanketflower</td>
<td>SLA (cm²/g)</td>
<td>9.24–14.5</td>
<td>11.8</td>
<td>1.38</td>
<td>7.51</td>
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<td>Seed Weight (mg)</td>
<td>1.51–2.85</td>
<td>2.11</td>
<td>0.286</td>
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<td>Aboveground Biomass (g)</td>
<td>0.658–2.42</td>
<td>1.46</td>
<td>0.512</td>
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<td>Flower Count</td>
<td></td>
<td>1–3</td>
<td>1.84</td>
<td>0.765</td>
</tr>
<tr>
<td>Prairie smoke</td>
<td>SLA (cm²/g)</td>
<td>9.56–12.7</td>
<td>10.8</td>
<td>0.988</td>
<td>3.66</td>
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<td></td>
<td>Seed Weight (mg)</td>
<td>0.609–1.34</td>
<td>0.922</td>
<td>0.188</td>
<td>2.13</td>
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<tr>
<td></td>
<td>Aboveground Biomass (g)</td>
<td>0.680–2.18</td>
<td>1.27</td>
<td>0.412</td>
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<tr>
<td></td>
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<td>0–3</td>
<td>1.59</td>
<td>1.02</td>
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<tr>
<td>Threadleaf phacelia</td>
<td>SLA (cm²/g)</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<tr>
<td></td>
<td>Seed Weight (mg)</td>
<td>0.179–0.362</td>
<td>0.296</td>
<td>0.052</td>
<td>0.298</td>
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<td></td>
<td>Aboveground Biomass (log; g)</td>
<td>0.174–0.984</td>
<td>0.349</td>
<td>0.249</td>
<td>0.016</td>
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<td></td>
<td>Flower Count</td>
<td>18–83</td>
<td>35.3</td>
<td>18.2</td>
<td>1.84</td>
</tr>
</tbody>
</table>

Notes: We used the inverse of insolation because of an opposing effect of elevation and insolation found during preliminary data exploration. Data transformation noted. +/– denote the direction of the relationship (positive or negative). Boldface indicates variables with significant results.
obscuring adaptive responses and (or) an inability to respond to variations in environmental conditions (that is, low plasticity). Also possible is that even greater environmental variation is required to prompt a trait response, or that relationships with the environments are nonlinear, which we could not assess here given sample size constraints. Additional fitness parameters important to plant establishment could be analyzed, including seed viability and germination rates.

For the other 3 species, we observed responses in selected traits that may be explained by the preferred microhabitats of each species within the heterogeneous grassland landscape. Blanketflower SLA was smaller in high insolation areas (Figure 2; Table 2). Smaller SLA is associated with slower growing leaves that have increased thickness and density with less transpiring leaf surface and therefore reduced water loss in drier soils (Shipley and others 2006; Poorter and others 2009). A similar trend was observed in cutleaf daisy but was primarily driven by the 2 low-elevation sites that had lower insolation. SLA in these 2 Asteraceae species also changed with elevation, but responses differed and for cutleaf daisy depended on insolation (Table 1); SLA increased with elevation in blanketflower and decreased in cutleaf daisy. The underlying reason for this difference is uncertain but may be related to their preferred habitats. Cutleaf daisy tended to grow on exposed, high insolation sites including high-elevation ridgelines, while blanketflower preferred the more north-facing, lower insolation, high-elevation slopes. Cutleaf daisy also produced larger seeds at higher elevation sites that were drier and hotter (Table 2). This increase in seed size may be a response to stress because larger seeds contain more resources that can produce faster-growing, larger seedlings with higher colonizing ability (Westoby and others 1992; Coomes and Grubb 2003). Blue-eyed Mary had more flowers at higher elevation sites that tended to have increased shrub cover.

Overall, traits differed in variability and so did plant species (Figure 2), but the drivers of this variability remain unknown. SLA and seed weight, both heritable traits (Westoby and others 1992; Leishman 2001; Shipley 1995; Morecroft and Woodward 1996; Scheepens and others 2010) correlated with gradients in elevation and insolation in some species in ways that support previous findings (Qi and others 2015; Poorter and others 2009). We do not know if this trait variation is related to local adaptation or plasticity as it requires reciprocal transplant experiments (Gibson and others 2016) and longer-term monitoring of survival and fitness of seeds collected along elevational and insolation gradients.

**WHAT ARE THE IMPLICATIONS FOR RESTORATION AND SEED COLLECTION?**

Many practitioners use seed-transfer zones to guide seed collection and procurement, but the level of environmental variation...
and local adaptation within a single seed-transfer zone is often unknown and could affect restoration success (Bischoff and others 2006; Davidson and Germino 2020). Our study provides some insights into these two unknowns. First, the surveyed area was similar in size to many restoration projects, with 90% of our sites falling in a single Generalized Provisional Seed Zone (Bower and others 2014). We observed substantial variation in abiotic and biotic properties within that seed zone, however, and transferring plants along these gradients may yet reveal fitness consequences. Conducting a reciprocal transplant study using seed collected along these elevation and insolation gradients will help to elicit potential fitness consequences. Second, despite the substantial environmental variation, we found perennial species across the breadth of these environments and annual plants also flowered and set seed across these areas, which indicates broad environmental tolerance in the short term. Third, the capacity to establish does not appear to be broadly plastic or adaptive because only a few species and trait combinations correlated with environmental gradients (although there may be longer-term fitness differences that could impact restoration success). Overall, we found that environmental variables and plant traits differed substantially on a small spatial scale, both within and between species, but plant trait patterns were not consistently correlated across species. Systematically collecting seeds along elevation and insolation gradients can provide a breadth of plant traits to test trait-by-environment interactions in a reciprocal transplant study and may improve the likelihood of restoration success.

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<table>
<thead>
<tr>
<th>Species</th>
<th>Size</th>
<th>Type</th>
<th>Price per 1000</th>
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<tr>
<td>Bitternut Hickory</td>
<td>12-18&quot;</td>
<td>Seedlings</td>
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<tr>
<td>Tulip Poplar</td>
<td>18-24&quot;</td>
<td>Seedlings</td>
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<td>12-18&quot;</td>
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<td>Black Walnut</td>
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<td>Silky Dogwood</td>
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<td>River Birch</td>
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<td>Swamp White Oak</td>
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<td>Black Chokeberry</td>
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<td>$920.00</td>
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