The Extent, Drivers, and Consequences of Intraspecific Variation in Plant Functional Traits

Rachel M. Mitchell

A dissertation

submitted in partial fulfillment of the

requirements for the degree of

Doctor of Philosophy

University of Washington

2013

Reading committee:

Jonathan Bakker, Chair

Janneke Hille Ris Lambers

Soo-Hyung Kim

Program Authorized to Offer Degree:

School of Environmental and Forest Sciences

© Copyright 2013

Rachel M. Mitchell

University of Washington

Abstract

The Extent, Drivers, and Consequences of Intraspecific Variation in Plant Functional Traits

Rachel M. Mitchell

Chair of the Supervisory Committee:
Associate Professor Dr. Jonathan Bakker
School of Environmental and Forest Sciences

Classifying species and communities by their collection of functional traits (traits that indirectly or directly quantify plant fitness) rather than by species identity and richness alone is increasingly being used to identify global patterns in plant and ecosystem function, to identify abiotic filters which shape community composition, and to redefine plant strategies. Typically, studies using plant functional traits have relied on trait means or community level trait averages to quantify functional change. Recent research, however, suggests that intraspecific variation in plant functional traits is extensive, and may be of importance for a number of ecological processes.

My dissertation research focused on quantifying the extent, drivers, and consequences of intraspecific variation in plant functional traits. I conducted a study in Pacific Northwest grasslands which found that, after applications of carbon in the form of either sugar or activated carbon, perennial forbs, but not grasses, suffered significant decreases in cover and biomass. These results indicated that perennial forbs, as a functional group, were more susceptible to carbon addition; however some species of forb were less affected than others. One possible explanation for this difference was that some species exhibited greater variation in functional traits that were affected by carbon addition, and this variation may have allowed some individuals to pass unaffected through the treatment filter.

To test this hypothesis, I developed a study to investigate the extent of intraspecific variation in two commonly measured plant functional traits thought to reflect growth strategy, specific leaf area (SLA) and rosette diameter, within *Hypochaeris radicata,* a globally distributed perennial herb. I simultaneously examined the extent of intraspecific variation in plant functional traits, and the efficacy of methods used in the literature to quantify this variation. I collected trait measurements on 241 individuals from 10 populations spanning a 480 km transect. I found significant variation in plant traits: there was a 27-fold variation in SLA, and a 34-fold variation in rosette diameter. Furthermore, the method of analysis used to quantify these traits affected the interpretation of these results, as inclusion of intraspecific variation may violate the underlying assumptions of some commonly applied statistical tests (e.g. ANOVA), and thus this variation is frequently reduced through corrective measures.

Variation in plant traits can be driven by biotic, as well as abiotic factors. To determine whether and how plants shift their traits in response to biotic conditions, I measured SLA and plant height for a suite of 8 forbs in an experimental grassland. Traits were collected from individuals in one of three treatments: high forb cover, balanced grass/forb cover, or high grass cover. Using a combination of permutational analysis of variance and linear mixed models, I found that plants shifted traits in terms of both average expression and trait variation in response to the biotic community, but that the direction of the shift was highly idiosyncratic. In addition, the suite of plants shifted their overall niche breadth and niche spacing in both SLA and plant height to limit similarity in the face of high competition from dominant grasses.

Based on the results of the previous experiments, I then set out to determine whether this large variation was beneficial to *Hypochaeris radicata* in stressful abiotic conditions, and whether the observed variation was due to plasticity or adaptation. I collected seeds from three

populations (25 maternal lines from each) that differed in growing conditions and trait expression. Maternal lines also differed in their trait expression; using PCA, maternal lines were ranked by seed weight and rosette diameter, and lines with low, intermediate, and high trait values were selected for each treatment. Plants were grown in the greenhouse under three treatments: ambient, drought, or shade conditions. I collected trait data for SLA, rosette diameter, chlorophyll content, and leaf shape, as well as calculating relative growth rate and devotion to above- and below-ground tissues. Analyses of these data using hierarchical linear mixed models revealed that, for plant diameter, maternal line of origin explained a significant portion of observed variation (30%), but for all other traits, population of origin and maternal line explained very little of the observed variation (<10%). Furthermore, trait variation in the field was not predictive of trait variability in the greenhouse. These results indicate that, although there may be local genetic adaptation, plant trait expression is largely driven by plasticity to prevailing abiotic conditions.

In summary, I found that plant functional traits are highly variable and can respond to both abiotic and biotic drivers. In addition, I found that for *H. radicata*, variation in plant traits was largely driven by plasticity.

TABLE OF CONTENTS

List of Figures ii
List of Tables v
Acknowledgements vi
Chapter 1: A General Overview of Intraspecific Functional Trait Research 1
Chapter 2: Carbon Addition as a Technique for Controlling Exotic Species in Pacific Northwest Prairies 3
Chapter 3: Quantifying and Comparing Intraspecific Functional Trait Variability: A Case Study with *Hypochaeris radicata* 28
Chapter 4: Functional Trait Shifts in Response to Community Composition 55
Chapter 5: Sources and Drivers of Intraspecific Plant Trait Variation 55
Chapter 6: Synthesis 101
References 104
Appendix A: Supplementary Materials for Chapter 3 116

List of Figures

Figure 2.1 Aboveground biomass (mean + standard deviation) for A) all plants (total), B) forbs, and C) grass in plots treated with activated carbon (AC), sugar, or left untreated (control) in 2008 and 2009. PERMANOVA results are summarized in Table 1. Significant carbon effects are indicated by uppercase letters. 23

Figure 2.2 Spring (mean + standard deviation) of A) forb cover and B) grass cover in plots treated with activated carbon (AC), sugar, or left untreated (control) in 2008 and 2009. PERMANOVA results are summarized in Table 1. Significant carbon effects are indicated by uppercase letters. Significant Carbon x Year interactions were followed by analysis of each year individually; within years, lowercase letters indicate treatments that differ. 24

Figure 2.3 Spring and summer (mean + standard deviation) total cover in plots treated with activated carbon (AC), sugar, or left untreated (control) in 2008 and 2009. PERMANOVA results are summarized in Table 1. Significant Carbon x Year interactions were followed by analysis of each year individually; within years, lowercase letters indicated treatments that differ. Spring results were only marginally significant ($P = 0.09$). 25

Figure 2.4 Species richness (mean + standard deviation) for plots treated with activated carbon (AC), sugar, or left untreated (control) in A) spring and B) summer. PERMANOVA results are summarized in Table 1. Significant carbon effects are indicated by uppercase letters. 26

Figure 2.5 A) Plant basal area and B) bare ground (means and standard deviations) for plots treated with activated carbon (AC), sugar or left untreated (control) in spring 2008 and 2009. PERMANOVA results are summarized in Table 1. Significant Carbon x Year interactions were followed by analysis of each year individually; within years, lowercase letters indicate treatments that differ. 27

Figure 3.1 Techniques used to quantify or compare intraspecific variation drawn from peer-reviewed literature published between January 2007 and October 2012. Dark bars indicate quantitative statistical tests, and light bars indicate techniques which compare descriptive statistics. Sixty-four studies are included here but some studies used multiple techniques and were tallied multiple times. 50

Figure 3.2 Trait averages (+ SD) and coefficients of variation for SLA (top) and rosette diameter (bottom) among 10 populations. Populations are numbered from north to south. P-value and Uppercase letters denote significantly different groups as determined by PERMANOVA, and italicized lowercase letters denote significantly different groups as determined by PERMDISP. Note that the scale of the y-axis differs among panels. 51

Figure 3.3 Effect sizes (magnitude of difference from the estimated grand-mean, shown by the dashed horizontal lines) and 95% credible intervals for SLA (top) and rosette diameter (bottom) among 10 populations. Populations are numbered from north to south. Populations differ significantly if their credible intervals for that statistic do not overlap. 52

Figure 3.4 Point estimates and 95% credible intervals for the mean (left) and standard deviation (right) for SLA (top) and rosette diameter (bottom). Populations differ significantly if their credible intervals do not overlap. 53

Figure 3.5 Posterior distributions (solid lines) of SLA for the regional population (top panel) and 10 populations of *Hypochaeris radicata*. The vertical lines in each graph indicate the literature-based trait estimates from fertilized pot-grown plants (thick dashed line), field grown plants (dot-dashed line), unfertilized pot-grown plants (thin dashed line) and the LEDA Traitbase (dotted line). 54

Figure 4.1 Bivariate means and dispersions for each species in each seeding treatment. Treatment means are indicated with symbols; treatments dispersion is indicated by solid (forb-rich), long-dashed (mixed) or short-dashed (grass-rich) lines outlining the range of values. Significantly different means are indicated by different uppercase letters (A, B, or C) and significantly different dispersions are indicated by different lowercase letters (x, y, and z). The y-axis for *Ranunculus* is truncated to improve readability. 75

Figure 4.2 Violin plots showing height means and distributions for each species in each treatment. A violin plot is a combined boxplot and kernel density plot. Here, we show trait means solid points, and the data density (variation) estimated by kernel method. Significantly different ($\alpha = 0.05$) means are indicated by different uppercase letters (A, B, or C) and significant differences in dispersion are indicated by different lowercase letter (x, y, or z). 76

Figure 4. 3 Violin plots showing SLA means and distributions for each species in each treatment. Trait means are indicated by solid points. Significantly different ($\alpha = 0.05$) means are indicated by different uppercase letters (A, B, or C) and significant differences in dispersion are indicated by different lowercase letter (x, y, or z). 77

Figure 5.1 Collection locations for the 3 populations used in this study. 94

Figure 5.2 Mean + SD of field-based measurements for the three populations of origin. Significant differences ($\alpha = 0.05$) in average trait values are indicated by different uppercase letters and significant differences in variance are indicated by different lowercase letters..... 95

Figure 5.3 Principal Components Analysis of SLA, plant diameter and seed mass for maternal lines from GL, SP, and UB. Populations were analyzed together, but are shown in separate graphs for clarity. PC 1 explained 49% of observed variation. Filled symbols indicate maternal "high", "medium" and "low" lines from each population used in the study, while open symbols indicate maternal lines included in figure 5.2 but not grown in this study. 96

Figure 5.4 Proportion of total random effects variance explained by population of origin, maternal line, and residual variance (representing variance between individuals) for the five measured traits. 97

Figure 5.5 Mean (+ SD) values of A) final diameter, B) leaf length: width, C) root:shoot ratio, D) SLA, and E) final relative chlorophyll content for each maternal line (individual bar, arranged from low to high along based on scores from PC 1) in each population (unique color; defined in Table 5.1) for each treatment. Significant treatment differences ($\alpha = 0.05$) are indicated by different letters. 98

Figure 5.6 Change in average trait values for diameter (figures A and B) and relative chlorophyll content (Figures C and D) for individual maternal lines and populations. Variation among individuals within maternal lines and populations is not shown to maintain clarity. 99

Figure 5.7 Coefficients of Variation for SLA and plant diameter measured in the field and under various abiotic conditions (ambient, 50% drought, 80% shade) in the greenhouse for each population of origin..... 100

List of Tables

Table 2.1. Results of PERMANOVA models testing the effects of Block, Carbon addition, Year, and the Carbon x Year interaction on biomass, cover, richness, composition, bare ground and plant basal area in spring and summer. The pseudo-*F* (*F*) and permuted *P*-values (*P*) are reported for each factor along with the R^2 for the overall model. Significant effects ($\alpha = 0.05$) are in bold. Composition was a multivariate response and analyzed using Bray-Curtis distances; all other responses were univariate and analyzed using Euclidean distances. Each model was assessed using 9999 permutations. 21

Table 2.2. Temperature and precipitation data for Coupeville, Whidbey Island, Washington. Long-term normals are from 1971-2000 and are reported as mean (standard deviation). Spring is defined as March-May, and summer as June-August (Western Regional Climate Center). 22

Table 3.1 Application, assumptions, strengths, and weaknesses of statistical methods used to quantify intraspecific variation. 48

Table 4.1 Results of PERMDISP and PERMANOVA testing the effect of seeding treatment, species identity, and the interaction between treatment and species identity on bivariate traits, height, and SLA. Significant effects ($\alpha = 0.05$) are bold. 72

Table 4.2 Summary of trait responses, by species, to a changing biotic community. Traits were examined together (bivariate) or separately (height, SLA), and could be unchanged or differ in mean and/or variance. Graphical results are in Figures 4.1- 3, and statistical results in Table 4.1. 73

Table 4.3 Average niche breadth (square root of the residual term) and separation (square root of the species identity term) for eight forb species. 74

Table 5.1 Average annual rainfall and land-use history for the collection sites of the three study populations. 93

Table S.1 Collection site name, map location (Fig. 3), identifying code and total number of samples collected. 119

Acknowledgements

I would like to acknowledge the contributions of a number of people in the completion of my dissertation. My advisor and committee chair, Jon Bakker, has been invaluable throughout my graduate education. He has offered insight and feedback on the entire research process, from daydream to manuscript. I am also grateful for my other advisors, Janneke Hille Ris Lambers, Soo-Hyung Kim, Sarah Reichard, Martha Groom, and Regina Rochefort, who have provided support and guidance throughout this project.

In addition, a number of fellow students have helped with the completion of this work. Andrea Blin and Brandon Neuhaus spent hours weighing and measuring in the lab and field, and provided entertaining conversation at the same time. Without their efforts, this dissertation would still be in progress. I would also like to thank Eric Delvin. His tremendous efforts to restore Puget Sound prairies provided the data that were used in Chapter 4.

I would also like to acknowledge my friends and family. My parents, Michael and Jeanne Mitchell, and my sister, Amanda Mitchell, have been supportive and inquisitive and they, along with many friends, have provided a valuable sounding board for thoughts and ideas related to my research project. Without their support and encouragement, I never would have completed this project.

Finally, I would like to thank my husband, Dr. Ty Robinson. Ty completed his own PhD in Astronomy and Astrobiology in 2012, making him the ideal partner during my own graduate adventures. Not only could he perfectly understand my experiences and frustrations, but he was always willing to help in the field or in the greenhouse, despite his moderate dislike of the outdoors and his own busy schedule. Without him, and his fantastic gin and tonics, this project would have never been undertaken or completed.

Chapter 1: A General Overview of Intraspecific Functional Trait Research

In 1987, the concept of functional ecology was introduced (Calow 1987) as unique sub-discipline of ecology focused on how individuals, species, communities and ecosystems alter abiotic and biotic processes through their distinct biological functions. Following this introduction, plant ecology has embraced functional trait-based methods (Cadotte et al. 2011). In describing plants by their functional traits, which indirectly or directly measure fitness, rather than their taxonomy, a fuller understanding of plant community ecology has emerged. Understanding of plant strategy (Laughlin et al., 2010; Westoby, 1998), community assembly (Diaz et al. 1998, Maharjan et al. 2011), diversity (Petchey and Gaston 2002 and global trends in plant function (Diaz et al., 2004; Moles & Westoby, 2003; Moles et al., 2009; Wright et al., 2005) have been extended due in part to a trait based approach.

Much of the research about functional traits has focused on differences between species, and variation between individuals has been intentionally minimized through commonly applied measurement protocols (Cornelissen et al. 2003b). However, recent research has shown that some traits can vary substantially within species (Karley and Hawes 2008, Lecerf and Chauvet 2008, Violle et al. 2009). For example, leaf mass and wood density vary significantly among individuals and populations of *Nothofagus pumilio* (Fajardo and Piper 2011), while measurements in tropical forests have shown high intraspecific variation in leaflet traits (Hulshof and Swenson 2010). Physiological and functional traits, such as leaf nitrogen and specific leaf area, also show substantial variation (Scheepens et al. 2010, Baruch 2011, Long et al. 2011).

Although intraspecific variation is increasingly recognized as important (Albert et al. 2012, Violle et al. 2012b), many questions about the extent, drivers and consequences of intraspecific variation remain unanswered. In this dissertation, I examine when and how plant

traits exhibit variation, and how this observed variation impacts plant growth, survival, and coexistence. These studies deepen our understanding of the drivers and importance of intraspecific trait variation.

In the first study, I examined how plant functional groups (grass, forbs, or legumes) responded to carbon addition to the soil surface in an exotic-dominated grassland. Based on the results of this study, I then simultaneously quantified the extent of trait variation and evaluated commonly applied statistical methods using for trait comparisons using 10 populations of the common grassland invader, *Hypochaeris radicata*. After quantifying this variation, I then examined whether trait values shift in response to biotic drivers, and whether this shifting might lead to coexistence in an experimental grassland community. Finally, I determined whether observed trait variability is driven primarily by phenotypic plasticity to prevailing abiotic conditions, or adaptation and genetic factors in three geographically distinct populations of *H. radicata*.

Note to the reader: Chapter 2 has been published in a peer review journal (Mitchell and Bakker 2011)*, and Chapter 3 is currently in review for publication in a peer reviewed journal. Chapters 4 and 5 are each intended as a separate manuscript for publication. Therefore, each chapter includes an abstract and published chapters include a stand-alone references section. Formatting differs from chapter to chapter based on the requirements of the target journals.*

Chapter 2: Carbon Addition as a Technique for Controlling Exotic Species in Pacific Northwest Prairies

Abstract

Competition for soil resources is a driving force in plant community ecology. Amendments that alter soil resources could reduce exotic plant invasion and favor native species. We tested the effects of two types of carbon addition, sugar and activated carbon (AC). Sugar stimulates the microbial community and can temporarily reduce plant available nitrogen (N), while AC adsorbs plant available N and other organic and allelopathic compounds. Our study site was a grassland dominated by exotic species in central Whidbey Island, Washington. Six experimental plots were utilized. Sugar (1000 g m^{-2}; 42% C) and AC (420 g m^{-2}; 100% C) were applied in a split-plot design in spring 2008. Aboveground biomass, spring and summer plant cover, species richness, community composition, plant basal area and bare ground were tracked for two growing seasons. Sugar reduced forb biomass and spring forb cover in both years, but did not affect the abundance of grasses. Sugar also reduced spring species richness in both years and plant basal area in the first year. AC reduced forb biomass in both years but affected no other variables. These results indicate that sugar and, to a lesser extent, AC could be valuable tools for reducing the abundance of exotic forbs in Pacific Northwest prairies ecosystems. As non-toxic alternatives to herbicides, they could provide a window of opportunity in which desirable species could be established during restoration activities in areas where herbicide use is undesirable or prohibited.

Introduction

How plants compete for resources is a fundamental question in ecology (Goldberg et al. 1997, Tilman 1999). Resource levels are spatially and temporally dynamic, and can affect the outcome of competitive interactions (Tilman and Wedin 1991, Wedin and Tilman 1993). Consequently, intentional alterations of resource availability have been suggested as a management tool to reduce or prevent exotic plant invasion (Seabloom 2003, Corbin and D'Antonio 2004, Iannone et al. 2008). Alterations that favor native over exotic species could contribute to the restoration of ecosystems in which exotic plant species are a significant concern (Zink and Allen 1998, Kulmatiski and Beard 2006, Mangold and Sheley 2008).

In many temperate ecosystems of the northern hemisphere, elevated levels of soil nitrogen (N) are an obstacle to increasing native diversity (Maron and Connors 1996, Kolb et al. 2002, Abraham et al. 2009) and ecosystems with a high diversity of native species are more resistant to invasion by exotic plants (Dukes 2001, Pokorny et al. 2005, Sheley and Carpinelli 2005, Hooper and Dukes 2010). Fertilization (Carpenter et al. 2008), grazing (Hobbs 1996), nitrogen fixation by exotic species (Liao et al. 2008) and atmospheric deposition can all increase the level of N present in soils (Bobbink 1998). Changes in soil N have been shown to decrease native plant diversity while favoring invasive species (Suding et al. 2005, Vinton and Goergen 2006, Clark 2007).

Pacific Northwest prairies are an extremely endangered, historically N-poor North American ecosystem. This unique ecosystem provides habitat for several rare and endangered species, including the streaked horned lark (*Eremophila alpestris strigata*), Taylor's checkerspot butterfly (*Euphydryas editha taylori*), island marble butterfly (*Euchloe ausonides insulanus*) and golden paintbrush (*Castilleja levisecta*). Presently, these prairies occupy less than 3% of their

former acreage (Chappel et al. 2000). Remaining prairie fragments are often heavily invaded by exotic plant species (Dunwiddie et al. 2006) and have been degraded by agriculture and forest development.

Carbon addition to the soil surface has been shown to be one of the most reliable methods for altering soil C:N ratios (Perry et al. 2010, but see Biederman and Whisenant 2009) and recent studies in grassland ecosystems have demonstrated that carbon addition disproportionately affects exotic species (Averett et al. 2004, Blumenthal et al. 2009, Rashid and Reshi 2010) due to their higher requirement for soil N (Perry et al. 2010). Using carbon addition as a restoration technique in Pacific Northwest prairies to change C:N ratios could allow us to reduce or eliminate the establishment and spread of exotic species (Eschen et al. 2007).

When carbon is added as sugar, it stimulates soil microbial activity and temporarily reduces plant available N (Morghan et al. 1999). Dextrose, a simple monosaccharide that has been shown to behave similarly to sugar, can interact with other abiotic factors like water availability to reduce success of invasive species (Blumenthal et al. 2009).

Soil C:N ratios can also be altered by the addition of activated carbon (AC). AC has an extremely high surface area to volume ratio and can adsorb many times its own weight in organic compounds, including N. The method of N immobilization caused by AC is very different from that caused by sugar. One potential advantage of AC is that it also immobilizes a range of organic compounds, including potentially allelopathic compounds (Kabouw et al. 2010). AC is not widely used in restoration and its effect on exotic invaders of Pacific Northwest Prairies is not known, though charcoal, a naturally occurring type of AC, would have been present in these ecosystems historically (Boyd 1999, Weiser and Lepofsky 2009).

We hypothesized that amendments that alter soil C:N ratios will have a strong negative effect on exotic species. We specifically addressed three questions: 1) Does carbon addition affect the abundance and cover of exotic plant species? 2) Do sugar and AC perform similarly? 3) How long do effects of carbon addition last?

Methods

This experiment was performed at the Prairie Overlook site at Ebey's Landing National Historical Reserve on Whidbey Island, Washington. Nine plots were established at the Prairie Overlook by the National Park Service and some have undergone limited planting with native species. Six of the nine plots were used for this experiment. The site is part of Ebey's Prairie, and comprised the largest prairie-type ecosystem on Whidbey Island. Historically this prairie was maintained by frequent burnings by Native Americans (Weiser and Lepofsky 2009) and was likely dominated by perennial fescues such as Roemer's fescue (*Festuca roemeri)* and red rescue (*F. rubra)*. This area is characterized by glacial outwash soils, which range from coarse- to fine-textured material and are well drained. Fire has been suppressed since approximately 1850, and the prairie has been converted to agricultural use. Presently, the site is dominated by exotic grasses such as soft brome (*Bromus hordeaceus*), orchard grass (*Dactylis glomerata*) and exotic forbs such as hairy cat's ear (*Hypochaeris radicata*) and narrow-leaf plantain (*Plantago lanceolata*). State-listed noxious weeds such as Scotch broom (*Cytisus scoparius)* and ox-eye daisy (*Leucanthemum vulgare*) are also present at the site, but are actively controlled by the National Park Service.

Six 10x15 m whole plots at the Prairie Overlook were utilized in this experiment. Each plot received one of three pre-treatments before the experiment began: 1) treated with herbicide, tilled, and planted with Roemer's Fescue 2) treated with herbicide, tilled, and left unplanted 3)

treated with herbicide, mowed, but not tilled or planted for a total of two plots per each pre-treatment.

Carbon addition treatments were assigned following a split-plot design. Each 10x15 m whole plot was subdivided into 3 treatment plots, each 5x10 m. Each treatment plot received one of three carbon addition treatments: 1) sugar (1000 g m^{-2}; 42% C), 2) AC (420 g m^{-2}; 100% C), or 3) control (no carbon addition). Carbon was added in three equal applications over a 5-week period in spring 2008, with total application rates equal to the above values.

Eight 0.5x1 m quadrats per treatment plot were censused for species identity, plant basal area, percent cover and substrate. Data were collected in spring (to capture spring ephemeral species) and summer (to capture peak biomass) in 2008 and 2009. Each summer, above-ground biomass was clipped from four 10x100 cm strips per treatment plot. The biomass was sorted by functional group (grass or forb), dried at 60°C for 10 days, and weighed. The total richness was combined for adjacent pairs of quadrats to yield richness m^{-2}.

The experiment had a split-plot design with 6 whole plots and 3 treatment plots per whole plot, for a total of 18 experimental units. There was not sufficient replication of pre-treatments to test their effect statistically.

Our response variables were grass, forb and total biomass, species richness in spring and summer, community composition (multivariate; cover of every species on every plot) in spring and summer, plant basal area and percent bare-ground. Spring and summer data were analyzed separately to assess C addition effects on both spring ephemeral species and during summer peak biomass and cover. We used repeated measures ANOVAs with whole plots included as a fixed blocking term. We focused on the Carbon main effect and the Carbon x Year interaction term. Significant Carbon x Year interactions were followed by analyses of individual years to identify

which carbon treatments differed within a given year. Analyses were conducted using PERMANOVA, a non-parametric, permutational multivariate analysis of variance. This technique makes no distributional assumptions about the data and can be applied to uni- and multivariate data (Anderson 2001, McArdle and Anderson 2001). We used the PERMANOVA+ add-on for the PRIMER-E software package for these analyses (Anderson et al. 2008). Euclidean distances were used for analyses of biomass, basal area and percent bare-ground, and Bray-Curtis distances for analyses of community composition. Rare species (those occurring on <5% of plots) were deleted from multivariate community data, and the data were Wisconsin standardized (relativized by species maxima then plot total). Statistical significance was assessed using 9,999 permutations, with $\alpha = 0.05$.

Results

Sugar and AC addition significantly reduced forb biomass over both treatment years, but did not affect grass or total biomass (Figure 2.1; Table 2.1). Sugar addition significantly reduced forb cover in the spring, particularly during the first year (Figure 2.2), but not in the summer (Table 2.1). Total cover was lower in sugar-treated plots than other treatments during the first year, causing significant Carbon x Year interactions for total cover treated with sugar in both seasons. Pairwise comparisons indicated that in 2008, the effect from sugar was only marginally significant in spring (P=0.09) but significant in summer (P =0.004) and neither treatment had a significant effect in 2009 (Figure 2.3).

Sugar significantly reduced spring species richness (Figure 2.4; Table 2.1) while AC had no effect. Summer richness did not differ among carbon treatments in either year. Neither spring nor summer composition was affected by carbon amendment (data not shown).

Carbon amendment did not significantly affect the amount of bare ground present in the plots in spring or summer (Figure 2.5). There was a significant Carbon x Year interaction for plant basal area in the spring as basal area was lower in plots treated with sugar during the first year but did not differ among treatments in the second year (Figure 2.5; Table 2.1).

Discussion

This study supports the idea that carbon addition, and particularly sugar addition, may be a useful restoration tool in prairie ecosystems. Carbon addition as a method of exotic plant control has been widely investigated in the literature (Perry et al. 2010) and a variety of techniques have been developed, including addition of sugar (e.g., Morghan and Seastedt 1999, Mangold and Sheley 2008), sawdust (e.g., Bleier and Jackson 2007), straw (e.g., Kaushik and Inderjit 2007), wood-waste (Biederman and Whisenant 2009), and activated carbon (e.g., Kulmatiski and Beard 2006).

Sugar lends itself to restoration for a variety of reasons. It is relatively inexpensive, and easily obtained from wholesalers. In addition, it easily dissolves in water allowing it to be sprayed or irrigated on to restoration sites. Sugar is also non-toxic and can be applied in areas where herbicide use is prohibited or undesireable. Sugar is believed to reduce levels of soil N by stimulating metabolism in the soil microbe community and is presumed to be short-lived in the environment, making it an ideal amendment for short-term restoration or weed reduction.

Activated carbon, on the other hand, is presumed to be long-lived in the environment; it will remain “active”, absorbing organic molecules until full, after which it will remain sequestered in the soil, potentially permanently altering soil C:N ratios. AC is commonly used in agriculture, and is thus easily obtained and relatively inexpensive. In addition, it can be applied in a variety of ways, including dry surface applications, sprayed as a water-based slurry or tilled

into soil. Because AC is 100% C, it is non-toxic and can be used in areas where herbicides are not permitted.

One of the main questions of this study was whether carbon addition affects abundance or cover of exotic species. Although carbon addition did not have any significant effects on composition or total productivity, there were significant reductions in forb biomass for two growing seasons after application. Sugar amendments also reduced total cover, cover of forbs, overall spring species richness and plant basal area on treated plots. These results indicate that treatments with sugar, and to a lesser extent AC, do indeed affect abundance and cover of exotic species. These results indicate that sugar addition could have the potential to limit or reduce plant-plant competition, a condition that would be beneficial during restoration efforts. Such a decrease in competition could create a window of opportunity for introduction of desirable native species that might otherwise be unable to establish in a heavily invaded ecosystem. Recent research in Puget lowland prairies has indicated that treatment with sugar favors growth of transplanted native grasses and forbs (Kirkpatrick et al., this issue), making this an especially promising result. Combining sugar treatments with establishment of a diverse mixture of native prairie species could lead to improved native establishment and survival even in the presence of exotic invaders.

We also wanted to determine the longevity of carbon amendment effects on the plant community. Recent research indicates that addition of carbon to the soil surface is the most reliable method for altering C:N ratios (Perry et al. 2010) and that, regardless of the C source used, alterations to the soil lose effectiveness rapidly after cessation of treatment (Prober and Lunt 2009). Rapid loss of carbon effectiveness was not supported in our study, as some significant effects remained detectable in the second growing season after application.

Kirkpatrick et al. (2010), however, has demonstrated that these effects do diminish over time, and exotic species can experience a recovery and rebound within four years of sugar application, reaching or exceeding their original abundance. These findings suggest that sugar is relatively short-lived in the environment, and long-term reapplications may be necessary to fully control weeds. One potential advantage of the short-lived nature of sugar effects is that applications could be timed to weed or native phenology, or used in a targeted fashion during early stages of plant invasion as a preventative measure with low environmental impact.

We were also interested in whether or not sugar and AC, which alter soil C:N ratios in different ways, have similar effects on the plant community. AC had a more limited effect on the plant community than sugar did. This may indicate that AC takes longer to affect the plant community than sugar, that it affects a different suite of plant species (e.g., spring ephemerals), or that the method of application (dry addition to the surface) did not adequately introduce it to the system. The AC used in this study was of very fine particle size (<150 μm) and our study site experiences very high wind, which may have reduced the amount of AC penetrating the soil surface. Recent research has found that carbon amendments tilled directly into the soil are accessed more rapidly than those applied to the surface (Biederman and Whisenant 2009), which may explain the lack of observed effects for AC. This fact may make AC of limited use in areas where desirable plants are present, such as prairie remnants. However, it may be of utility in tilled fields or as a longer-lasting addition to applications of short-lived carbon amendments, as AC affects soil N through adsorption, rather than through stimulation of the microbial community. This duel-pronged approach my more effectively alter soil C:N ratios than sugar or AC alone.

AC may also offer other ecosystem services. AC, created from charcoal, is similar to biochar, a carbon source created by pyrolysis of plant material. Biochar can be either "inactive" (having a low porosity and ability to adsorb chemicals) or "activated" (highly porous and adsorptive). Biochar has been suggested as a method of increasing soil carbon sequestration (Glasser et al. 2002) and activated biochar has been shown to adsorb allelopathic chemicals (Kabouw et al. 2010), compounds which are often excreted by a number of aggressive invaders (Callaway and Ridenour 2004). Altering soil C:N ratios through the addition of AC or activated biochar could serve the dual purpose of exotic species reduction and increased soil storage of C.

Although we found that neither treatment affected grasses, a number of studies have found grasses to be most heavily impacted by carbon addition (e.g. Averette 2004, Eschen et al. 2007). It is possible that the rates used in this experiment were not high enough to have any effect on the grass community, as some research has shown that higher rates (e.g., 3346 g m^{-2} (Blumenthal et al. 2003)) of application yield greater results. Our study used identical rates to those in Eschen et al. 2006, who found a significant change in biomass allocation among grasses, but ours produced the opposite effect; grasses were unaffected, while forb biomass and cover were decreased. This may be due to the differences inherent in a field- versus greenhouse-based study. At present, no general recommendation for application rates is available for carbon addition (Perry et al. 2010), and a wide variety of rates have been used in previous studies (e.g., 84 – 3346 g m^{-2} (Blumenthal et al. 2003)).

Neither sugar nor AC showed any significant effect on species composition, total biomass, or the amount of bare ground present during the course of this experiment. These results indicate that carbon addition in the form of either sugar or AC likely narrowly targets forb species, while leaving grasses, the dominant contributors to biomass (Figure 2.1) unaffected.

This may be due to an increased need for N in herbaceous species compared to grasses, or a difference in timing of uptake of soil N. However, it seems unlikely that carbon addition causes complete mortality of affected forb species, as composition did not differ between treatment and control plots, and bare ground did not increase in response to carbon addition.

Temperature and precipitation differed in 2008 and 2009; 2009 was an anomalously hot and dry growing season (Table 2.2). It is very likely that these strong abiotic stresses overwhelmed any effect of carbon amendment in 2009. In addition, all significant main effects on the plant community were detected during spring measurements. This indicates that growth and establishment of exotic species impacted by changes in soil resources in spring, rather than summer.

Future research directions should include testing a variety of application methods for both sugar and AC such as tilling, spraying as a slurry, or repeated applications over a number of growing seasons. In addition, the effects of carbon addition on different plant life-stages should be investigated. Presently, the effects of carbon application on native prairie species are not well understood. Kirkpatrick et al. (this issue) found that sugar addition to native species had no effect on standing cover, including spring ephemerals, but found enhanced performance of native transplants in sugar treated plots within the first year. Four years post-treatment exotic species had rebounded to higher than previous levels of cover. Further studies should be conducted at sites with an intact prairie community in order to determine whether these trends are ubiquitous throughout Puget prairies.

Further research into the restoration utility of sugar should include combining sugar or AC application with spraying of a grass-specific herbicide like Fusilade. Such a treatment would mitigate the weak carbon effects on grass species, significantly reducing competition from

dominant invaders. Following reduction of exotic species, a diverse mixture of native species could be introduced through seeding (where bare mineral soil is exposed) or through plugging. This treatment sequence would allow native species to establish during periods of low competition for space and resources. Further control of exotic species through carbon addition may be necessary to maintain native dominance and prevent exotic species from rebounding to higher than pre-treatment levels (Kirkpatrick et al. this issue).

In conclusion, our research indicates that surface applications of sugar in particular can significantly reduce the biomass and cover of exotic forbs while simultaneously reducing overall species richness and plant basal area. AC was of more limited use for weed control. Both sugar and AC demonstrated effects on the plant community one year after application. The general applicability of sugar treatment makes it a useful tool for restoration projects facing invasion by exotic forbs. Further research is needed to determine what effect this treatment might have on desirable native forbs and how it might be integrated into restoration treatments.

Acknowledgements

We thank Laura Blume, Ryan Haugo, Andrea Blin, and Brandon Neuhaus for assistance with this research as well as Regina Rochefort, Janneke Hille Ris Lambers, Sarah Reichard and Soo-Hyung Kim for their advice and guidance. This project was supported by the National Park Service under task agreement J8W07070010.

References

Abraham, J. K., J. D. Corbin, and C. M. D'Antonio. 2009. California native and exotic perennial grasses differ in their response to soil nitrogen, exotic annual grass density and order of emergence. Plant Ecology 201:445-456.

Alpert, P. and J.L. Maron. 2000. Carbon addition as a countermeasure against biological invasion by plants. Biological Invasions 2:33-40.

Anderson, M.J. 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecology 26: 32-46.

Anderson, M. J., R. N. Gorley and K. R. Clarke. 2008. PERMANOVA+ for PRIMER: Guide to software and statistical methods. PRIMER-E, Plymouth, United Kingdom.

Averett, J. M., R. A. Klips, L. E. Nave, S. D. Frey, and P. S. Curtis. 2004. Effects of soil carbon amendment on nitrogen availability and plant growth in an experimental tallgrass prairie restoration. Restoration Ecology 12:568-574.

Bakker, J. D. 2008. Increasing the utility of indicator species analysis. Journal of Applied Ecology. 45: 1829-1835.

Biederman, L. A., and S. G. Whisenant. 2009. Amendment placement directs soil carbon and nitrogen cycling in severely disturbed soils. Restoration Ecology no. doi: 10.1111/j.1526-100X.2009.00564.x.

Bleier, J. S., and R. D. Jackson. 2007. Manipulating the quantity, quality and manner of C addition to reduce soil inorganic N and increase C4:C3 grass biomass. Restoration Ecology 15:688-695.

Bobbink, R. 1998. The effects of air-borne nitrogen pollutants on species diversity in natural and semi-natural European vegetation. Journal of Ecology 86:717-738.

Boyd, R. 1999. Indians, Fire and the Land in the Pacific Northwest. Oregon State University Press, Corvallis, OR.

Blumenthal, D. M., N. R. Jordan, and M. P. Russelle. 2003. Soil carbon addition controls weeds and facilitates prairie restoration. Ecological Applications 13:605-615.

Blumenthal, D. M. 2009. Carbon addition interacts with water availability to reduce invasive forb establishment in a semi-arid grassland. Biological Invasions 11:1281-1290.

Chappell, C. B., M. S. Mohn Gee, B. Stephens, R. Crawford, and S. Farone. 2000. Distribution and decline of native grasslands and oak woodlands in the Puget Lowland and Willamette Valley ecoregions, Washington. *In* S.H. Reichard, P. Dunwiddie, J. Gamon, A. Kruckelberg and D. Salstrom (editors), Washington Native Plant Society, Seattle.

Clark, C. M., E. E. Cleland, S. L. Collins, J. E. Farigione, L. Gough, K. L. Gross, S. C. Pennings, K. N. Suding, and J. B. Grace. 2007. Environmental and plant community determinants of species loss following nitrogen enrichment. Ecology Letters 10:596-607.

Corbin, J. D., and C. M. D'Antonio. 2004. Can carbon addition increase competitiveness of native grasses? A case study from California. Restoration Ecology 12:36-43.

Dukes, J. S. 2001. Biodiversity and invasibility in grassland microcosms. Oecologia 126:563-568.

Dunwiddie, P., E. Alverson, A. Stanely, R. Gilbert, S. Pearson, D. Hays, J. Arnett, E. Delvin, D. Grosboll, and D. Marschner. 2006. The vascular plant flora of the south Puget Sound prairies, Washington, USA. Davidsonia 17:51-69.

Eschen, R., H. Muller-Scharer, and U. Schaffner. 2006. Soil carbon addition affects plant growth in a species-specific way. Journal of Applied Ecology 43:35-42.

Eschen, R., S. R. Mortimer, C. S. Lawson, A. R. Edwards, A. J. Brook, J. M. Igual, K. Hedlund,

and U. Schaffner. 2007. Carbon addition alters vegetation composition on ex-arable fields. Journal of Applied Ecology 44:95-104.

Goldberg, D., and A. Novoplansky. 1997. On the relative importance of competition in unproductive environments. Journal of Ecology 85:409-418.

Haubensak, K.A. and I. M. Parker. 2004. Soil changes accompanying invasion of the exotic shrub *Cytisus scoparius* in glacial outwash prairies of western Washington (USA). Plant Ecology 175:71-79.

Hegarty, J., D. Zabowski, and J. D. Bakker. 2011. Use of soil properties to determine the historical extent of two western Washington prairies. Northwest Science 85:

Hobbs, N. T. 1996. Modification of ecosystems by ungulates. Journal of Wildlife Management. 60:695-713.

Hooper, D. U., and J. S. Dukes. 2010. Functional composition controls invasion success in a California serpentine grassland. Journal of Ecology 98:764-777.

Iannone, B. V., and S. M. Galatowitsch. 2008. Altering light and soil N to limit *Phalaris arundinacea* reinvasion in sedge meadow restorations. Restoration Ecology 16:689-701.

Kaushik, S., and Inderjit. 2007. *Oryza sativa* straw restricts *Phalaris minor* growth: allelochemicals or soil resource manipulation? Biology and Fertility of Soils 43:557-563.

Kirkpatrick, H. E. and K. C. Lubetkin. 2011. Response to sucrose addition by native and introduced plant species in Puget lowland prairies. Northwest Science 85:

Kolb, A., P. Alpert, D. Enters, and C. Holzapfel. 2002. Patterns of invasion within a grassland community. Journal of Ecology 90:871-881.

Kabouw, P., M. Nab, and N. M. van Dam. 2010. Activated carbon addition affects substrate pH and germination of six plant species. Soil Biology and Biochemistry 42:1165-1167.

Kulmatiski, A., and K. H. Beard. 2006. Activated carbon as a restoration tool: Potential for control of invasive plants in abandoned agricultural fields. Restoration Ecology 14:251-257.

Liao, C. Z., R. H. Peng, Y. Q. Luo, X. H. Zhou, X. W. Wu, C. M. Fang, J. K. Chen, and B. Li. 2008. Altered ecosystem carbon and nitrogen cycles by plant invasion: a meta-analysis. New Phytologist 177:706-714.

Mangold, J. M., and R. L. Sheley. 2008. Controlling performance of bluebunch wheatgrass and spotted knapweed using nitrogen and sucrose amendments. Western North American Naturalist. 68:129-137.

Maron, J. L., and P. G. Connors. 1996. A native nitrogen-fixing shrub facilitates weed invasion. Oecologia 105:302-312.

McArdle B.H and M.J. Anderson. 2001. Fitting multivariate models to community data: a comment on distance-based redundancy analysis. Ecology 82: 290-297.

Morgan, J. P. 1994. Soil impoverishment: a little-known technique holds promise for establishing prairie. Restoration Management Notes: 12:55-56.

Morghan, K. J. R., and T. R. Seastedt. 1999. Effects of soil nitrogen reduction on nonnative plants in restored grasslands. Restoration Ecology 7:51-55.

National Park Service. 2006. Ebey's Landing National Historical Reserve Final General Management Plan and Environmental Impact Statement. National Park Service, Seattle.

Perry, L. G., D. M. Blumenthal, T. A. Monaco, M. W. Paschke, and E. F. Redente. 2010. Immobilizing nitrogen to control plant invasion. Oecologia 163:13-24.

Pokorny, M. L., R. L. Sheley, C. A. Zabinski, R. E. Engle, J. J. Svejcar, and J. J. Borkowski. 2005. Plant functional group diversity as a mechanism for invasion resistance. Restoration

Ecology 13:448-459.

Prober, S. M., and I. D. Lunt. 2009. Restoration of *Themeda australis* swards suppresses soil nitrate and enhances ecological resistance to invasion by exotic annuals. Biological Invasions 11:171-181.

Rashid, I., and Z. Reshi. 2010. Does carbon addition to soil counteract disturbance-promoted alien plant invasion? Tropical Ecology 51:339-345.

Seabloom, E. W., W. S. Harpole, O. J. Reichman, D. Tilman. 2003. Invasion, competitive dominance, and resource use by exotic and native California grassland species. Proceedings of the National Academy of Sciences of the United States of America. 23:13,384-13,389.

Shabin, J. and J. H. Myers. 2010. Relationships between Scotch broom (*Cytisus scoparius*), soil nutrients and plant diversity in the Garry oak savannah ecosystem. Plant Ecology 207:81-91.

Sheley, R. L., and M. F. Carpinelli. 2005. Creating weed-resistant plant communities using niche-differentiated non-native species. Rangeland Ecology and Management 58:480-488.

Suding, K. N., S. L. Collins, L. Gough, C. Clark, E. E. Cleland, K. L. Gross, D. G. Milchunas, and S. Pennings. 2005. Functional- and abundance-based mechanisms explain diversity loss due to N fertilization. Proceedings of the National Academy of Sciences of the United States of America 102:4387-4392.

Tilman, D., and D. Wedin. 1991. Dynamics of nitrogen competition between successional grasses. Ecology 72:1038-1049.

Tilman D. 1999. The ecological consequences of changes in biodiversity: a search for general principles. Ecology 80:1455-1474.

Vinton, M. A., and E. M. Goergen. 2006. Plant-soil feedbacks contribute to the persistence of *Bromus inermis* in tallgrass prairie. Ecosystems 9:967-976.

Vitousek, P. M., J. D. Aber, R. W. Howarth, G. E. Likens, P. A. Matson, D. W. Schindler, W. H. Schlesinger, and G. D. Tilman. 1997. Human alteration of the global nitrogen cycle: Sources and consequences. Ecological Applications 7:737-750.

Wedin, D., and D. Tilman. 1993. Competition among grasses along a nitrogen gradient – initial conditions and mechanisms of competition. Ecological Monographs 63:199-229.

Weiser, A., and D. Lepofsky. 2009. Ancient land use and management of Ebey's Prairie, Whidbey Island, Washington. Journal of Ethnobiology 29:184-212.

Zink, T. A., and M. F. Allen. 1998. The effects of organic amendments on the restoration of disturbed coastal sage scrub habitat. Restoration Ecology 6:52-58.

Table 2.1 Results of PERMANOVA models testing the effects of Block, Carbon addition, Year, and the Carbon x Year interaction on biomass, cover, richness, composition, bare ground and plant basal area in spring and summer. The pseudo-*F* (*F*) and permuted *P*-values (*P*) are reported for each factor along with the R^2 for the overall model. Significant effects ($\alpha = 0.05$) are in bold. Composition was a multivariate response and analyzed using Bray-Curtis distances; all other responses were univariate and analyzed using Euclidean distances. Each model was assessed using 9999 permutations.

	Block (df = 5, 25)		Carbon (df = 2, 25)		Year (df = 1, 25)		Carbon x Year (df = 2, 25)		R^2
	F	*P*	*F*	P	*F*	P	*F*	P	
Biomass									
Total	13.1	**<0.001**	1.7	0.205	0.2	0.634	1.6	0.231	0.257
Biomass Forb	7.0	**<0.001**	6.0	**0.005**	1.0	0.331	0.2	0.808	0.339
Biomass Grass	14.8	**<0.001**	0.1	0.924	3.5	0.067	0.8	0.453	0.240
Cover									
Spring Total	3.1	**0.024**	1.4	0.256	3.1	0.095	3.6	**0.044**	0.464
Summer Total	4.9	**0.002**	2.3	0.125	81.9	**<0.001**	3.8	**0.038**	0.173
Spring Forb	6.8	**<0.001**	3.6	**0.044**	2.9	0.103	7.3	**0.002**	0.297
Summer Forb	10.2	**<0.001**	0.5	0.617	53.5	**<0.001**	2.6	0.090	0.184
Spring Grass	12.7	**<0.001**	0.3	0.738	13.3	**0.001**	0.6	0.583	0.241
Summer Grass	16.5	**<0.001**	1.6	0.217	185.8	**<0.001**	1.0	0.398	0.083
Richness									
Spring	7.8	**<0.001**	5.0	**0.015**	9.3	**0.005**	0.3	0.756	0.297
Summer	3.2	**0.025**	2.3	0.115	224.7	**<0.001**	1.2	0.305	0.091
Bare Ground									
Spring	2.3	**0.063**	2.2	0.129	2.3	0.145	0.2	0.843	0.571
Summer	1.4	0.239	0.3	0.788	8.3	**0.005**	0.01	0.926	0.609
Basal Area									
Spring	6.2	**<0.001**	0.2	0.824	21.27	**<0.001**	6.5	**0.006**	0.275

Table 2.2 Temperature and precipitation data for Coupeville, Whidbey Island, Washington. Long-term normals are from 1971-2000 and are reported as mean (standard deviation). Spring is defined as March-May, and summer as June-August (Western Regional Climate Center).

	Normal (1971-2000)		2008		2009	
	Spring	Summer	Spring	Summer	Spring	Summer
Average Temperature (C°)	9.6 (±1.2)	15.6(±0.9)	9.1	15.3	9.5	16.9
Total Precipitation (cm)	4.5(±1.9)	2.7(±1.7)	5.5	3.4	4.7	1.1

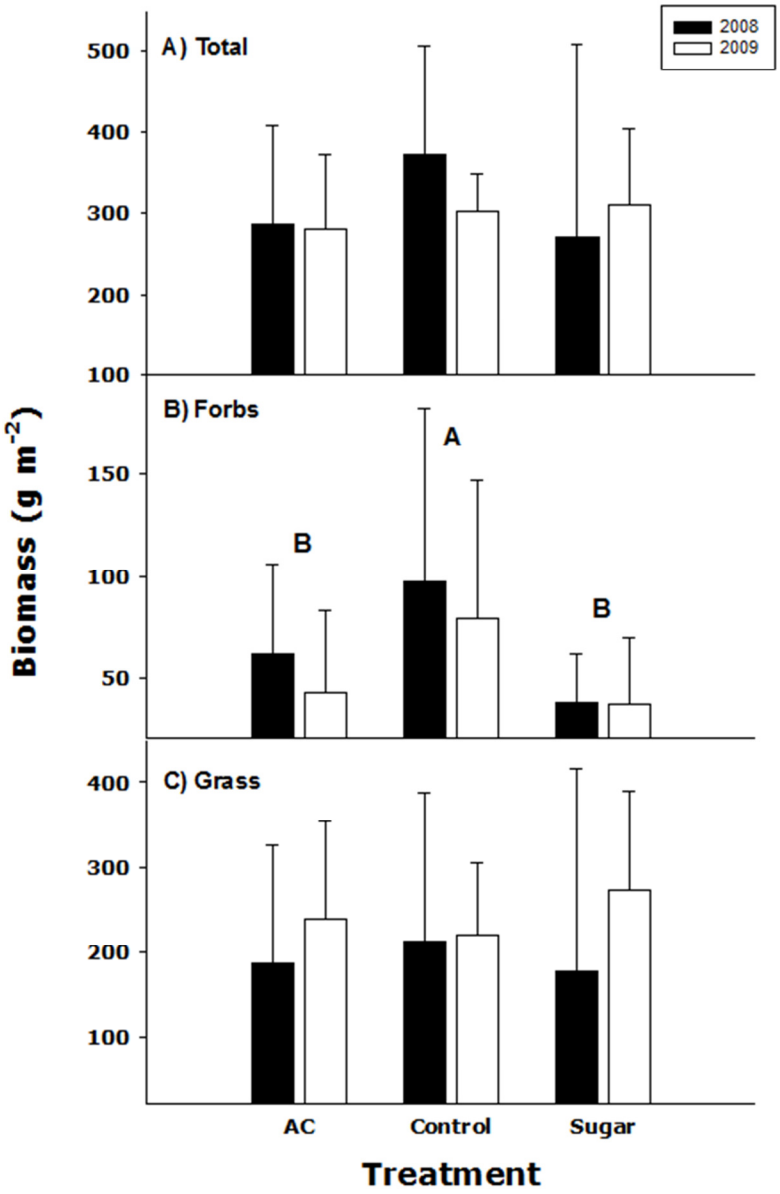

Figure 2.1 Aboveground biomass (mean + standard deviation) for A) all plants (total), B) forbs, and C) grass in plots treated with activated carbon (AC), sugar, or left untreated (control) in 2008 and 2009. PERMANOVA results are summarized in Table 1. Significant carbon effects are indicated by uppercase letters.

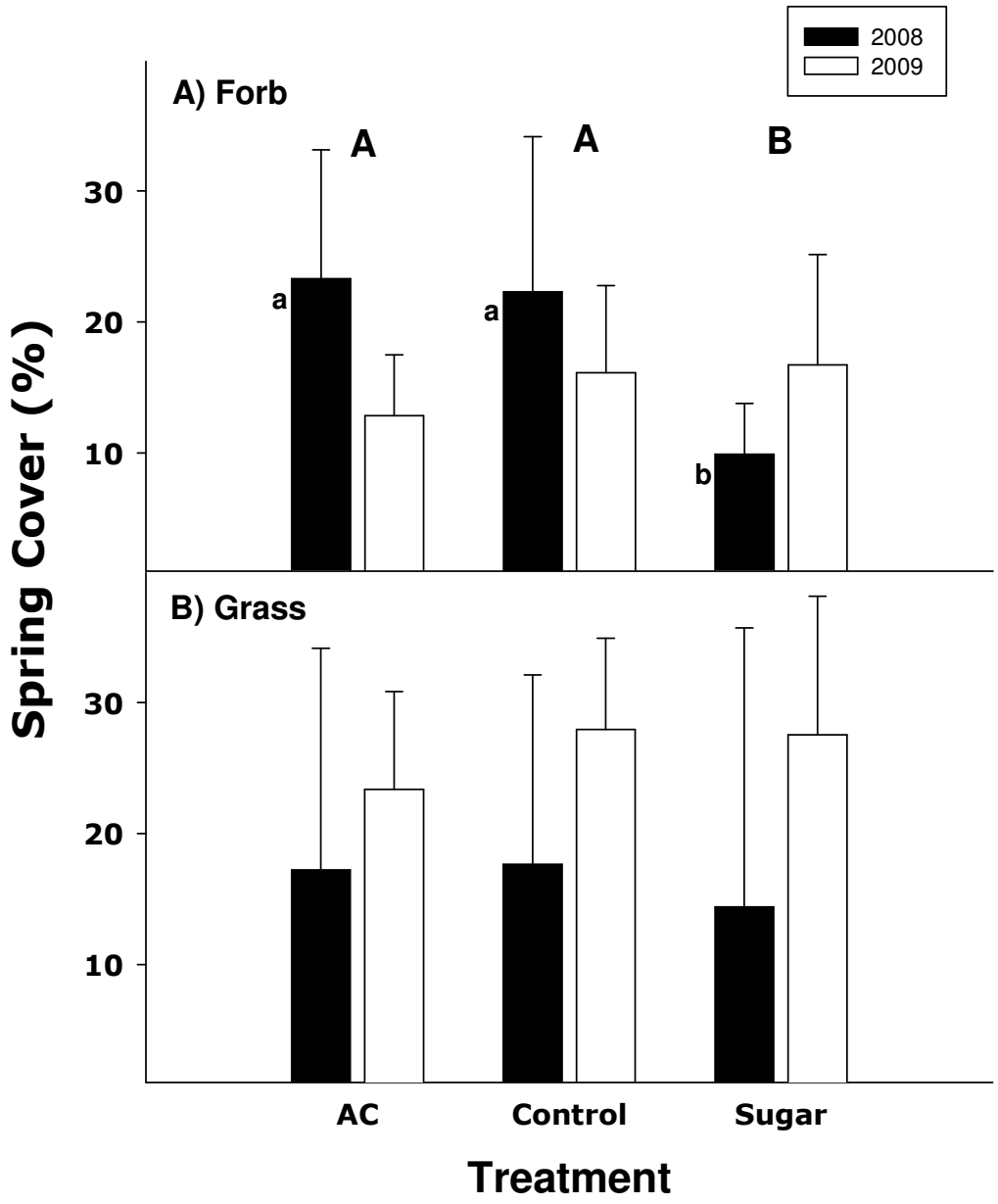

Figure 2.2 Spring (mean + standard deviation) of A) forb cover and B) grass cover in plots treated with activated carbon (AC), sugar, or left untreated (control) in 2008 and 2009. PERMANOVA results are summarized in Table 1. Significant carbon effects are indicated by uppercase letters. Significant Carbon x Year interactions were followed by analysis of each year individually; within years, lowercase letters indicate treatments that differ.

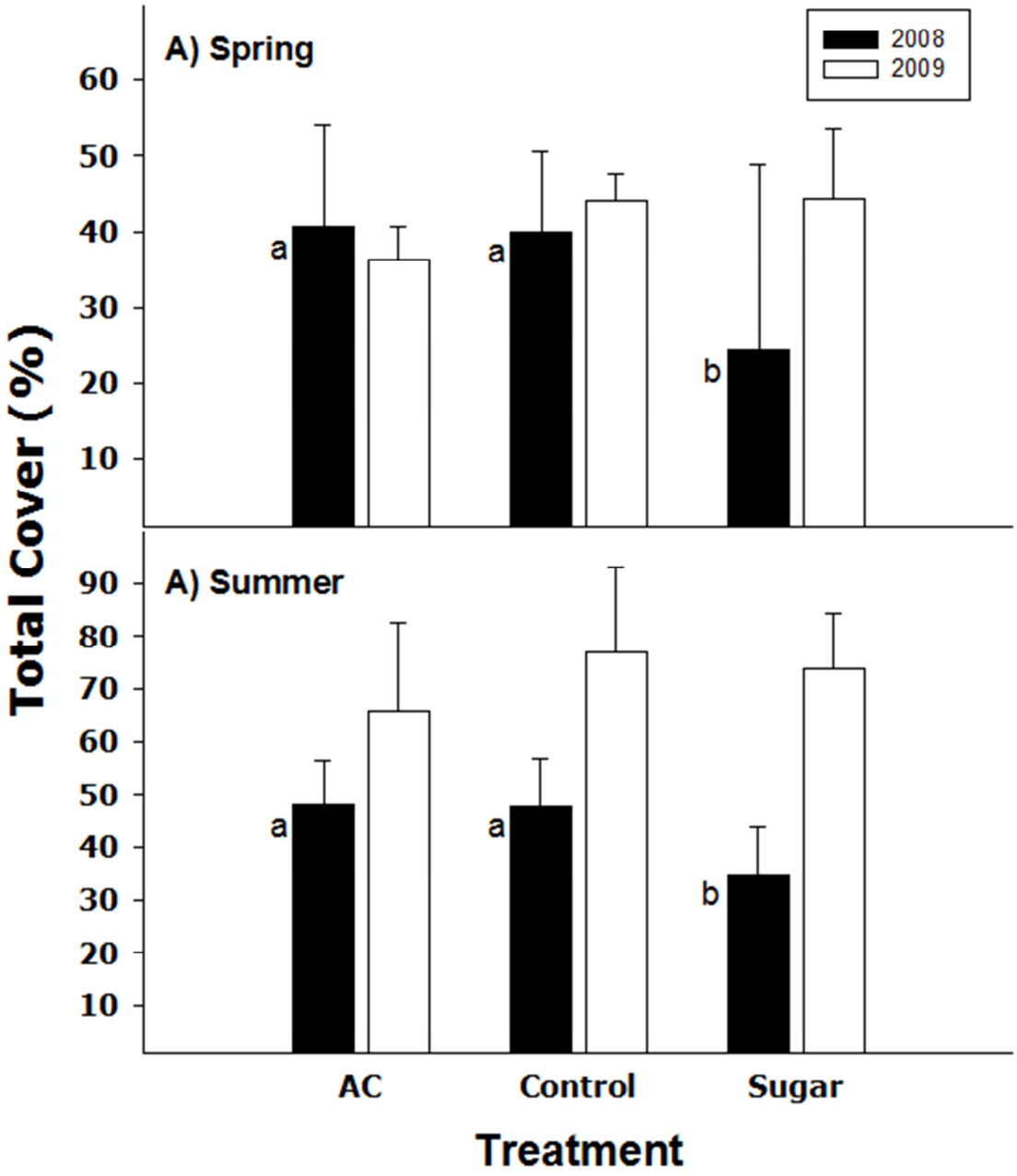

Figure 2.3 Spring and summer (mean + standard deviation) total cover in plots treated with activated carbon (AC), sugar, or left untreated (control) in 2008 and 2009. PERMANOVA results are summarized in Table 1. Significant Carbon x Year interactions were followed by analysis of each year individually; within years, lowercase letters indicated treatments that differ. Spring results were only marginally significant ($P = 0.09$).

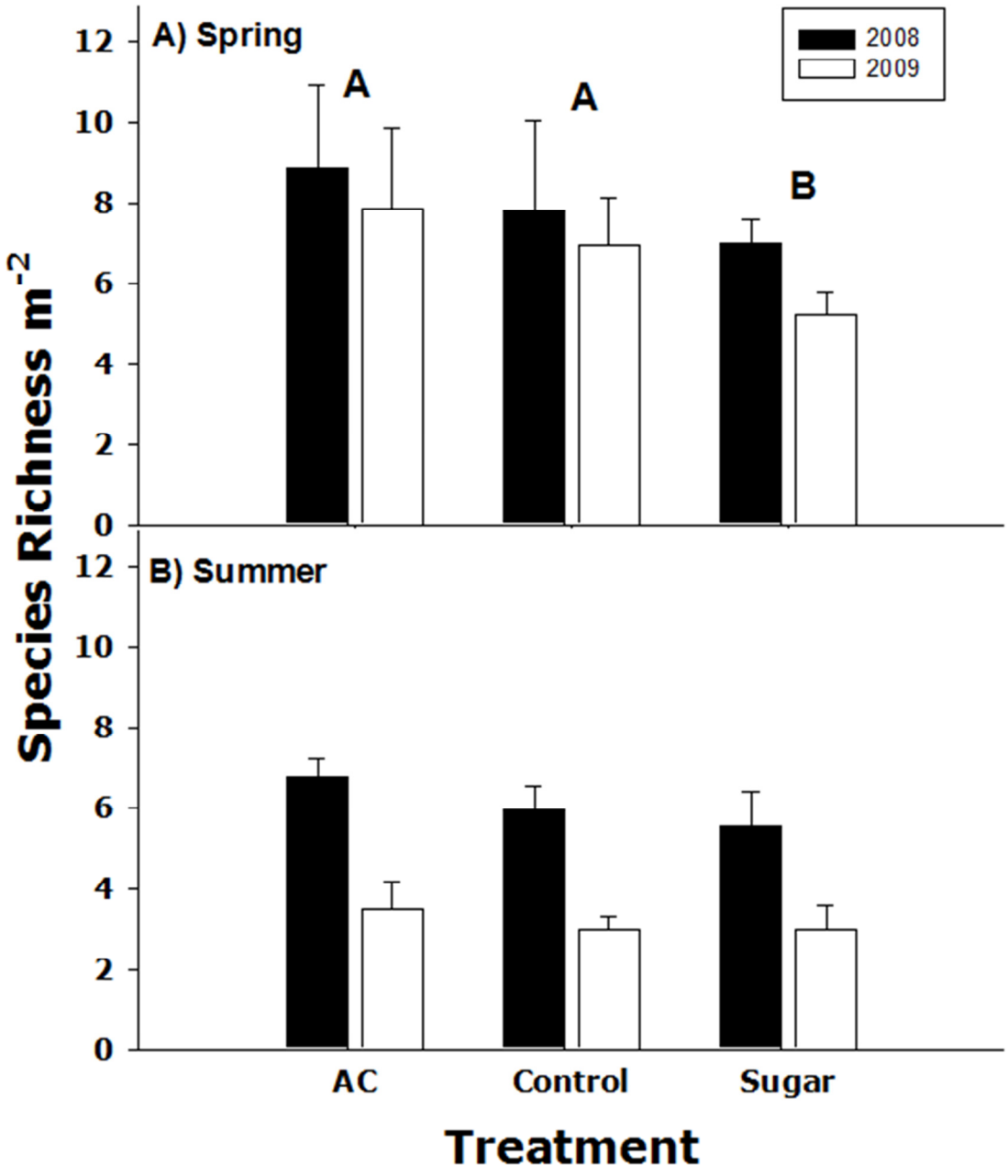

Figure 2.4 Species richness (mean + standard deviation) for plots treated with activated carbon (AC), sugar, or left untreated (control) in A) spring and B) summer. PERMANOVA results are summarized in Table 1. Significant carbon effects are indicated by uppercase letters.

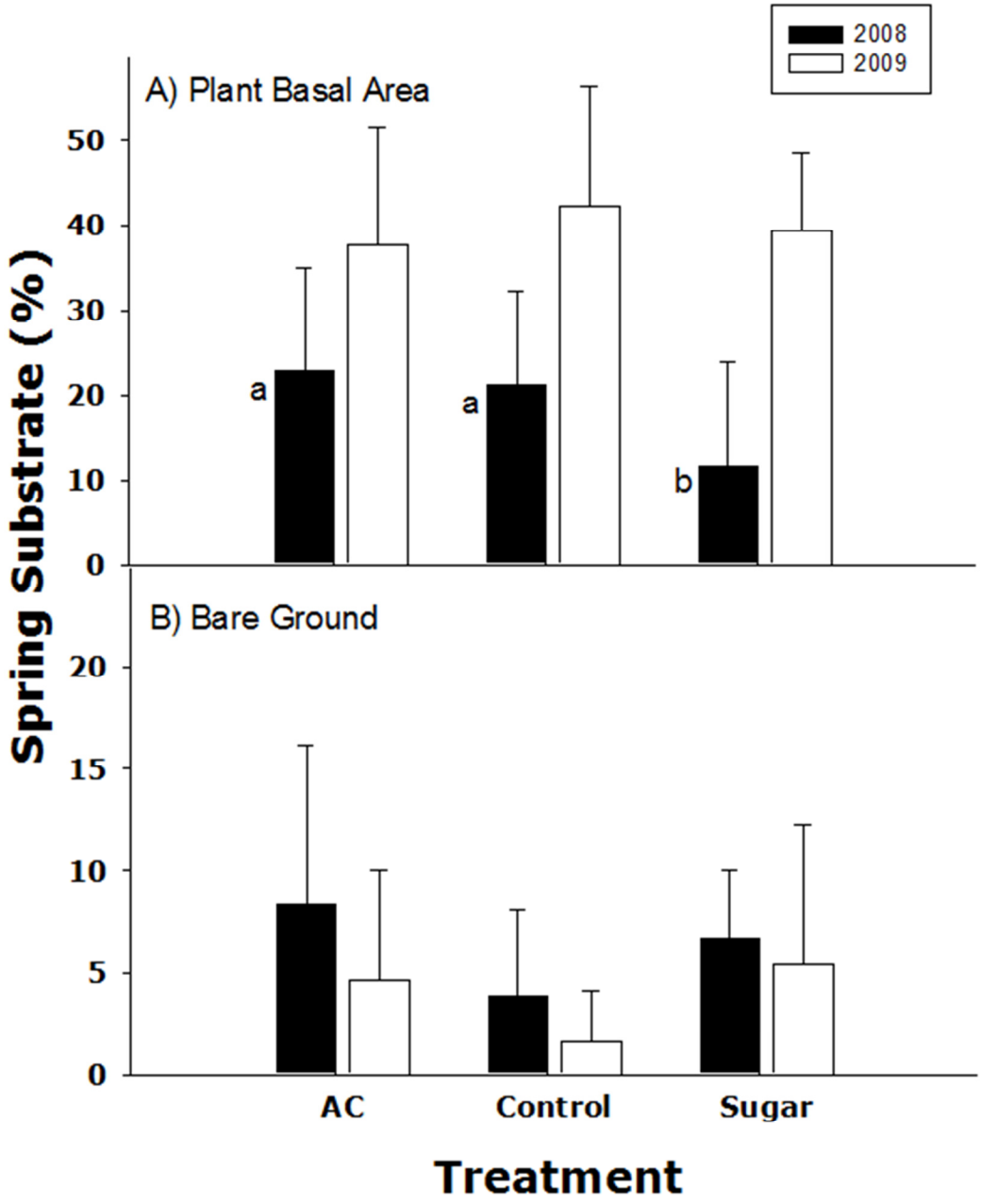

Figure 2.5 A) Plant basal area and B) bare ground (means and standard deviations) for plots treated with activated carbon (AC), sugar or left untreated (control) in spring 2008 and 2009. PERMANOVA results are summarized in Table 1. Significant Carbon x Year interactions were followed by analysis of each year individually; within years, lowercase letters indicate treatments that differ.

Chapter 3: Quantifying and Comparing Intraspecific Functional Trait Variability: A Case Study with *Hypochaeris radicata*

SUMMARY

1. Intraspecific trait variation is an important source of variability in populations, species, and communities, but the ways in which this variation is quantified and compared have not been rigorously assessed.
2. We reviewed the last 5 years of ecological literature quantifying intraspecific variation (64 published studies) in order to identify commonly applied statistical methods. Analysis of Variance techniques (n=43) were the most commonly applied. Levene's tests (n=14), linear techniques (both general and generalized models) (n=12) and mixed effects modeling (n=9) were also used. Qualitative comparisons of plant phenotype using descriptive statistics (n=10) and coefficients of variation (n=8) were also applied. Bayesian analysis was used in a single study.
3. We compared the efficacy and interpretation of analysis of variance, tests for homogeneity of variance, qualitative comparisons, mixed effects models, and Bayesian hierarchical modeling in a case study measuring variation in specific leaf area and rosette diameter using 10 populations (n=241 individuals) of *Hypochaeris radicata*. We also examined whether database- and literature-based trait values provided a good estimates for measured populations.
4. Our results indicate intraspecific variation is substantial. Significant differences existed in both means and variation across populations for both measured traits, and the choice of statistical technique influenced the interpretation of these results. There was a 27-fold variation in specific leaf area (1.7 to 46.1 mm^2/mg) and a 34-fold variation in rosette diameter (1.7 cm to 59.1 cm). Only Bayesian estimates were able to simultaneously

quantify and compare variation within and across populations, as well as estimating trait values and variation on a larger, regional scale. Literature-based trait values had poor fit for 4 of 10 populations, and differed from the estimated regional trait distribution.

5. *Synthesis.* Intraspecific variation in measured plant functional traits is significant. Although both classical and Bayesian techniques yielded similar results, Bayesian techniques were more sensitive to differences in intraspecific variation, could simultaneously examine variation within and across populations, could estimate regional trait distributions, and did not require that the assumption of heterogeneity of variance be met. Bayesian techniques, and hierarchical models in particular, represent a powerful analytical tool for studies of intraspecific variation.

Key-Words: hierarchical Bayesian analysis, intraspecific variation, mixed effects models, PERMANOVA, PERMDISP, plant functional traits, rosette diameter, specific leaf area

INTRODUCTION

Over the past several decades, plant ecology has moved from a reliance on strictly taxonomic classification of species towards the inclusion of functional trait-based methods (Calow 1987, Cadotte et al. 2011). In describing plants by their functional traits, which indirectly or directly measure fitness, rather than their taxonomy, a fuller understanding of plant community ecology has emerged. Understanding of global trends in plant traits (Diaz et al., 2004; Moles & Westoby, 2003; Moles et al., 2009; Wright et al., 2005), the effects of abiotic filters on trait community assembly (Diaz et al. 1998, Maharjan et al. 2011), and definitions of plant strategies (Laughlin et al., 2010; Westoby, 1998) have all been extended due in part to a functional trait based approach. Definitions of diversity have also expanded to include the array of functional traits within ecosystems and landscapes (Petchey and Gaston 2002).

Much of the research about functional traits has focused on differences between species. This emphasis on interspecific variation is reflected in a commonly applied definition of a "useful" trait in community ecology: one which shows high reproducibility, or low variation, within a species (McGill et al. 2006). While traits such as photosynthetic pathway are fixed and do not vary within species, recent research has shown that other traits can vary substantially (Karley and Hawes 2008, Lecerf and Chauvet 2008, Violle et al. 2009, Albert et al. 2010b), and that this trait variation can have important implications. Trait variation influences species coexistence (Jung et al. 2010a, Clark 2010a, Pruitt and Ferrari 2011, Long et al. 2011), other trophic levels (Karley and Hawes 2008, Ruhnke et al. 2009, Lankau 2011), and ecosystem functions, (Pontes et al. 2007, Lecerf & Chauvet 2008) and is an important factor in the calculation of functional diversity (Cianciaruso et al. 2009, Albert et al. 2012).

Although defined generically as the variation within a species, intraspecific variation can be studied at different levels, e.g. within and across populations. While much trait variation can be linked to genetic differences (Begg et al. 2012), the levels of variation can also reflect different mechanisms. Variation within populations may reflect seasonal differences (Al et al. 2005, Prior et al. 2005, Nouvellon et al. 2010) and ontogeny (Smith et al. 2011). Variation among populations may reflect climatic and resource gradients (Thuiller et al. 2004, Baruch 2011, Long et al. 2011), disturbance history (Kyle and Leishman 2009, Da Silveira Pontes et al. 2010, Mason et al. 2011), and niche partitioning due to differing community structure (Gubsch et al. 2011, Roscher et al. 2011).

The fact that intraspecific variation can be studied at different levels requires careful decisions about how data are collected, analyzed, and interpreted. However, despite increased interest in intraspecific variation, the way in which this variation is quantified has not been rigorously assessed. In this paper, our objectives were to compare the efficacy and interpretability of methods used to quantify intraspecific variation, and to assess the utility of database derived trait values. We conducted a literature review documenting current methods of quantifying and comparing intraspecific variation, and then used six methods to quantify variation within and among populations of *Hypochaeris radicata*, a widespread weed. We focused on two traits, and posed three questions: 1) How much trait variation exists within and between populations of *H. radicata*? 2) Do different analytical techniques deliver different results or interpretations, and what are their strengths and limitations? 3) Are literature-based trait means an accurate representation of trait values? We hypothesized that significant intraspecific variation exists within and across populations of this species, and that different

analytical techniques would yield different results. Furthermore, we hypothesized that the predicted high variation in plant traits would make literature-based trait means inaccurate.

CHARACTERIZING INTRASPECIFIC VARIATION

We performed a literature review to identify the analytical methods used to quantify and compare intraspecific variation. These studies typically sought to directly quantify the intraspecific variation displayed within a population, compare traits across populations within species, or both. We searched the Thompson-Reuters Web of Science database (Thompson-Reuters, USA) for the term "intraspecific variation" (including wildcard terms such as vari*, var* and intra*) in articles published in peer reviewed journals between January 2007 and October 2012. The full text of each article was searched for these terms. Studies were then reviewed to determine whether they were appropriate in scope and focus. We limited our review to studies that focused on plants, and were drawn from plant ecology, plant evolution, forestry and agricultural journals. Sixty-four articles fit these criteria.

We reviewed each article to determine the method or methods used to analyze or interpret intraspecific variation. Most studies included a statistical test, usually an analysis of variance (ANOVA) or other linear models of some sort (43 and 12 of 64, respectively). Fourteen studies explicitly stated that a Levene's test was used when appropriate, and nine studies applied mixed effects models. One study used Bayesian analysis of variance. Roughly one third of studies (20 of 64) did not conduct statistical tests of intraspecific variation but summarized it using descriptive statistics such as standard errors, standard deviations, or differences in average size. Eight studies compared coefficients of variation (Fig. 3.1).

When evaluating the suitability of these techniques, it is important to recognize their assumptions, strengths, and weaknesses (Table 3.1). Descriptive statistics used to characterize intraspecific variation include the standard deviation (SD), standard error (SE), and coefficient of variation (CV). While these measures provide useful information about the amount of variation present in populations, they are sensitive to sampling errors and methodological problems. Qualitative comparisons and reporting of plant trait values may provide a record of useful information for other trait-based analytical approaches. However, if these summaries are not accompanied by statistical tests it is impossible to determine whether intraspecific trait variation was significant.

Linear models were the most common method of analyzing intraspecific variation. Classical ANOVA compares populations based on the ratio of variation among and within populations. While this approach identifies differences among populations, mean trait values do not necessarily indicate, or correlate with, the degree of variation expressed in each population, and no inference about variation within populations can be made from trait means. In addition, ANOVA and linear models assume that residuals of the data are normally distributed; deviations from this assumption can bias the results of such tests. Other distributional forms can be analyzed via generalized linear models, or via permutational tests such as PERMANOVA (Anderson 2001) that make no assumptions about the distributional form of the data. Finally, these techniques assume homogeneity of variance, yet heterogeneous variance among populations may be one way that intraspecific variation is manifested.

It is well known that statistically significant ANOVA results can indicate differences among populations in mean values, heteroscedasticity, or both. Differences in variance among populations can be detected with a test of homogeneity of variance (e.g. Levene's test), though

this test is typically used diagnostically rather than inferentially and is frequently not reported in the literature (only 14 of 64 studies reported diagnostic testing for equal variance). In addition, while this method can indicate which populations differ in variance, it does not provide a quantitative measure of the distribution of measured traits. A permutational equivalent of Levene's test has been developed (PERMDISP; (Anderson 2004) that tests for differences in dispersion among populations, although this test suffers the same weaknesses of the traditional Levene's test.

Mixed effects models relax many of the assumptions of more commonly applied techniques, are appropriate for complex, non-normal data, and include hierarchical models that can accommodate and estimate variation across multiple levels of organization (Zuur et al. 2009). The use of these models is growing in popularity in ecological studies (Bolker et al. 2009), and mixed effects models have been suggested for quantifying intraspecific trait variation (Albert et al. 2011). While mixed effects models do allow for an estimation of variance, they are unable to provide parameters for comparison between individual populations; such comparisons are necessary when identifying more- or less-variable populations.

Bayesian analysis of variance was used in a single study (Fig. 3.1). Bayesian parameter estimation and hierarchical models provide a flexible tool for addressing many ecological questions (Ellison 2004). They can simultaneously estimate trait distributions and variation within and across populations, as well as estimate trait distributions for the entire sample region. In addition, Bayesian parameter estimation is not constrained by assumptions of homogeneity of variance. An additional unique feature of Bayesian techniques is that prior information can be incorporated into models in the form of an "informative" prior probability. The ability to use prior information – in the form of previously acquired data, expert opinion, or literature-based

values - can strengthen models and reduce the time and cost associated with data collection (Kuhnert et al. 2010). However, informative prior probabilities also draw the largest criticisms from those skeptical of Bayesian techniques (Lele and Dennis 2009), and should be implemented cautiously and appropriately (Choy et al. 2009).

CASE STUDY

Hypochaeris radicata is a short-lived perennial herb species common in both intact and degraded grassland ecosystems throughout Europe, North America, Australia, New Zealand and Japan. It forms basal rosettes with oblong-lanceolate leaves and produces several upright, branching flower stalks during a long flowering period – June through October in the Pacific Northwest. *H. radicata* is self-incompatible (Pico et al. 2004) and its seeds are wind dispersed up to a few hundred meters (Mix et al. 2006).

Trait Measurements

In Summer 2011 and Spring 2012, specific leaf area (SLA) and basal rosette diameter were measured on 241 individuals in 10 populations, including one greenhouse grown population. Populations were at least 1 km apart, and spanned a 480 km range from Corvallis, Oregon to Coupeville, Washington. Field populations were located in unmowed areas that received full to partial sun, and included urban parks, roadsides, and glacial-outwash grasslands. In this article, populations are numbered from north to south in terms of their collection locations (see Fig. S1 and Table S1 in Appendix A).

SLA is the ratio of leaf area to leaf mass. It is a widely used functional trait (Wilson et al. 1999) because it is easily measurable for a variety of plant species, and is correlated with

exploitation of available resources, productivity, leaf life-span, and leaf quality (Reich et al. 1992, Westoby 1998). Plants with low SLA typically have longer-lived leaves and slower overall relative growth rates (Poorter 1990) because SLA influences the total amount of area available for photosynthetic capture. High SLA plants, in contrast, typically have short-lived leaves with high photosynthetic efficiency (Poorter 1998). SLA is also an important determinant of decomposition rates and nutrient return (Laughlin 2011); high SLA leaves break down more quickly. SLA is partially determined by genetic factors (Scheepens et al. 2010), but can also be affected by soil nutrient availability (Knops and Reinhart 2000) and light availability (Meziane and Shipley 1999). A mature, fully exposed, undamaged leaf was collected from each individual. Leaves were stored in whirl-pak plastic bags at 4°C for no more than 48 hours and then rehydrated following Cornelissen et al. (2003). After rehydration, leaves were scanned in a flatbed scanner at 200 dpi, dried at 60°C for seven days, and weighed. One-sided leaf area was calculated from the scanned images using ImageJ version 1.45 (available at http://rsb.info.nih.gov/ij). SLA was calculated as leaf area (mm^2) divided by dry leaf mass (mg).

Basal rosette diameter was used as a measure of plant competitive ability. Plant height is more commonly used in this sense (Westoby 1998), but is less relevant for *H. radicata* because this species has a very flat, impenetrably dense growth form, and competes for light via physical exclusion rather than height growth. Devotion of resources to rosette diameter is assumed to be analogous to resource devotion to vertical height for other species: larger sizes represent greater access to light and greater carbon assimilation. For each individual, rosette diameter was calculated as the average of two measurements in perpendicular directions.

We searched the literature and the LEDA Trait Database (Kleyer et al. 2008) for published data about SLA and rosette diameter in *H. radicata*. We obtained four published mean

values for SLA, three from Mokany & Ash (2008) and one from Kleyer et al. (2008). We did not find any literature-based values for rosette diameter. Literature-based values were compared with the posterior distributions produced from a Bayesian ANOVA of our field-measured values.

Statistical Analysis

Based on our literature review, we selected six methods of characterizing intraspecific variation: qualitative comparisons, including comparisons of coefficients of variation, ANOVA-based techniques, tests of homogeneity of variance, linear mixed models, and Bayesian ANOVA. Qualitative comparisons were simple summaries of the data in terms of the extreme values, means, and standard deviations. Coefficients of variation were calculated as the standard deviation divided by the mean for each trait in each population.

SLA was log-normally distributed while rosette diameter was normally distributed. Levene's test on transformed data indicated that both traits exhibited non-homogeneous variance between populations. Since data did not meet this key assumption of ANOVA, we used permutational analysis of variance (PERMANOVA) with post-hoc pairwise comparisons to test for differences among populations. Homogeneity of variance was tested using PERMDISP, a permutational analog to Levene's test. These analyses was conducted using the PERMANOVA+ add-on to PRIMER-E (Clarke & Gorley, 2006), with the Euclidean distance measure, type III sums of squares, and 9,999 permutations.

We fit linear mixed effects models using REML estimation with population designated as a random effect to quantify variation between and within populations. SLA data were transformed to meet assumptions of normality and each trait was modeled separately. Analyses

were conducted using the function "lmer" in the lme4 package for R 2.14.2 (R Development Core Team 2011).

Bayesian ANOVA provides a means of estimating the probability distributions of parameters of interest, including means and standard deviations. Our analysis methods are summarized here but more detailed methods are provided in Appendix S1, since we found this technique to be particularly powerful yet rarely used. In its most basic form, Bayesian statistics can be summarized as:

$$\text{Posterior distribution } \alpha \text{ Likelihood} \times \text{Prior distribution} \quad \textbf{(eqn 1)}$$

From the posterior distribution, point estimates of common moments, such as the mean, median, and standard deviation can be quantified, and distributions of parameters of interest can be directly compared with a variety of techniques. We used hierarchical random effects Bayesian ANOVAs (Gelman et al. 2004) with non-homogenous variance and non-informative priors. We assumed that SLA was log-normally distributed for the *i*th individual in the *j*th population with a population level mean (θ_j) and variance($\sigma^2_{[j]}$):

$$SLA_{i,j} \sim ln\ N\ (\theta_j, \sigma^2_{[i]})\ i=1,\ldots,n_j;\ j=1,\ldots,10 \quad \textbf{(eqn 2)}$$

We assumed that diameter was normally distributed with a population level mean (θ_j) and population variance ($\sigma^2_{[j]}$):

$$Diameter_{i,j} \sim N(\theta_j, \sigma^2_{[j]})\ i=1,\ldots,n_j;\ j=1,\ldots,10 \quad \textbf{(eqn 3)}$$

Analyses were conducted using Markov Chain Monte Carlo simulation techniques with Gibb's sampling as implemented in OpenBUGS (version 3.2.2, Lunn *et al.* 2009) through R (version 2.14.2). Three chains were run for 5000 iterations. After visual inspection for convergence, the first 500 iterations were discarded as burn-in. Differences within and across population means were evaluated by comparing effect sizes. Differences between mean and standard deviation

parameters were evaluated by comparing 95% credible intervals (CIs). Effect sizes were calculated as the differences between posterior parameter estimates of the mean for each population and the estimated grand-mean for the regional population. Populations were considered to differ significantly from the regional population if their CI did not bound zero, and from other populations if their CIs did not overlap. Posterior distributions were constructed for each population and for the regional population. For brevity, only the posterior distributions for SLA are shown below; posterior distributions for diameter are available in the supporting material (Fig. S2).

Results

Qualitative comparisons revealed large variation in both traits. SLA varied 27-fold among individuals, ranging from 1.7 to 46.1 mm^2/mg, while rosette diameter varied 34-fold, ranging from 1.7 to 59.1 cm. Populations differed 4-fold in mean SLA and 5-fold in mean diameter (Fig 3.2). Coefficients of variation (*CVs)* varied more strongly among populations for SLA (range: 0.06 to 0.31) than rosette diameter (range: 0.21 to 0.45).

There were significant differences among populations in both SLA and diameter (Fig. 3.2) when analyzed using PERMANOVA. Pairwise comparisons identified four groups of populations for SLA. Differentiation among populations was stronger for diameter: four populations (1, 4, 6 and 8) were different from each other and all other populations. There was no significant difference in variance for SLA, but diameter was significantly different *($P < 0.0001$*; PERMDISP). Populations 1, 4, 6 and 8 – the same ones identified as different via PERMANOVA - were significantly different from one another, and from the other populations (Fig. 3.2).

Mixed models indicated that, for SLA, population identity explained 58% of observed variation, while 42% of variation was due to differences among individuals. For diameter, the same trend was observed: 65% of observed variation was due to differences in populations, while 35% of variation was due to differences among individuals.

Bayesian ANOVA indicated that there was high intraspecific variation in SLA around the grand mean. Populations 2 and 4 had a significantly higher mean SLA than the grand mean, while populations 3 and 9 had significantly lower mean SLAs (Fig. 3.3). Estimates of SD for SLA demonstrated large differences among populations, as population 3 had a large estimated SD, while populations 4, 5, 6 and 9 had low estimated SD (Fig 3.4). We used our posterior distributions to compare estimated values with literature-based values for SLA. Four populations (1, 3, 7, and 9) had poor overlap with any of the published values, indicating that literature-based values poorly represented population-level mean trait values (Fig. 3.5).

Bayesian ANOVA also indicated that intraspecific variation for rosette diameter was high among populations (Fig. 3.3). Populations 4 and 6 had larger diameters than the grand mean, while populations 1 and 8 had smaller diameters than the grand mean. Mean values for diameter differed strongly among populations (Fig. 3.4). Estimates of SD for rosette diameter demonstrated a significantly different SD for only populations 2 and 8, although there was high uncertainty in the estimates for 6 and 10 (Fig. 3.4).

DISCUSSION

Our results demonstrate substantial intraspecific variation for commonly measured plant functional traits. Both SLA and rosette diameter showed high variation across populations (as evidenced by significant differences in mean values) and within populations (as evidenced by

significant differences in estimated standard deviations). This variation is unaccounted for when a single mean value is used for each species, as is common in many ecological studies. However, this variation also likely influences how plants function and therefore the failure to account for it may bias estimates of community-level traits and ecosystem functions.

The magnitude of variation in these functional traits is surprising considering that population samples were collected on small spatial scales, and that, due to relatively long dispersal distances and proximity to major roadways, gene-flow between measured populations is possible. Even populations that were geographically very close (i.e. < 1 km apart) showed significant differences in both means and variation in functional traits, indicating that measuring traits *in situ* is likely to be critical when quantifying intraspecific variation.

Choosing Analytical Methods

The choice of analytical method used to quantify intraspecific variation can strongly influence our understanding of its magnitude and importance. Qualitative techniques, while helpful in showing how much variation is present, are generally unsatisfactory as they do not provide insight as to whether the observed variation differs significantly among populations.

Although popular, and capable of identifying differences among populations, ANOVA-based techniques require assumptions that are biologically unrealistic for some traits. When these assumptions are met, ANOVA provides limited insight into how or why populations differ, though its utility is strengthened when accompanied by a test of homogeneity of variance. Furthermore, because these techniques assume that variance across populations is identical, when they are used sampling efforts are designed and data transformations are used that deliberately minimize variation, effectively eliminating it from analysis. PERMANOVA and

PERMDISP are less influenced by these assumptions and therefore are more robust techniques than classical ANOVA for quantifying intraspecific variation. Mixed effects models can provide a great deal of insight into how variance is partitioned across levels of organization, and, like Bayesian methods, can be implemented hierarchically to estimate trait values on a regional level. While results from mixed effects models can shed some light on the extent of intraspecific variation, this technique was not able to directly quantify variation within populations, or how traits are distributed around population means.

The Bayesian and PERMANOVA approaches identified the same populations as differing from one another. However, the ability to not only estimate variance among populations but also to directly estimate variance within populations is a key strength of the Bayesian approach. The point estimates and credible intervals for standard deviations provide nuanced information regarding trait distributions that is not provided through any of the other techniques. However, Bayesian ANOVA is relatively uncommon, in part because it is computationally intensive and because the underlying framework differs substantially from more commonly applied statistical methods. In addition, it may require more extensive data collection (although all statistical techniques can be biased by low sample size), and lacks the significance tests and values commonly used to interpret statistical tests. Overall, however, we suggest that this technique is best for quantifying intraspecific variation within and across populations, and for incorporating this variation into larger ecological studies.

The Importance and Strength of a Bayesian Approach

Recent work has called for an emphasis on distribution-based techniques for estimating and incorporating intraspecific variation (Albert et al. 2011; Violle et al. 2012) into community

and regional level analyses and predictive models. Variation within and across populations likely has a measurable effect on both species and communities (Jung et al. 2010a, Crutsinger et al. 2010).

At the species level, posterior probability distributions can be compared directly within and among populations. They can also be used as prior distributions in future analyses examining changes in trait distribution (e.g., increased or decreased variation) in response to changes in abiotic conditions or community composition. The posterior distribution for a given trait could be used to make predictions about a population or species' ability to cope with changing conditions.

At the community level, posterior probability distributions can be used to compare amongst species, identify their degree of overlap with respect to a given functional trait, and develop predictions about the outcome of species interactions. It is common at present to calculate community-level trait averages by weighting the mean trait value for each species in a community by its relative abundance (e.g. Shipley, Vile, & Garnier 2006). Community-weighted trait distributions should more accurately represent the functional traits of a community since they can include different distributional forms for each species. Such complete modeling of trait distributions, rather than the reliance on trait means, will allow for better quantification of critical ecosystem function concepts such as functional richness, evenness, and divergence (Mason, Mouillot, & Lee, 2005).

Trait Selection

In our case study, conclusions about which populations differ from one another depended on which trait we focused on. We focused on two traits that correlate with plant function. The

decision of which trait or suite of traits to focus on for a given taxa is likely to influence conclusions about intraspecific variation, functional diversity, and plant function in general. Identifying key traits that are critical to the question, species, or ecosystem function of interest is critical.

The measured populations showed a very large range of values for SLA, including very low SLAs similar to those found in conifer needles. These low values do not appear to be measurement errors as they were consistently measured in populations which occur on glacial outwash soils, or during spring conditions (primarily Population 3). There are a number of potential explanations for this low trait value, including limitation for sunlight, soil nutrients or growing degree days, or differences in leaf ontogeny not easily assessed in the field. Regardless, they are part of the range of potential SLA values for this species and further highlight the value of quantifying intraspecific trait distributions.

We considered variation in one trait at a time, but a species' ability to cope or adapt to changing conditions may be driven by a suite of functional traits, making a univariate approach incomplete. We suggest that future studies explore multivariate Bayesian techniques to identify how variation in a suite of traits influences species' ability to cope and adapt to changing conditions.

Future Directions

It was beyond the scope of this study to investigate the causes of the observed variation in traits, but local adaptation to microsite conditions through genetic variation or phenotypic plasticity, and differences in traits due to ontogeny, are potential explanations for the different amounts of variation present. Abiotic gradients have been shown to induce plastic responses in

plant traits in cork oak (*Quercus suber*) (Ramirez-Valiente et al. 2010), Patterson's curse (*Ecium plantagineum*) (Sharma and Esler 2008), *Metrosideros polymorpha* (Cordell et al. 1998) and many other species (Davidson et al. 2011). Genetic variation in populations has also been shown to create wide phenotypic and functional trait variation in some species (Pezzani and Montaña 2006, Leger et al. 2009, Scheepens et al. 2010, Bilton et al. 2010, Nakamura et al. 2011) which can have cascading effects on surrounding communities and ecosystem services (Crutsinger et al. 2010). Further study, likely in a greenhouse or common garden setting, would be necessary to tease out the factors influencing the observed differences in traits and variation.

Regardless of the source of observed trait variation, the extent of intraspecific variation present in a population may indicate a species' or population's ability to adapt to changes in abiotic conditions, cope with invaders (or become invasive itself), or adapt to changes within the community. For example, species or populations that demonstrate higher intraspecific variation in traits related to stress tolerance (e.g. water-use efficiency, growth rate) may be able to better cope with shifts due to climate change, while those with lower variation may be driven locally extinct. Species or populations with high variation in certain functional traits may be more likely to become invasive when introduced to new habitats, or may be able to compete or cope with newly introduced species. If high intraspecific trait variation indicates an increased ability to cope with biotic and abiotic stress, this may be of critical importance to conservation biology, as efforts to preserve and expand variable populations may be of key importance in a shifting environment. In contrast, populations with low variability may be at higher extinction risk, and require additional protection or assistance. Further research into the role of intraspecific variability in mediating species and population responses to biotic and abiotic stress should be conducted.

Regional-level trait estimates can be used to quantify species-level niche-breadth, and may be useful from a conservation stand-point. For example, species with low variation may have a greater risk of extinction due to global change. The effects of climate change, habitat disturbance, land use, or conversion on species or ecosystems could also be quantified using model updating. More complex, community-level hierarchical models can be used to partition sources of variation between intra- and interspecific sources and across multiple levels of organization.

CONCLUSIONS

Although intraspecific variation in important plant functional traits can be high, and likely has ecological consequences, techniques to adequately quantify this variation are not yet consistently implemented. Permutational ANOVA was reasonably successful in detecting differences among populations, particularly if combined with a permutational test of dispersion within populations. Of the methods we considered, however, we suggest that Bayesian parameter estimation is most appropriate for quantifying intraspecific variation. The ability to directly estimate, incorporate prior knowledge about, and statistically compare variation, as well as the opportunity to use estimated parameters for future predictions, represents an important step in moving towards a more holistic and predictive view of functional ecology.

Measurements of intraspecific variation should be incorporated into future analyses of plant communities, particularly at small geographic scales, studies focused on response traits, or those focused on a single species (Albert et al. 2011). Future research should focus on quantifying the extent and importance of intraspecific variation within and across plant populations, communities and biomes. The ability of a species to expand or contract its trait

variability in response to changing conditions should be quantified, and the effect of increased or reduced trait variation in regards to fitness should be evaluated.

ACKNOWLEDGMENTS

We thank Eric Delvin, Tom Kaye, Danielle Santamaria and Ty Robinson for their assistance with this research, and Janneke Hille Ris Lambers, Soo-Hyung Kim, Sarah Reichard, Regina Rochefort, and Daniel Laughlin for their advice and guidance. We also thank Lauren Sandhu and two anonymous referees for suggestions that improved this manuscript. Portions of this project were supported by the National Park Service under task agreement J8W07070010.

Table 3.1 Application, assumptions, strengths, and weaknesses of statistical methods used to quantify intraspecific variation.

Method	Application	Assumptions	Strengths	Weaknesses
Descriptive Statistics	Comparison of means, standard deviations, standard errors, and ranges	Measured values, standard deviations or standard errors are representative population and/or treatments effects	Provide information on values of traits of interest	Anecdotal; no statistical test
Coefficient of Variation	Calculates the ratio of the standard deviation to the mean, quantifying data dispersion	Suitable only for non-negative values on a ratio scale	Directly quantifies and allows for direct comparison of variance	Cannot be used for some data; no statistical test
Homogeneity of Variance	Test for equality of variance; typically used diagnostically	Dependent on method used	Direct test for equality of variance	Underlying data distribution cannot be quantified
- PERMDISP	Test for data dispersion from the mean using distance-based measures.	Appropriate distance measures are used to construct the dissimilarity matrix	A direct test for data dispersion around the mean, including significance and pair-wise comparisons.	Choice of distance measure is critical, and can bias outcomes. Underlying data distribution cannot be inferred
Analysis of Variance	Test for differences between means based on sums of squares	Normally distributed data; equal variance; independence of errors	Can indicate differences between populations	Does not directly test whether populations differ in trait means and/or variation around the mean
- PERMANOVA	Test for differences between means using sums of squares and permutation tests	Weak assumption: homogenous variance	No distributional assumption; can detect differences between populations	Underlying data distribution cannot be inferred from this test. Does not directly test whether populations differ in trait means and/or variation around the mean

Linear Techniques				
- General Linear Models - Generalized Linear Models	Relate a continuous response to linear predictors	Normally distributed data; equal variance; independence of errors	Can indicate differences between populations or along gradients	Does not directly test whether populations differ in both trait means and variation around the mean
- Mixed Effects Models	Relate non-normal, non-continuous responses to linear predictors	Multiple families of distributions; independence of errors; interpretation of variance dependent on distributional form	Can indicate differences between populations	Variance specified, and is typically not quantified directly.
	Relate non-normal, non-continuous responses to non-normal and non-continuous predictors	Incorporate both random and fixed effects; Multiple families of distributions; independence of errors; interpretation of variance dependent on distributional form	Indicates differences between populations; hierarchical estimation of population parameters	Cannot directly estimate variance for a given population
Bayesian Techniques	Derives a posterior probability distribution for a variable based on a prior probability, and a likelihood function; estimation of parameters for direct comparison,	Prior and posterior data must have some assumed and assigned distributional structure	Directly estimates both mean values and variance directly from data; allows for direct comparison of variance terms; allows for updating of distributions based on new data; assumptions about distributions can be assessed directly	Sensitive to improper or incorrect prior distributions; classic significance tests are not typically applied, computational intensive

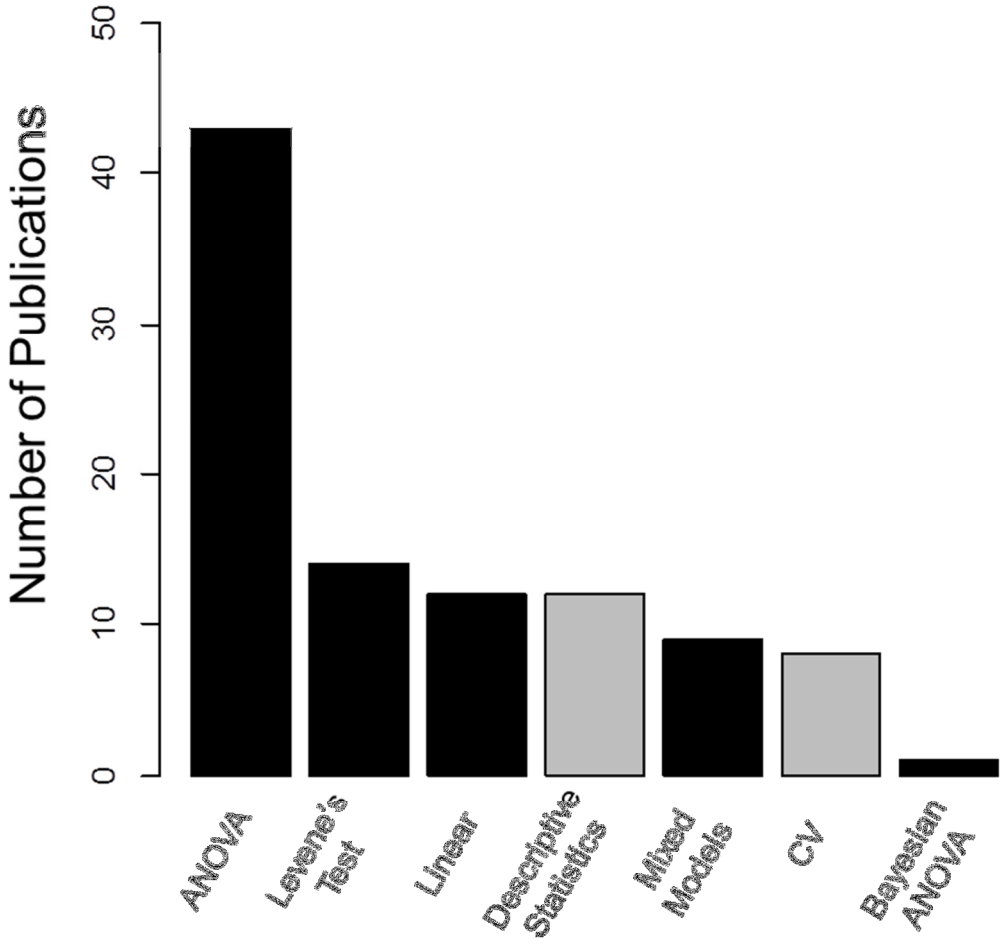

Figure 3.1 Techniques used to quantify or compare intraspecific variation drawn from peer-reviewed literature published between January 2007 and October 2012. Dark bars indicate quantitative statistical tests, and light bars indicate techniques which compare descriptive statistics. Sixty-four studies are included here but some studies used multiple techniques and were tallied multiple times.

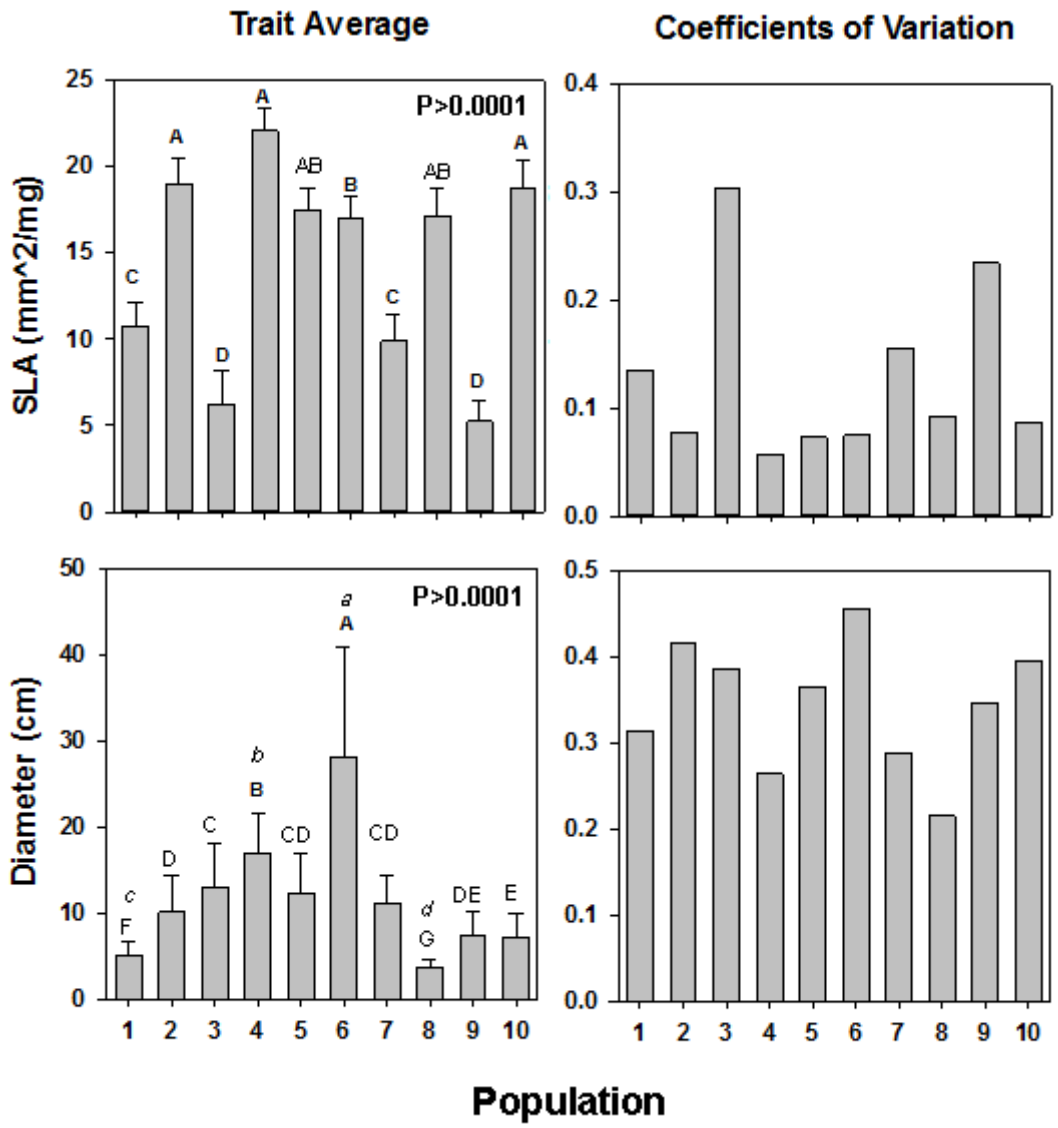

Figure 3.2 Trait averages (+ SD) and coefficients of variation for SLA (top) and rosette diameter (bottom) among 10 populations. Populations are numbered from north to south. P-value and Uppercase letters denote significantly different groups as determined by PERMANOVA, and italicized lowercase letters denote significantly different groups as determined by PERMDISP. Note that the scale of the y-axis differs among panels.

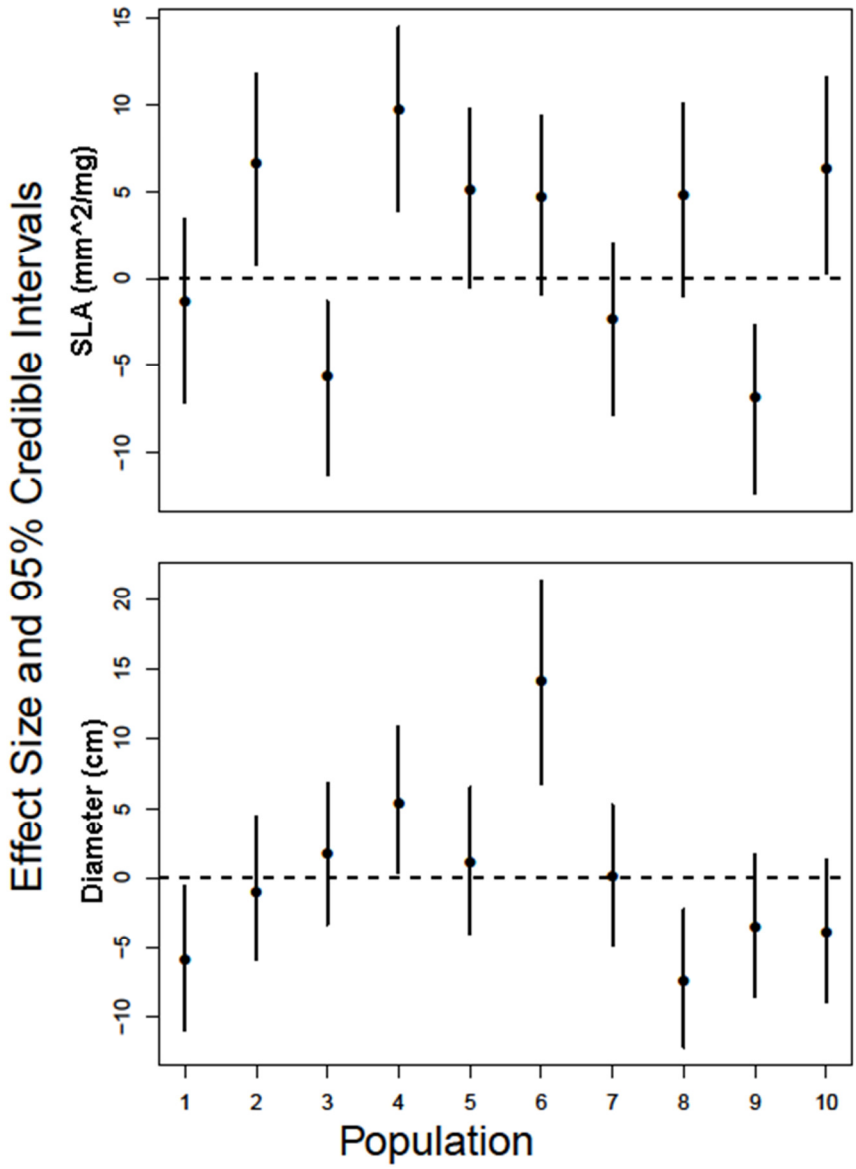

Figure 3.3 Effect sizes (magnitude of difference from the estimated grand-mean, shown by the dashed horizontal lines) and 95% credible intervals for SLA (top) and rosette diameter (bottom) among 10 populations. Populations are numbered from north to south. Populations differ significantly if their credible intervals for that statistic do not overlap.

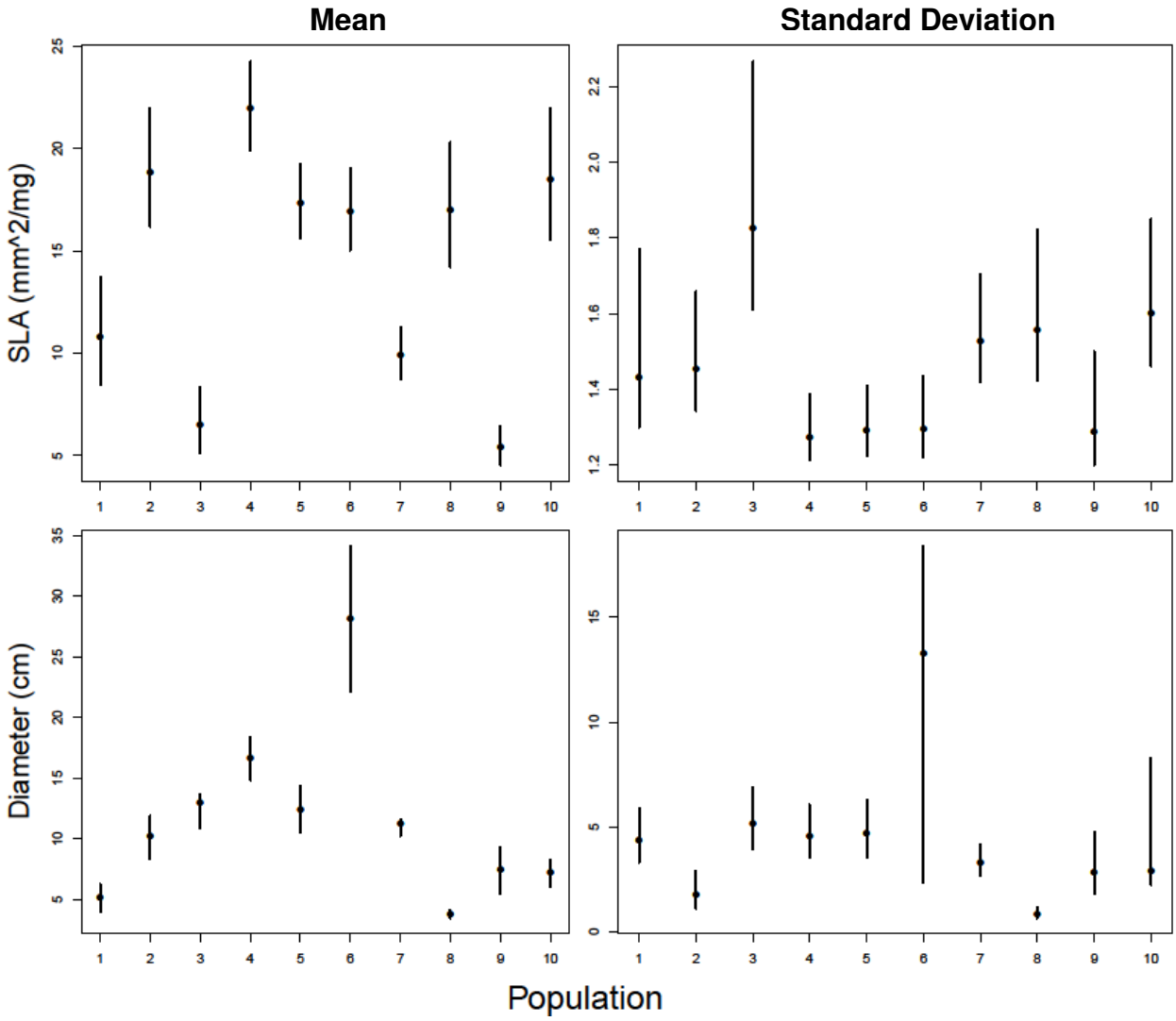

Figure 3.4 Point estimates and 95% credible intervals for the mean (left) and standard deviation (right) for SLA (top) and rosette diameter (bottom). Populations differ significantly if their credible intervals do not overlap.

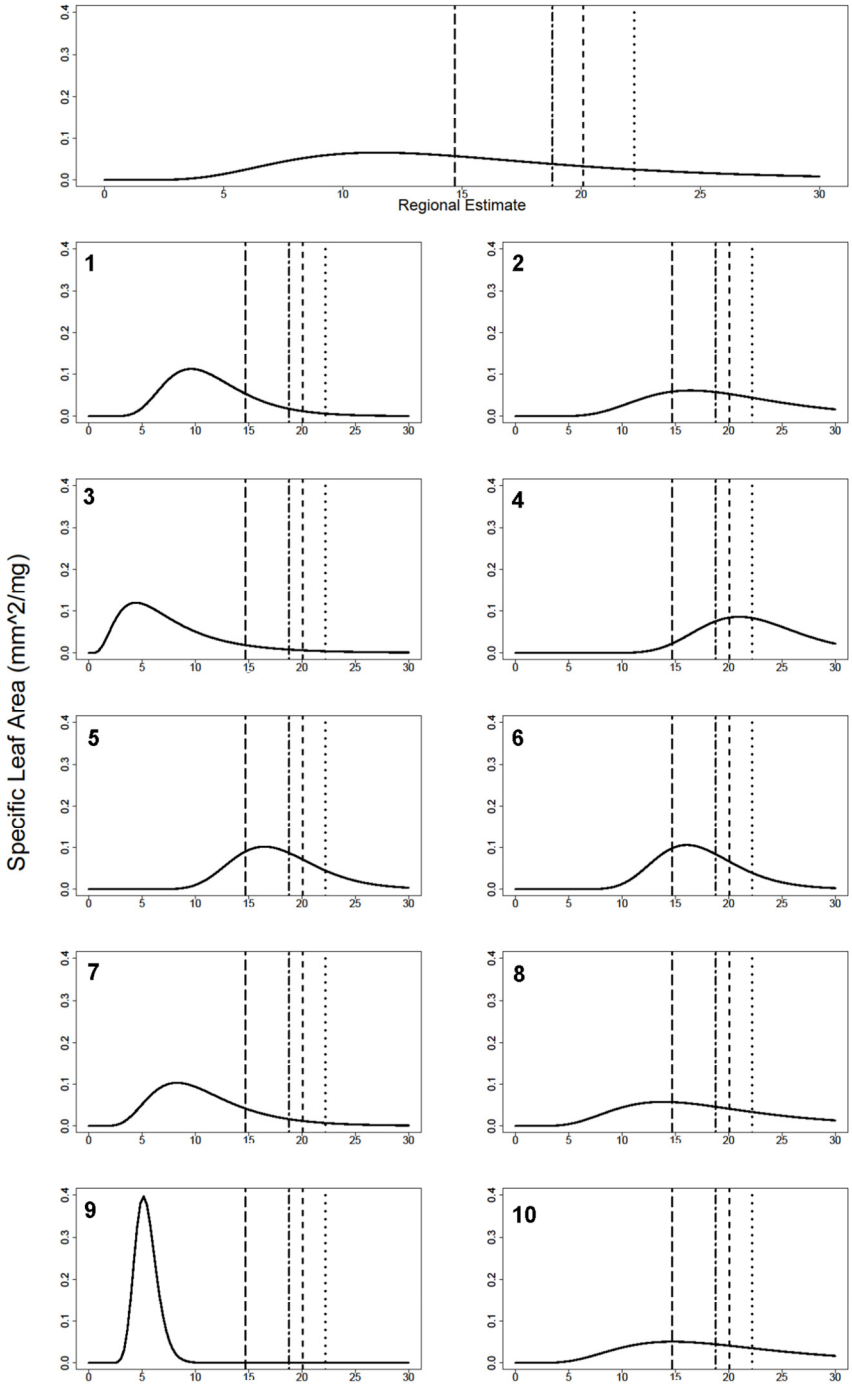

Figure 3.5 Posterior distributions (solid lines) of SLA for the regional population (top panel) and 10 populations of *Hypochaeris radicata*. The vertical lines in each graph indicate the literature-based trait estimates from fertilized pot-grown plants (thick dashed line), field grown plants (dot-dashed line), unfertilized pot-grown plants (thin dashed line) and the LEDA Traitbase (dotted line).

Chapter 4: Functional Trait Shifts in Response to Community Composition

ABSTRACT

During community assembly, plant functional traits are hypothesized to undergo filtering at two levels: by prevailing abiotic conditions, followed by competition from competitors. While trait shifting in response to abiotic conditions is well documented, whether and how plant species shift their traits in response to the biotic community is less studied. Species can shift traits in terms of mean trait response, or in terms of the distribution of traits within a population. In the face of increased competition, species may shift both trait means and distributions toward an "optimum" trait value. As a result, niche-space becomes more packed, and species must limit similarity to reduce competitive interactions through reduction of overall trait breadth. We investigated trait shifting in two axes of the "Leaf-Height-Seed" plant strategy scheme for eight species of forbs in an experimental grassland. These species were grown under uniform abiotic conditions in diverse communities that differed in the proportion of grass seed sown into the plot: grass-rich, mixed, and forb-rich. Specific Leaf Area (SLA) and plant height (diameter for one rosette-forming species) were measured for 25 individuals per species in each treatment. We examined differences among species and treatments in each trait, and for the bivariate trait space. Species trait variation and trait averages were compared using PERMDISP and PERMANOVA, and evidence of limiting similarity was quantified using linear mixed models and variance component analysis. While height and SLA shifted in terms of means, variances, or both for most species, there was no consistent pattern between community types and the direction of shifting. Examination of within-species and between-species variance components demonstrated that species in the grass-rich treatment exhibited increased niche-packing for both traits, but there was no indication of limiting similarity (i.e. niche spacing did not increase with increased niche

packing). These results highlight that trait shifting is highly idiosyncratic, and that such responses need to be incorporated into studies at both the species and community level.

INTRODUCTION

The distribution of functional traits within biological communities is driven by two overarching processes: environmental filtering and biotic interactions. Environmental filtering is hypothesized to act directly on plant functional traits, eliminating individuals that do not have traits amenable to survival. Indeed, abiotic filtering has been shown to create global-scale patterns in plant trait expression (Diaz et al. 1998), and such patterns can be used to predict plant geography (Swenson and Weiser 2010) and ecosystem services along abiotic gradients (Lavorel et al. 2011). Such studies have largely assumed that variation of plant functional traits within species was negligible, but recent research determined that this variation can be substantial (Mitchell and Bakker n.d., Albert et al. 2010b, 2010a) and can influence species coexistence (Jung et al. 2010a, Clark 2010a, Pruitt and Ferrari 2011, Long et al. 2011), ecosystem function (Pontes et al. 2007, Lecerf & Chauvet 2008) and community assembly (Siefert 2012). The consideration of intraspecific trait variation has been shown to significantly alter conclusions about relationships between trait values and species variation at the community level (Cianciaruso et al. 2009, Messier et al. 2010), and incorporation of intraspecific variation has improved species abundance models (Laughlin et al. 2012).

Although abiotic filtering alters both community- and species-level trait distributions, the role of biotic interactions in shaping these distributions is less understood. Interactions between species are largely assumed to affect community structure and trait distribution through competition and limiting-similarity (Weiher and Keddy 1995). Abiotic filters generally reduce community-level trait dispersion relative to the regional species pool (Keddy 1992), while competition for limiting resources is expected to lead to increased trait dispersion within the community to limit the similarity between species (Fargione and Tilman 2005, but see Schöb et

al. 2012 for evidence of facilitation) due to inherent tradeoffs in plant growth and acquisition (Westoby 1998). For example, species experiencing a neighborhood community composed of tall individuals may shift growth towards traits that facilitate light-harvesting, and reduce allocation to traits for nutrient storage or reproduction (Gubsch et al. 2011). Such trait shifting may cause species to become increasingly similar in terms of average trait values, increasing competitive interactions. In response to increased intra- and interspecific competition, species may reduce their overall niche breadth and increase spacing between similar species (limiting similarity). Such shifts would result in increased niche packing and maintenance of biodiversity, which in turn may increase those ecosystem processes that have been linked to biodiversity (e.g. productivity (Tilman et al. 1996) and invasion resistance (Kennedy et al. 2002)). Although increasing community-level biodiversity, especially by increasing the proportion of functionally similar species, is expected to affect traits in a predictable way (i.e. shifting to limit similarity), empirical tests of the effects of biodiversity on plant trait variation, and especially intraspecific trait variation, remain uncommon (Burns and Strauss 2012).

We examined the effect of biotic interactions on the trait distributions of a guild of functionally similar forbs in an experimental grassland that had homogenous abiotic conditions (i.e. flat, tilled, and herbicided soils). We focused on these forbs for a number of reasons. First, they are often treated as a single "functional group" within community ecology, and are assumed to respond similarly to abiotic and biotic conditions (Lavorel et al. 1997). Second, forbs comprise much of the plant diversity in the system. Finally, although forb species diversity is great, they contribute relatively little to overall above-ground productivity, are unlikely to be the primary competitors for limiting resources, and thus are likely to be impacted by changes in the biotic community (e.g. grass abundance) that affect resource availability.

We focused on two major axes of the "Leaf-Height-Seed" trait-based strategy scheme: Specific Leaf Area (SLA) and plant height (Westoby 1998). This scheme allows the strategy of any species to be compared by identifying its position in three-dimensional space described by the plant's SLA, Height, and seed mass. Because of its relative ease of collection and its correlation with exploitation of available resources, productivity, leaf life-span, and leaf quality (Reich et al. 1992, Westoby 1998), SLA is a widely used functional trait (Wilson et al. 1999). Height is a measure of plant competitive ability; larger plant height gives greater access to light and greater potential to assimilate carbon.

We tested three hypotheses. First, we hypothesized that trait distributions for the measured species would shift in response to increased grass presence, and that this shift would be directional; traits would become less variable in response to increased grass competition. Second, we hypothesized that trait means would shift in response to grass competition, with plants becoming taller and with higher SLA as grass competition increased. Finally, we hypothesized that there would be evidence for limiting similarity (e.g. decreased niche breadth and increased niche spacing) for the species of interest as competition from grass increased.

METHODS

Study Site

This study was conducted within a larger prairie restoration experiment at the Glacial Heritage Preserve, Thurston County, Washington. Plots (6.32 m x 6.32 m) were established in 2008 on a tilled old-field site. The plots sampled for this study received a solarization site treatment: extant vegetation was killed with glyphosate, the soil was tilled, and clear plastic was spread across the surface for 3 summer months. Plots were then seeded with one of three seeding mixes, keeping the overall rate at ~630 seeds per m^2, but altering the portion of grass to

forb seeds: forb-rich (2:98), mixed (25:75), or grass-rich (50:50). The seeding mixture included four species of grass and 22 forbs. Seeds were from wild populations or seed increase beds.

The site received a controlled burn in Autumn 2011 to stimulate native prairie plants and to control invasive grasses. *Festuca roemeri,* a tall (~0.5 m), perennial bunchgrass, is the dominant native grass at the site, and provides the matrix for the community. Plant identity and cover were monitored in spring 2012, at which point forb cover and richness paralleled the initial seeding mixes, although some seeded species failed to establish and some unseeded species had colonized treatment plots. The forb-rich plot had a 1:3 ratio of grass to forb cover, with 22 forb and 3 grass species present. The mixed plot had an average of 1:2 ratio of grass to forb cover grass cover, with 19 forb and 4 grass species, and the grass rich plot had an average of 1:1.33 ratio of grass to forb cover, with 18 forb species and 4 grass species.

Species selection

We focused on eight species which occurred in all seeding treatments: *Eriophyllum lanatum*, *Ranunculus occidentalis*, *Plectritis congesta*, *Epilobium ciliatum, Hypochaeris radicata, Cerastium arvense, Rumex acetosella,* and *Geranium molle*. Hereafter, plants will be referred to only by their genus. Four of these species (*Eriophyllum, Ranunculus, Plectritis, Cerastium*) were seeded into plots, while the other colonized the plots naturally. *Plectritis* is an annual species, while *Geranium* exhibits a variable life-history, and can be annual, biennial, or perennial. The remaining plants are perennial.

Trait Measurement

Plant trait data were collected in Summer 2012 from 25 individuals of each species in each treatment. Plants were sampled only if they were at least a meter from the plot edge. Two traits were measured: Specific leaf area (SLA) and height (basal rosette diameter for

Hypochaeris; see below). These functional traits are considered to be orthogonal components of the Leaf-Height-Seed plant strategy scheme (Westoby 1998).

For SLA, a mature, fully exposed, undamaged leaf was collected from each individual. Leaves were stored in whirl-pak plastic bags at 4°C for no more than 48 hours and then rehydrated following Cornelissen et al. (2003). After rehydration, leaves were scanned in a flatbed scanner at 200 dpi, dried at 60°C for a minimum of seven days, and weighed on a Mettler AE 163 analytical scale. One-sided leaf area was calculated from the scanned images using ImageJ version 1.45 (available at http://rsb.info.nih.gov/ij). SLA was calculated as leaf area (mm^2) divided by dry leaf mass (mg).

Plant height was measured as the shortest distance from the ground to the highest photosynthetic structure without stretching in healthy adult plants exposed to full sunlight (following Cornelissen et al. (2003)). One species, *Hypochaeris,* is a rosette forming species, and therefore height was not a meaningful measure of competitive ability (Mitchell and Bakker n.d.). Instead, we measured the diameter of the rosette as an index of its ability to occupy space and capture light. Rosette diameter was calculated as the average of two measurements taken in perpendicular directions.

Statistical Analysis

We sought to detect shifts in trait averages and distributions for SLA and height, as well as the combined bivariate trait space. Both traits were normally distributed within species. We used permutational analysis of variance (PERMANOVA) to test for differences amongst species, treatments, and species x treatment interactions. Like standard ANOVA, a significant PERMANOVA does not indicate whether populations differ in means, variance, or both. We used PERMDISP, a permutational analog to the Levene's test (Anderson 2001), to assess

dispersion of data around a multivariate centroid or univariate mean. Both tests were conducted using the PERMANOVA+ add-on to PRIMER-E (Clarke & Gorley, 2006), with the Euclidean distance measure, type III sums of squares and 9,999 permutations. A significant PERMDISP test indicates that treatments differ in variance, while a significant PERMANOVA test without a significant PERMDISP test indicates treatments differ in mean trait values. Significant results for both tests indicate that treatments differ in variance, but may also differ in mean value. Non-significant results for both tests indicate that a trait did not differ among treatments. We used visual inspection of means and post-hoc (Tukey's HSD) contrasts to determine how data dispersion differed by treatment for each species, and how average trait values differed by treatment for each species.

To quantify whether traits shifted in terms of trait niche and trait spacing for the combined guild of eight species, we used linear mixed models with the "lme4" package in R. We fit separate models for each seeding treatment, using species identity as a random factor. We chose to treat species identity as a random factor for this portion of the analysis because we assumed niche breadths and niche spacing could be generalized to the larger population of forbs. Variance components were analyzed to examine both niche breadth and niche separation between the eight species following Dimitrakopoulos and Schmid (2004). Niche breadth was quantified as the square root of the residual (within species) variance components, while niche separation was quantified as the square root of the between-species variance component (explained with the "species identity" term). Larger values of variance components indicate greater niche breadth and greater niche-separation, respectively.

Results

Bivariate Trait Analysis

Analyses of bivariate data indicated that there were significant shifts ($P < 0.05$) in both trait dispersion (PERMDISP) and means (PERMANOVA in the absence of significant PERMDISP results) in response to seeding treatments (Table 4.1, Figure 4.1).

Geranium, *Hypochaeris* and *Rumex* exhibited no significant shifting in terms of either bivariate means or dispersion in response to seeding treatments. *Cerastium* and *Plectritis* exhibited no significant difference in dispersion between treatments, but there were significant differences in bivariate means between the grass-rich and other treatments for both species. *Epilobium, Eriophyllum,* and *Ranunculus* exhibited significantly different variances and visual inspection indicated that they also differed in trait averages. There was no clear directional pattern in trait shifting; some species shifted in terms of means only while others shifted both means and variances. Interestingly, no species shifted only in terms of variance. Trait shifting is summarized in Table 4.2.

Univariate Trait Analysis

Height

Univariate analysis of plant height/diameter indicated that there were significant differences ($P<0.05$) in both trait means and dispersion (Table 4.1, Figure 4.2). All possible combinations of mean and variance shifting were observed, and changes were not directional.

Geranium exhibited no significant differences in terms of trait means or dispersion between treatments. *Hypochaeris* and *Rumex* differed only in terms of dispersion. For *Hypochaeris*, individuals in the mixed treatment had the highest average dispersion from the centroid (5.1 cm) while *Rumex* in the forb-rich treatment had the highest dispersion from the centroid (5.1 cm). *Cerastium* differed only in terms of trait means, with individuals in the mixed treatment being tallest and those in the grass-rich being shortest. *Epilobium*, *Eriophyllum*,

Plectritis and *Ranunculus* differed significantly in both trait dispersion and trait means. Trait shifting is summarized in Table 4.2.

SLA

Univariate analysis of SLA indicated that there were significant differences ($P<0.05$) in both trait means and dispersions (Table 4.1, Figure 4.3). All species exhibited trait shift in means, variances, or both, and changes were not directional.

Cerastium, Rumex, and *Epilobium* differed only in terms of trait dispersion. *Cerastium* individuals in the grass-rich treatment exhibited greater average dispersion (6.0 mm^2/mg), than those in the forb-rich (2.6 mm^2/mg) and mixed (2.5 mm^2/mg) treatments. For *Epilobium* individuals in the forb-rich treatment had significantly higher average dispersion (7.2 mm^2/mg) than those in the mixed (3.4 mm^2/mg) or grass-rich treatments (2.6 mm^2/mg). *Hypochaeris* and *Ranunculus* differed only in terms of average trait values. *Hypocheris* individuals in the grass-rich treatment had the highest SLA (24.1 mm^2/mg), and individuals in the forb-rich treatment had the lowest SLA (21.3 mm^2/mg), while *Rununculus* individuals in the mixed treatment had significantly higher SLA (18.1 mm^2/mg) than those in the forb- or grass-rich treatments (17.0 and 16.1 mm^2/mg). *Eriophyllum*, *Geranium*, and *Plectritis,* had significantly different means and variances. Trait shifting is summarized in Table 4.2.

Niche Breadth and Separation

Results for niche breadth and separation differed for SLA and height (Table 4.3) although we lacked replication to determine whether these differences were statistically significant. For height, niche separation (square root of between-species variance) was much lower in the grass-rich treatment (3.1) than in other treatments (5.5 (forb) and 5.3 (mixed)). Niche width (square

root of within-species variance) was also lower in the grass-rich (5.0) treatment than in other treatments (6.0 (forb) and 5.3 (mixed)).

For SLA, niche separation was highest for plants in the mixed seeding treatment (5.0), and lowest for those in the grass-rich treatment (3.8), while the forb-rich was slightly lower than the grass-rich treatment (4.7). Similarly, niche width was widest in the mixed treatment (6.5) and narrowest in grass-rich treatment (5.2), with the forb-rich treatment intermediate (5.8).

DISCUSSION

The importance of intraspecific trait variability is increasingly recognized (Violle et al. 2012a) in processes like community assembly (Jung et al. 2010b, Bilton et al. 2010, Laughlin et al. 2012), plant-herbivore interactions (Karley and Hawes 2008, Wurst et al. 2008), plant-microbe interactions (Kabouw et al. 2010, Lankau 2011), response to abiotic conditions (López et al. 2009, Fajardo and Piper 2011) and patterns of plant strategy (Frenne et al. 2011). In our study, we focused on two axes of the "Leaf-Height-Seed" plant strategy scheme, SLA and height, to assess whether and how plants were shifting functional traits in response to changes in the biotic community. These traits have been demonstrated to be well-correlated with other frequently measured plant traits, and represent fundamental trade-offs in plant strategy (Westoby 1998). Plants cannot simultaneously horde soil-derived resources in long-lived, low SLA leaves, and devote resources to stem or leaf tissue for light acquisition and competition (height). Soil resources are limiting resources in grassland habitats and access to light is directly impacted by the surrounding vegetation. Thus, both are likely to be impacted by changes in the proportion of forbs or grasses present in a community.

Our results demonstrate that there are substantial shifts in plant functional traits in response to changes in the biotic community, but contrary to our hypothesis, these shifts were

highly idiosyncratic despite the fact that these plants share similar attributes within the ecosystem. There was no consistent pattern in trait shifting in response to increasing grass cover for bivariate trait-space or for univariate analyses, indicating that when and how a trait shifts in response to biotic conditions is species specific. These shifts may be due to local adaptation, trait plasticity, or a combination of both. This study was not designed to address which of these sources of variation were most common, and both are possible, as seeds used in the seeding treatments were collected from a variety of source populations, and the genetic provenance of volunteer species are unknown.

Results supported our hypotheses that plants would shift not only trait means in response to grass competition, but trait variance as well. Bivariate trait analyses indicated that, for at least some species, there was no significant change in trait-space in response to the biotic community, while other species shifted in terms of trait-space, and trait means, or a combination of both (Table 4.2).

For plant height, a trait which captures resource allocation to competitive, supportive structures, we expected plants to increase trait averages and decrease trait variation in response to increased grass competition. Instead, we saw that species behave idiosyncratically across all treatments (Figure 4.3). For example, *Cerastium* exhibited decreased height in response to increased grass competition, while *Epilobium* had decreased height only in the mixed treatment. In the forb-rich treatment, *Hypochaeris* displayed the lowest variability and *Rumex* the highest. *Geranium* shifted neither means nor trait dispersion in response to grass competition.

SLA, which captures extraction of soil resources for dedication to light-harvesting structures, displayed a similar lack of pattern. As grass competition increased, we expected species to exhibit higher SLA (that is, lower allocation of resources to light capture) as allocation

is shifted to competitive support structures (height or rosette diameter). *Ranunculus* displayed the highest average SLA in the mixed treatment, while *Hypochaeris* displayed the highest SLA in the grass-rich treatment. *Cerastium* had the highest dispersion in grass-rich treatments, while *Epilobium* had the highest dispersion in forb-rich treatments.

Such results make generalizations about trait shifting at the species level very difficult. Although traits are expected to shift in similar ways across all species in response to filtering (Diaz et al. 1998), trait responses to the biotic community do not appear to support this hypothesis. These results indicate that, while abiotic conditions may homogenize traits in a predictable way, biotic conditions do not. Instead of trait means and variation being directionally driven by biotic competition, the traits shifted idiosyncratically, likely reflecting differing species-level resource extraction and coexistence strategies (Gubsch et al. 2011). Idiosyncratic trait shifting may facilitate coexistence by allowing for greater niche-packing without competitive exclusion.

Results at the community level support this idea. For both height and SLA, niche-breadth and niche separation were narrowest in the grass-rich treatment (Table 4.3). This supports the idea that, at the community level, populations can shift trait distributions to reduce niche breadth and increase overall niche packing to reduce competition, maintaining biodiversity when resources (in this case, space and light) are limiting. However, contrary to the "limiting similarity" hypothesis, individual species do not appear to be reducing niche-width to avoid extirpation, as niche separation was lowest in the grass-rich treatment. These results may instead support the idea of "lumpy coexistence" amongst similar species (Scheffer and Van Nes 2006) due to competitive interactions driving existing species towards a similar realized niche.

Although not statistically tested, niche-breadth and niche-spacing were greatest for height in the forb-rich treatment, as predicted. However, SLA exhibited a slightly different pattern: the mixed treatment, rather than the forb-rich treatment, exhibited the greatest niche breadth and least separation between species. There are a number of possible explanations for these results. SLA is a less dynamic trait than plant height. While plants can shift resource allocation to supporting structures in response to changing light environments, plant leaves, once constructed, are less able to shift in terms of tissue allocation. Thus, the overall pattern of niche breadth and separation for this trait may be a legacy of earlier abiotic or biotic conditions not captured by this study. In addition, resource availability is likely to be more heterogeneous in the mixed seeding treatment, as both intra-guild and grass competition are likely to be more spatially heterogeneous. Plants in this intermediate community may have greater access to diverse soil resources, allowing wider niche-breadth and greater niche spacing despite increased grass-competition.

We did not examine the effects of the plant community on the third axis of the L-H-S scheme, primarily because seed collection times at the site differ by species, and plants that are producing seeds have typically begun senescing, making tissue collection for trait measurement difficult. Seed mass has been shown to have high intraspecific variability (Michaels et al. 1988, Hendrix and Sun 1989) and to respond strongly to abiotic conditions (Dainese and Sitzia 2013). It is extremely likely that seed mass would exhibit shifting in response to the biotic community in terms of both means and variance at both the species and community level. Like both plant height and SLA, it is likely that such shifts would be highly idiosyncratic, as seed mass may be affected by temperature, soil nutrients, or maternal effects (Roach and Wulff 1987, Dainese and Sitzia 2013). Future studies should examine shifting in this dimension of the L-H-S scheme.

Although the influences of abiotic filters between treatments were minimized for this study (flat, well-tilled site preparation), the abiotic conditions at the study site still impacted observed trait shifting. Not all species that were seeded into each treatment plot established, and other species were able to colonize the plots. This suggests that abiotic filtering had likely already acted to homogenize the traits of the plants measured within the plots. Furthermore, individuals who survived initial abiotic filtering are still subject to fluctuations in weather and resources that may also shape trait expression. In regions with stronger abiotic filters (e.g. strong microsite differences, drought), trait shifting in response to the biotic community may be limited, as abiotic filters may more strongly influence trait expression.

In addition, our research community was relatively species-poor (~30 species per plot). In communities that are more species rich, patterns of trait shifting are likely to be different. For example, species may shift more dramatically in species rich communities, as overall competition for resources is likely to be much higher. Conversely, trait shifting may be less important, as there is unlikely to be a single, dominant matrix species in a highly diverse community.

CONCLUSIONS

This study provides clear evidence that plant traits can shift trait distributions in terms of both average trait expression, and trait variation, in response to changes in the biotic community. This driver of functional trait variation has received relatively little attention, and this study indicates that, in addition to abiotic conditions, biodiversity and composition of the community should be considered in studies of plant traits. Like a few other studies investigating trait shifting, we found that species respond differently to changes in the biotic community (Gubsch et al. 2011, Roscher et al. 2011), and that no consistent pattern could be identified across all species (Díaz et

al. 2007, Albert et al. 2010b, Gubsch et al. 2011). This differential response of species to changes in the biotic community highlights the fact that reliance on "functional groups" or on published trait means are of limited utility when examining community level processes. Despite this idiosyncratic response to the biotic community, overall patterns of increased niche packing and reduced niche breadth still persisted, indicating that, at the community level, traits do shift in predictable ways.

These results have important management implications. Because we focused on the most widespread forb species across all treatments, it is not clear whether rare or genetically constrained species would exhibit similar trait shifting. If trait variation is driven largely by genetic variability, rare species with limited gene pools may not be able to shift appropriately in the face of changing biodiversity (e.g. in response to invasion or range shifting). Ensuring that populations of concern exhibit wide trait or genetic diversity may help such species to overcome perturbation.

In addition, we did not quantify trait shifting for all of the species in the community. The observed results, which are currently attributed to increasing competition from grass, could be due to changes in presence or abundance of other species not measured in this study. A more complete examination of trait shifting over time and at the community scale would strengthen our understanding of biotic drivers of trait expression. In addition, comparisons of trait shifting between high- and low-biodiversity communities should be conducted.

Future research should focus on quantifying trait shifting, niche breadth and niche spacing for multiple traits for the entire plant community. Temporal changes in traits and niches should also be quantified to further elucidate the role of trait variation in driving coexistence.

ACKNOWLEGEMENTS

We thank Eric Delvin, Peter Dunwiddie, and Ty Robinson for their assistance with this research, and Janneke Hille Ris Lambers, Soo-Hyung Kim, Sarah Reichard, Regina Rochefort, and Martha Groom for their advice and guidance.

Table 4.1 Results of PERMDISP and PERMANOVA testing the effect of seeding treatment, species identity, and the interaction between treatment and species identity on bivariate traits, height, and SLA. Significant effects (α = 0.05) are bold.

	Bivariate		Height		SLA	
	F	*P*	*F*	*P*	*F*	*P*
PERMDISP						
Treatment (Tr)	7.4	**0.001**	8.8	**0.0001**	2.5	0.14
Species (Sp)	9.4	**0.0001**	5.8	**0.0001**	9.9	**0.0001**
Tr x Sp	4.2	**0.0001**	3.4	**0.0002**	8.7	**0.0001**
PERMANOVA						
Treatment (Tr)	9.4	**0.0001**	15.5	**0.0001**	.09	.9058
Species (Sp)	42.5	**0.0001**	46.8	**0.0001**	30.5	**0.0001**
Tr x Sp	2.9	**0.0001**	3.6	**0.0001**	3.9	**0.0001**

Table 4.2 Summary of trait responses, by species, to a changing biotic community. Traits were examined together (bivariate) or separately (height, SLA), and could be unchanged or differ in mean and/or variance. Graphical results are in Figures 4.1- 3, and statistical results in Table 4.1.

Species	Bivariate	Height	SLA
Cerastium	Mean	Mean	Variance
Epilobium	Mean; Variance	Mean; Variance	Variance
Eriophyllum	Mean; Variance	Mean; Variance	Mean; Variance
Geranium	No change	No change	Mean; Variance
Hypochaeris	No change	Variance	Mean
Plectritis	Mean	Mean; Variance	Mean; Variance
Ranunculus	Mean; Variance	Mean; Variance	Mean
Rumex	No change	Variance	Variance

Table 4.3 Average niche breadth (square root of the residual term) and separation (square root of the species identity term) for eight forb species.

Treatment	Height	SLA
Forb		
Separation	5.5	4.7
Breadth	6.0	5.8
Mixed		
Separation	5.3	5.0
Breadth	5.7	6.5
Grass		
Separation	3.1	3.8
Breadth	5.0	5.2

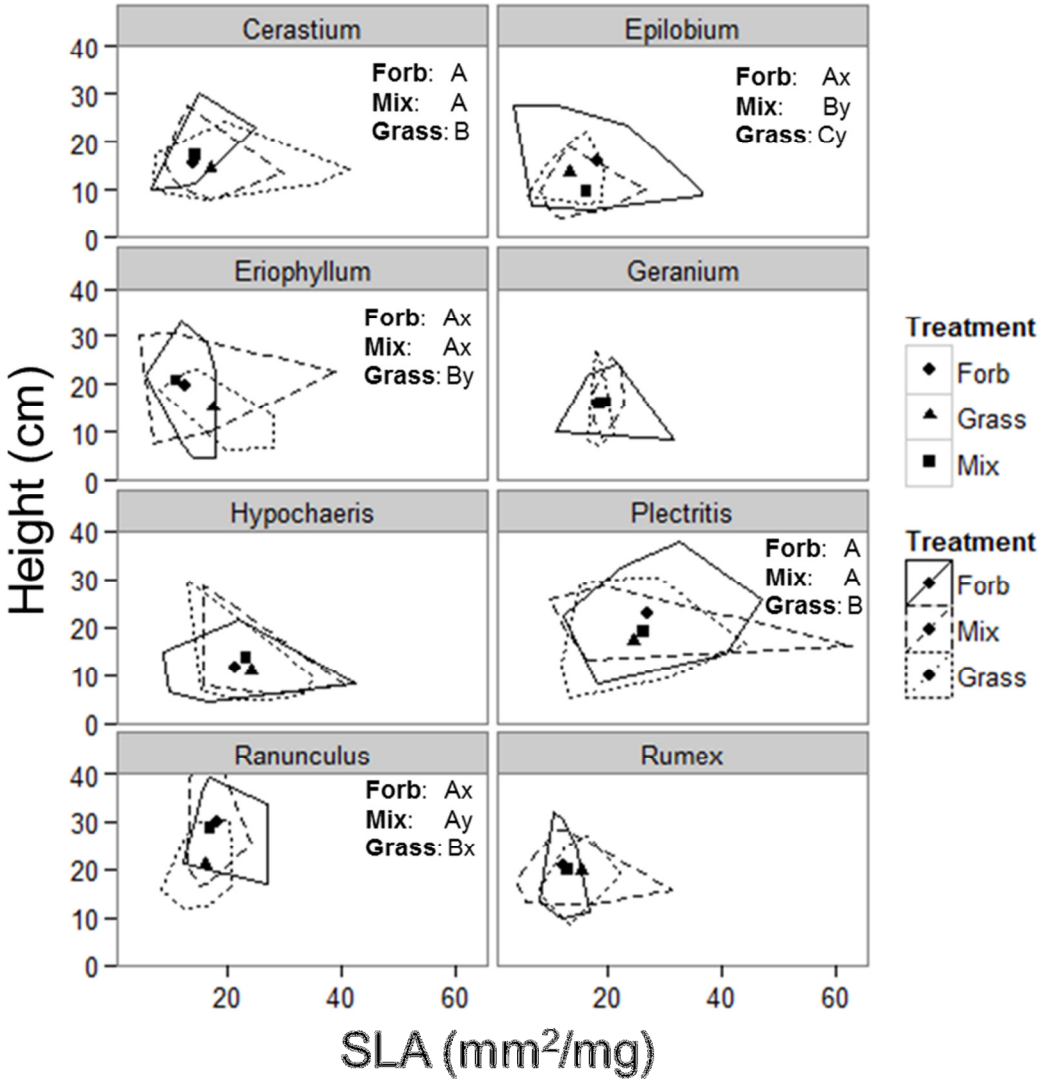

Figure 4.1 Bivariate means and dispersions for each species in each seeding treatment. Treatment means are indicated with symbols; treatments dispersion is indicated by solid (forb-rich), long-dashed (mixed) or short-dashed (grass-rich) lines outlining the range of values. Significantly different means are indicated by different uppercase letters (A, B, or C) and significantly different dispersions are indicated by different lowercase letters (x, y, and z). The y-axis for *Ranunculus* is truncated to improve readability.

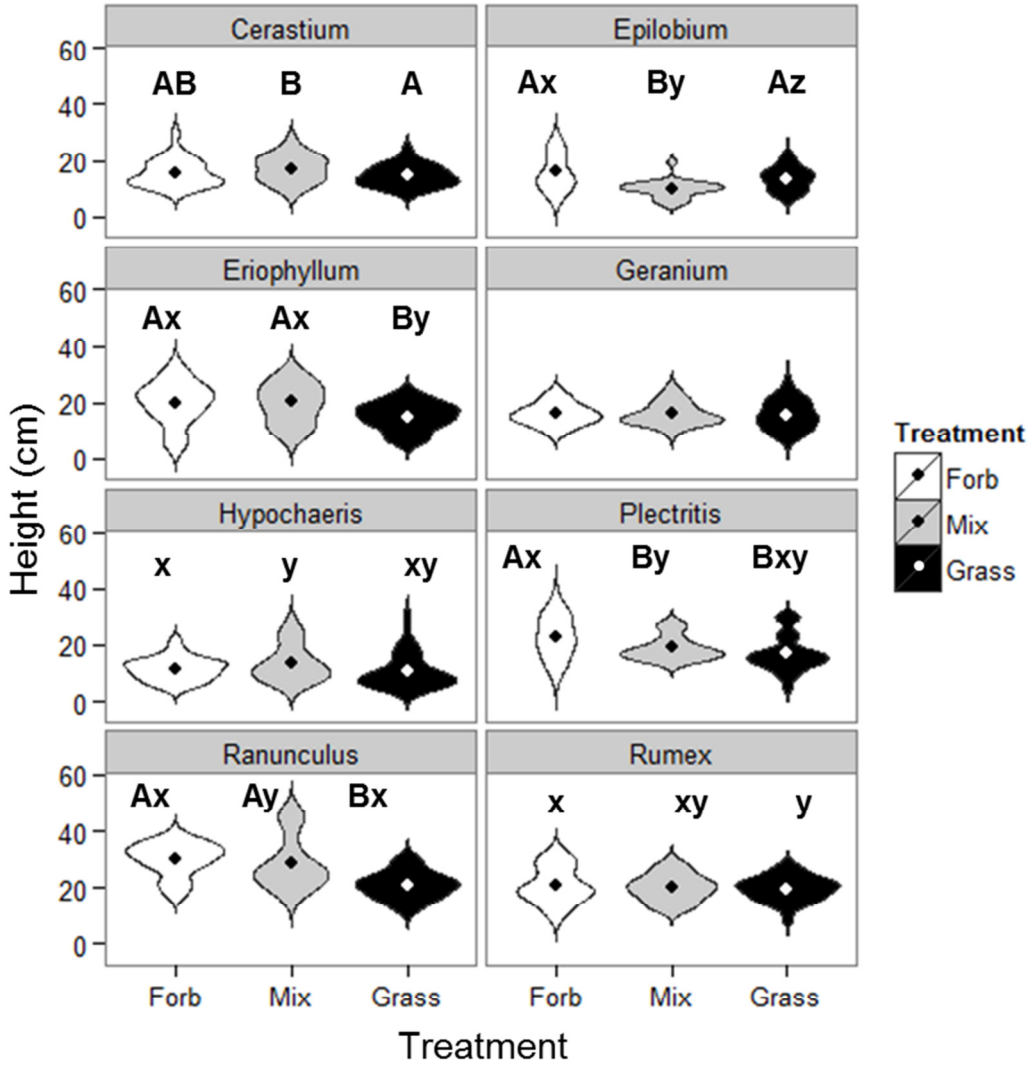

Figure 4.2 Violin plots showing height means and distributions for each species in each treatment. A violin plot is a combined boxplot and kernel density plot. Here, we show trait means solid points, and the data density (variation) estimated by kernel method. Significantly different ($\alpha = 0.05$) means are indicated by different uppercase letters (A, B, or C) and significant differences in dispersion are indicated by different lowercase letter (x, y, or z).

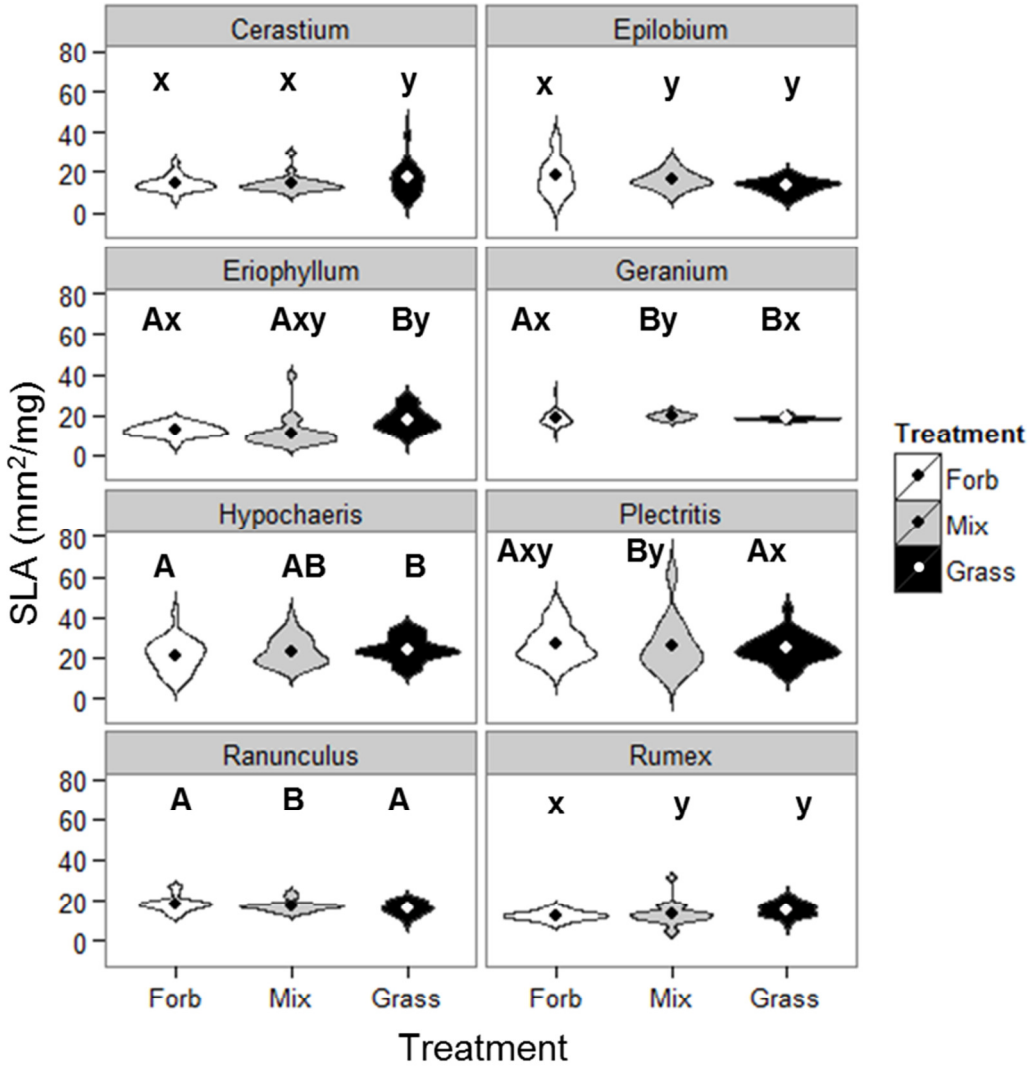

Figure 4. 3 Violin plots showing SLA means and distributions for each species in each treatment. Trait means are indicated by solid points. Significantly different ($\alpha = 0.05$) means are indicated by different uppercase letters (A, B, or C) and significant differences in dispersion are indicated by different lowercase letter (x, y, or z).

Chapter 5: Sources and Drivers of Intraspecific Plant Trait Variation

ABSTRACT

The importance of intraspecific variation in plant functional traits in structuring communities and driving ecosystem processes has been increasingly recognized. While intraspecific variation in plant functional traits has been studied for various plant species in response to a number of drivers, the mechanisms governing this observed variation are less well studied. Observed variation could be due to adaptation to local conditions, leading to distinct ecotypes, or to plasticity in observed traits. In addition, traits may vary with ontogeny, a source of variation that is often neglected. We investigated 1) whether intraspecific trait variation was primarily due to ecotypic differences or trait plasticity 2) Whether traits varied with ontogeny, and 3) whether field-based variation was predictive of greenhouse variation. We used three geographically and climactically distinct populations of the widespread weed *Hypochaeris radicata*. These populations differed significantly in trait variation for both rosette diameter and specific leaf area (SLA) in field-based measurements. We grew nine maternal lines from each population (27 maternal lines total) under three greenhouse conditions: control, drought, or 80% shade. Plant diameter and relative cholorphyll content were measured repeatedly throughout the experiment, and final plant diameter, leaf length:width ratio, root:shoot ratio, SLA, and final relative chlorophyll content were collected after five weeks. We used hierarchical mixed-models and variance components analysis to quantify differences in treatment effects and the contribution of population of origin and maternal line to observed variation. We compared coefficients of variation (CVs) between field and greenhouse populations to assess whether field variation was predictive of greenhouse variation. Overall, our results strongly indicate that observed variation in plant traits was driven primarily by plasticity. Shade and drought

significantly influenced all measured traits, and plant diameter was the only trait that had a significant proportion of random-effects variation explained by differences within populations (30%). All other traits showed negligible variation explained by either the population of origin or maternal line (1-9%). There were significant ontogenetic differences for both plant diameter and relative chlorophyll content. Variation for both maternal lines and populations increased over time, although variation between populations continued increasing at day 35 for relative chlorophyll content, and decreased for plant diameter. There was no evidence that trait variation measured in field-conditions was predictive of variation under greenhouse conditions for any population, and divergence from field measured variation showed no clear trends. Overall, our results indicate that observed intraspecific trait variation is due primarily to trait plasticity, rather than to adaptation to local conditions, that plant traits can differ significantly over the ontogeny of a plant, and that measures of plant trait variation taken in the field are not predictive of variation for populations grown under greenhouse conditions.

INTRODUCTION

Recent and growing interest in plant functional traits has led to a deepened understanding of critical ecological mechanisms. For example, trait-based approaches have identified differing processes of community assembly across spatial scales (Messier et al. 2010) and in temperate and tropical ecosystems (Myers et al. 2012), shaped understanding of abiotic drivers such as altitude (Swenson et al. 2011, Long et al. 2011), climate (Thuiller et al. 2004, Swenson and Weiser 2010), temperature (Hudson et al. 2011, Laughlin et al. 2011), and resource gradients (Fonseca et al. 2000) on community assembly and diversity, and identified global patterns of plant-trait expression (Diaz et al. 1998). In addition, trait-based approaches have led to a mechanistic understanding of correlations between biodiversity and ecosystem function (Díaz and Cabido 2001, Diaz et al. 2004, William et al. 2008).

Increasingly, the importance of intraspecific trait variation in shaping community assembly and ecosystem function has been recognized (Bolnick et al. 2011, Lepš et al. 2011, Albert et al. 2011, Violle et al. 2012a). Intraspecific variation in plant functional traits can be large, but is often ignored or its contribution miscalculated (Mitchell and Bakker n.d., Albert et al. 2010b, 2010a) despite playing an important role in structuring communities (Siefert 2012) and shaping ecosystem function (Lecerf and Chauvet 2008). For example, intraspecific variation in plant functional traits has been shown to structure communities along environmental gradients (Jung et al. 2010a), to drive diversity in forest ecosystems (Clark 2010b), and to enhance community resistance to plant invasion (Crutsinger et al. 2008).

Although intraspecific variation in plant functional traits is known to be important, the mechanisms governing observed trait variations are less well understood. Two processes are hypothesized to drive variation in trait expression: adaptation to local conditions, creating

distinct genetic ecotypes (Joshi et al. 2001), and phenotypic plasticity in response to prevailing abiotic conditions (Schlichting 1986). Although changes in intraspecific variation in plant functional traits have been observed in response to a wide range of environmental conditions (e.g., flooding (Jung et al. 2010a), fire (Moreira et al. 2012), and temperature (Fajardo and Piper 2011)), few studies have determined whether this observed variation can be attributed to ecotypic differences or phenotypic plasticity. Those studies that have investigated sources of intraspecific variation have, at times, found contrasting results, depending on the species and functional trait being investigated (Grassein et al. 2010, Bonser et al. 2010, Hansen et al. 2013). For example Firn et al. (2012) found significant plasticity in SLA for an exotic, invasive grass when compared to co-occurring native grasses. In cork oak, genetically distinct populations were found to display significantly different values for SLA, suggesting local adaptation to climate regimes, but populations also demonstrated high phenotypic plasticity (Ramírez-Valiente et al. 2010). In contrast, Robson et al. (2012) found that differences in SLA in European beech were primarily driven by genetic provenance. Confounding this examination is the fact that the amount of phenotypic plasticity expressed by a genotype or population can itself be treated as a functional trait, as variation has been shown to be both adaptive and with cost (Auld et al. 2010), although few studies have determined whether the degree of variability expressed by a population remains constant under new abiotic conditions.

If observed intraspecific trait variation is largely driven by genetic variability linked to adaptation, plant species with low variability and whose traits fall outside of abiotic or biotic filters may be extirpated from communities. Conversely, if trait variation is largely a plastic response independent of genetic diversity, even rare species with a few individuals within a community may survive filtering and persist in response to abiotic or biotic perturbations.

In addition to ecotypic or plastic sources of variation, plant trait expression may also differ over the lifespan of a plant. Previous research has indicated that the effects of ontogeny can differ depending on the trait being measured. Wood density, but not leaf mass per unit area, was strongly affected by ontogeny in tropical *Northofagus pumilio* (Fajardo and Piper 2011). SLA was found to decrease with age in quaking aspen (Smith et al. 2011) while Cornelissen et al. (2003a) found poor correlation between greenhouse seedlings and field-measured adults for 90 woody plants, indicating strong ontogenetic differences. Because traits can influence ecosystem function (Diaz et al. 2004), and temporal differences in plant trait expression may facilitate species coexistence and biodiversity maintenance (Violle et al. 2012a), determining how traits vary ontogenetically for the most commonly measured plant functional traits is of critical importance.

To test whether observed intraspecific trait functional trait variation is due to ecotypic differences or plastic responses, how trait expression is influenced by plant ontogeny, and whether variation in the field-based measurements predicts variability in greenhouse grown plants, we collected 25 maternal lines from three populations of the globally widespread weed *Hypochaeris radicata*. These populations, although from similar habitat types, differed in the degree of trait variation they expressed in plant diameter, specific leaf area (SLA), and seed mass. Individuals from selected maternal lines were grown in greenhouse conditions, but were subject to abiotic stresses commonly encountered in the field; namely, light limitation and drought stress. With these populations, we tested three hypotheses. First, we hypothesized that observed trait values (trait means) would exhibit some plastic responses, but that population of origin and maternal line of origin would explain the majority of observed variation. We also expected trait means and variability to change in response to ontogeny, and to increase with

increasing plant age. Finally, we expected variability measured in the field to be predictive of variability in the greenhouse.

METHODS

Study Species

Hypochaeris radicata is a short-lived perennial herb species common in both intact and degraded grassland ecosystems throughout Europe, North America, Australia, New Zealand and Japan. It forms basal rosettes with oblong-lanceolate leaves and produces several upright, branching flower stalks during a long flowering period – June through October in the Pacific Northwest. *H. radicata* is self-incompatible (Pico et al. 2004, Ortiz et al. 2006) and its seeds are wind dispersed up to a few hundred meters (Mix et al. 2006).

Experimental Design

Specific leaf area (SLA), plant diameter, and a mature seed-head (to collect average seed mass) were collected or measured from 20-25 plants (maternal lines) from each of three populations (Smith Prairie (SP), Glacial Heritage (GL) and Union Bay Natural Area (UB)) spanning a ~200 km range (Figure 5.1) for this experiment. Seeds were collected from individuals that were at least 3 meters from one another, to prevent sampling from closely related individuals. Populations occurred in grassland habitats on glacial outwash soil (SP and GL) or on mixed urban soil in UB. Collection locations differ in annual precipitation and land-use history (Table 5.1). Previous analyses of these populations indicate that there are significant differences in trait variability for specific leaf area (SLA) and rosette diameter (Mitchell and Bakker n.d.); follow-up analyses focusing specifically on these three populations indicated there were significant differences in trait dispersion and mean trait values for diameter (Figure 5.2A, P

< 0.0001) and significantly different trait dispersion for SLA (Figure 5.2B, P = 0.05). There was no significant difference in seed mass (Figure 5.2C).

In order to capture the range of traits expressed in each population, populations were analyzed using Principal Components Analysis (PCA), and maternal lines from each population were selected based on their score along PC 1, which explained 49% of the variation. Because average seed numbers from individual maternal lines were often less than 60, maternal lines could not be replicated across treatments. Instead, the three highest, three lowest, and three most central maternal lines along PC 1 were identified for each population, and a high, low and central maternal line was randomly assigned to each treatment (Figure 5.3).

Seeds from each of the selected maternal lines (3 populations x 9 maternal lines/population) were cold-stratified for 10 days and germinated at 25°C. Twenty germinants per maternal line (540 individuals total) were planted into 164 ml "conetainers" (Ray Leach) filled with Sunshine Mix #3 potting soil (Sun Gro Horticulture). Germinants were allowed to acclimate for seven days before treatments were applied.

Maternal lines were grown in the greenhouse under one of three treatments: ambient conditions, 80% shade (80% of light is blocked), and drought conditions (50% of winter precipitation). These treatment levels were selected to mimic marginal conditions where *H. radicata* is known to survive. Beginning on day seven, individuals from high, low and central maternal lines were sorted into one of the three treatments, and individuals were randomly arranged in pot racks to reduce edge effects. The ambient and shade treatments received weekly top-watering equivalent to average October-November rainfall across three collection sites (53 ml per week per conetainer), while the drought treatment received 50% of average winter rainfall (26 ml per week per conetainer). The drought treatment visibly induced plant stress; individuals

in the drought treatments became wilted between watering. In addition, each treatment received weekly 10-10-10 NPK fertilizer plus micronutrients per manufacturer specifications; fertilizer amounts were doubled in the drought treatment to account for reduced watering volume.

Mortality and measurements of plant diameter and relative chlorophyll content (using a SPAD 502 plus chlorophyll meter, Spectrum Technologies) were made every seven days, beginning on day seven (before treatment applications) for diameter measurements and on day 14 for SPAD measurements (as plant leaves were too small at day seven for data collection). After five weeks, leaf length and leaf width were measured for the largest leaf on each individual, and plants were destructively sampled. A single leaf from each individual was harvested, photographed and dried at 60°C and weighted using a Mettler AE 163 analytical scale. One-sided leaf area was calculated from the photographed images using ImageJ version 1.45 (available at http://rsb.info.nih.gov/ij). SLA was calculated as leaf area (mm^2) divided by dry leaf mass (mg). Above and belowground biomass were also harvested separately, dried at 60° C, and weighed, and root:shoot ratio were calculated.

Statistical Analysis

We used hierarchical linear mixed effects models and variance components analysis to quantify the influence of abiotic and genetic factors on observed traits. Population and maternal line were designated as random effects, with maternal line nested within populations. Shade, control and drought were treated as fixed effects. Models were fit with the "lme4" package in R (version 2.14.2), and p-values were generated using the "languageR' package. Correlation between dependent variables was checked; when variables had $r^2 < 0.55$ only one representative variable was tested. Models were fit for final diameter, leaf length:width ratio (larger numbers indicate more lanceolate leaves, lower numbers, more ovate leaves), root:shoot ratio, SLA, and final

relative chlorophyll content. Both SLA and diameter were log transformed to correct for normality and heteroscedacity.

Diameter and relative chlorophyll content were measured repeatedly, so separate analyses were done to examine ontogenetic effects. Individual plants were nested within maternal line in these models. To determine whether treatment and time had significant effects in repeat measure models, fixed factors (treatment and time) and their interactions were iteratively added, beginning with the null model. The best model was selected using Akaike's Information Criterion (AIC) and p-values for treatments and treatment x time interactions were generated using the "languageR" package. The proportion of random-effects variance attributable to either population of origin or maternal line was expressed as the percentage of total random-effects variance.

In order to determine whether field-based measurements of variability were predictive of greenhouse variability, we compared the coefficients of variation (CVs) for SLA and diameter in field populations and greenhouse populations for each treatment.

RESULTS

Population and Maternal Contributions to Trait Expression

Linear mixed effects models indicated that a surprisingly small amount of variation was explained by either population of origin or maternal line for most measured traits (Figure 5.4). Diameter was the only trait that showed a significant maternal contribution to observed variation; 30% of total random effects variation was attributable to variation among maternal lines, while only 3% of variation was attributable to differences between populations (Figure 5.4).

Plants in both the shade and the drought treatments were significantly different from plants in the ambient treatment in terms of diameter; individuals in the drought treatment had

smaller diameters, while plants in the shade treatment had larger diameters (Figure 5.5A). Both shade and drought had significant effects on leaf length:width ratios (Figure 5.5B); leaves in the shade treatment were significantly more lanceolate than those in the ambient and shade treatment. Plants in the shade treatment had significantly lower root:shoot ratios (Figure 5.5C) than those in drought or ambient treatments. For SLA, there was a significant treatment effect for both the shade and drought treatment, with plants in the ambient treatment having the lowest SLA and plants in the shade treatment having the highest SLA (Figure 5.5D). Relative chlorophyll content was significantly lower for plants in the shade treatment compared with those in the drought or ambient treatments (Figure 5.5E).

Ontogenetic effects

There were significant interactions between treatment and time for both relative chlorophyll content and plant diameter. Relative chlorophyll content was strongly affected by treatments; plants in the shade treatment had significantly lower chlorophyll content than the ambient treatment regardless of measurement time, while there were no significant differences between the drought and ambient treatment. Variation between maternal lines within populations decreased between days 14 and 28, but increased on day 35. Variation between populations showed an identical trend (Figures 5.6A and 5.6B). Plant diameters for individuals in the drought treatment differed significantly from ambient conditions only on days 28 and 35, while those in the shade treatment differed significantly at day 21 and at day 35. For plant diameter, variation for both maternal lines (Figure 5.6C) and populations increased over time (Figure 5.6D), although variation between populations decreased at day 35.

Predictiveness of field-measured variability

Comparisons of CVs from field-based and greenhouse-based measurements show that, overall, field-based measures are poor predictors of variability, particularly for plants grown under shade conditions, and that trends in variability differed by population of origin. (Figure 5.7). Plants grown under shade conditions consistently had higher variation in SLA than those grown under field-conditions for all populations, but SLA for plants in the drought and ambient treatments showed differing trends by population. Plants from GL had higher variability than field populations in both ambient and drought conditions, while plants from UB showed the opposite trend. Plants from SP showed no consistent trends in SLA variability.

For diameter, there were no consistent patterns in trait variability (Figure 5.7). Plants from GL had consistent variability across all treatments except shade, while both SP and UB had high variability in field populations, and reduced variability in greenhouse conditions.

DISCUSSION

We set out to determine whether observed intraspecific trait variation was driven predominantly by plasticity, or adaption to local conditions, how traits varied over the lifespan of a plant, and whether observed population variation in the field was predictive of trait variation in a greenhouse setting.

Our results indicate that, for *H. radicata*, plasticity is the predominant driver of observed trait variation. There were strong treatment effects for all measured traits, although shading treatments had a stronger effect than drought, no treatment was strong enough to induce significant mortality. These results indicate that *H. radicata* can tolerate a wide range of abiotic conditions, conditions that are typically omitted through commonly used trait collection procedures (Cornelissen et al. 2003b) which emphasize collection of sun-exposed mature leaves

to quantify plant functional traits. Our results demonstrate that plants in shade conditions can exhibit markedly different functional traits from those grown in sun conditions, particularly in traits like SLA. The contributions of these individuals are likely to influence the overall trait distribution of the plant community, which may in turn impact estimations of critical ecosystem processes like litter production and nutrient cycling (William et al. 2008). Omissions of individuals in partial or full-shade may have negative consequences for our understanding of trait mediated ecosystem processes and responses. However, not all species are capable of tolerating such a wide range of light conditions, and thus measurement of sun-exposed leaves is an appropriate technique for shade-intolerant species.

The variance explained by population of origin and maternal lines was surprisingly small for every measured trait. Only diameter had significant variation attributable to differences across maternal lines, and even this amount of variation (30%) was dwarfed compared to treatment effects. Virtually none of the observed variation could be attributed to population or maternal line for SLA or leaf shape (length:width ratio) (Figure 4), indicating that these traits are highly plastic. The very small amount of variation attributable to population or maternal sources across all treatments indicates that, for the traits measured in this experiment, plastic responses to prevailing conditions are the primary driver of observed variation.

Traits did show significant variation over time, indicating that ontogeny plays an important role in shaping trait expression. This source of variation is typically ignored in studies at the community level, as collection techniques typically focus on the "mature" plants and tissues (Cornelissen et al. 2003b) which can be difficult to distinguish in the field. Because plants of differing ontogenies may appear "mature" in the field, high intraspecific trait variation found in field populations could be partially attributed to differing ontogeny between individuals,

rather than being a byproduct of genetic or environmental conditions. Furthermore, ontogenetic variation in trait values and variability may be important in the structure and function of communities. For example, ontogenetic trait variation may lead to temporal niche partitioning, facilitating biodiversity maintenance through limiting similarity. In addition, such temporal variation may mediate impacts of abiotic filters. In our study, young plants were fairly homogenous, indicating that abiotic filtering is likely to have strong impacts on propagules. As plants aged, variation increased. This increased variation may reduce the effects of abiotic filtering on mature plants at the species level, as a wider variety of phenotypes are expressed, preventing local extirpation.

Our results indicate that high intraspecific trait variability in the field did not appear to predict variability in the greenhouse, at least for this species (Figure 5.6). This suggests that variability itself should not be treated as a species-specific trait. Despite the three measured populations originating from geographically distant locations and expressing differing levels of average trait values and variation in those locations, almost no variation in greenhouse populations was attributed to population of origin for any trait. This supports the idea that these traits are highly plastic, rather than manifestations of distinct ecotypes. The lack of quantifiable differences between populations could also be due to high genetic similarity across populations. Although these populations are geographically distant, invasion in North America by *H. radicata* is relatively recent and rapid, and these populations could experience genetic mixing due to their proximity to major roadways.

These results, taken together, indicate that abiotic conditions and ontogeny, and not adaptation and genetically distinct ecotypes, are responsible for driving trait expression and variation in field conditions. Any variability within or across maternal lines or populations was

reduced by the strong effects of the treatments, despite the presence of three geographically distinct populations, 27 distinct maternal lines, and 540 individuals. Although the results of this study strongly indicate that genetic factors are unimportant drivers of trait variation, it remains unclear whether this is true for all species and all traits. *Hypochaeris radicata* is globally widespread, and thus may be inherently more plastic or genetically diverse than species with more restricted distributions (Gitzendanner and Soltis 2000, Sultan et al. 2009). Plasticity may be a characteristic trait of widespread or dominant species, thus this research should be replicated with other species that are widespread, and species from restricted ranges, to determine the generality of this finding.

The treatments applied during this experiment represented strong abiotic filters for this species, but are within the range of conditions experienced by *H. radicata*. Studies involving a gradual light or moisture gradient could assess whether the importance of population of origin or plasticity changes depending on the strength of the abiotic filter, a question which was not addressed in this study. Finally, the effect of multiple abiotic filters and their interactions on trait expression could be tested in both the field and greenhouse settings.

CONCLUSION

Despite an increased focus on understanding and incorporating intraspecific variation in plant functional traits into larger, community- and ecosystem-level studies, the factors driving observed variation remain poorly understood. Our results suggest that observed intraspecific variation is largely driven by trait plasticity, and not genetic factors. Furthermore, there is little evidence that populations differ in the amount of plasticity they are capable of expressing, and variability measured in the field was not predictive of variability under imposed abiotic stress.

Finally, we found strong evidence of variation in plant traits due to ontogeny, a source of trait variation that is often ignored.

ACKNOWLEDGEMENTS

We thank Ty Robinson for assistance in the field, and Loretta Fisher for assistance in the lab. We also thank Janneke Hille Ris Lambers, Soo-Hyung Kim, Sarah Reichard, Regina Rochefort, and Martha Groom for their advice and guidance.

Table 5.1 Average annual rainfall and land-use history for the collection sites of the three study populations.

Population	Rainfall (cm)	Land Use
Glacial Heritage (GH)	129	Tilled Agriculture
Smith Prairie (SP)	65	Agriculture
Union Bay Natural Area (UB)	97	Reclaimed wetland

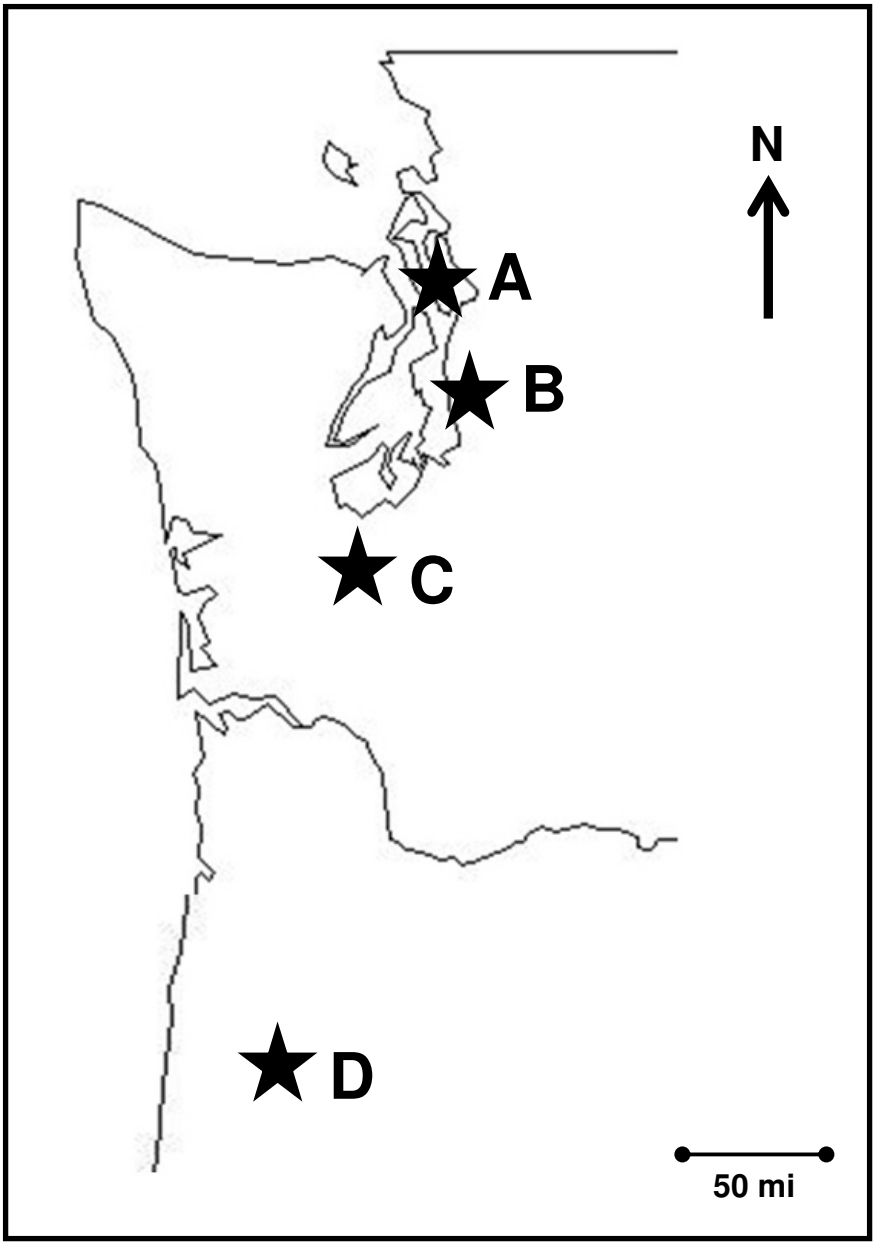

Figure 5.1 Collection locations for the 3 populations used in this study.

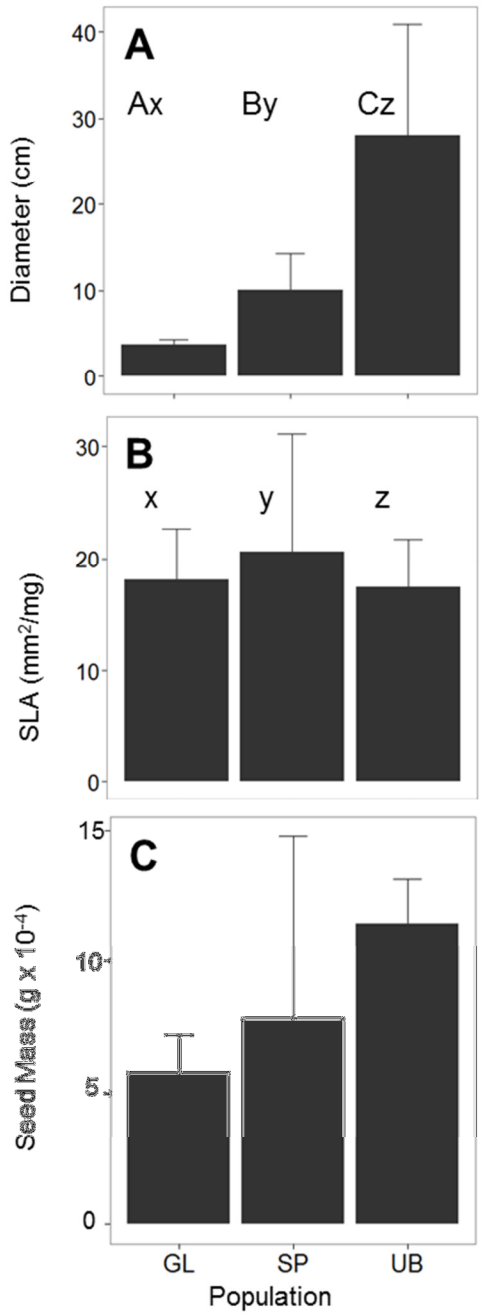

Figure 5.2 Mean + SD of field-based measurements for the three populations of origin. Significant differences ($\alpha = 0.05$) in average trait values are indicated by different uppercase letters and significant differences in variance are indicated by different lowercase letters.

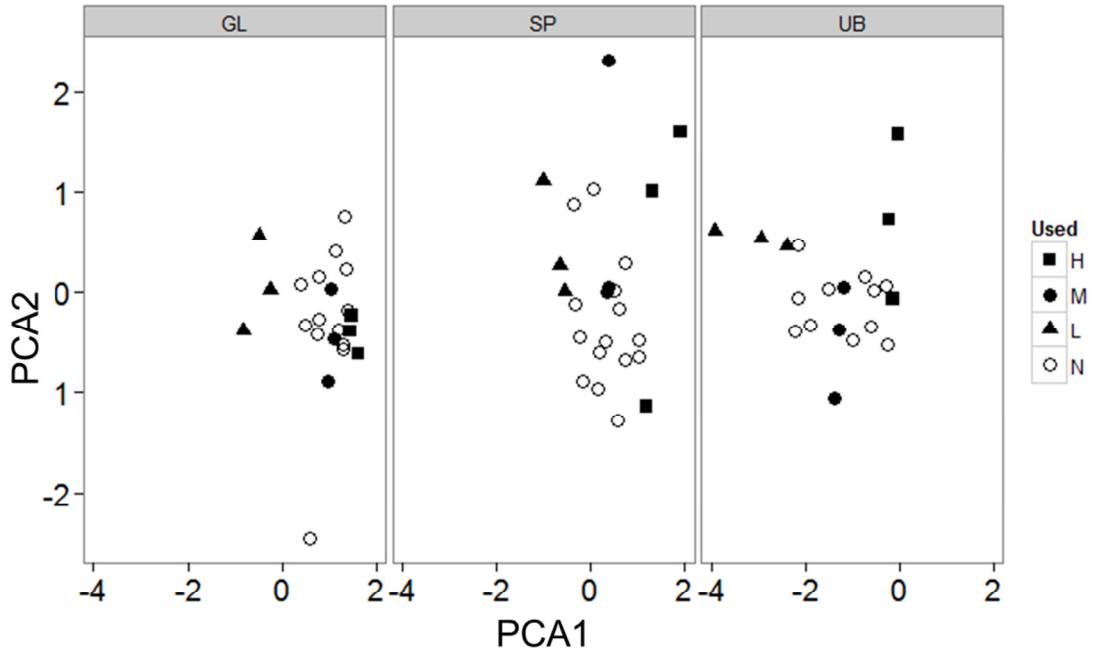

Figure 5.3 Principal Components Analysis of SLA, plant diameter and seed mass for maternal lines from GL, SP, and UB. Populations were analyzed together, but are shown in separate graphs for clarity. PC 1 explained 49% of observed variation. Filled symbols indicate maternal "high", "medium" and "low" lines from each population used in the study, while open symbols indicate maternal lines included in figure 5.2 but not grown in this study.

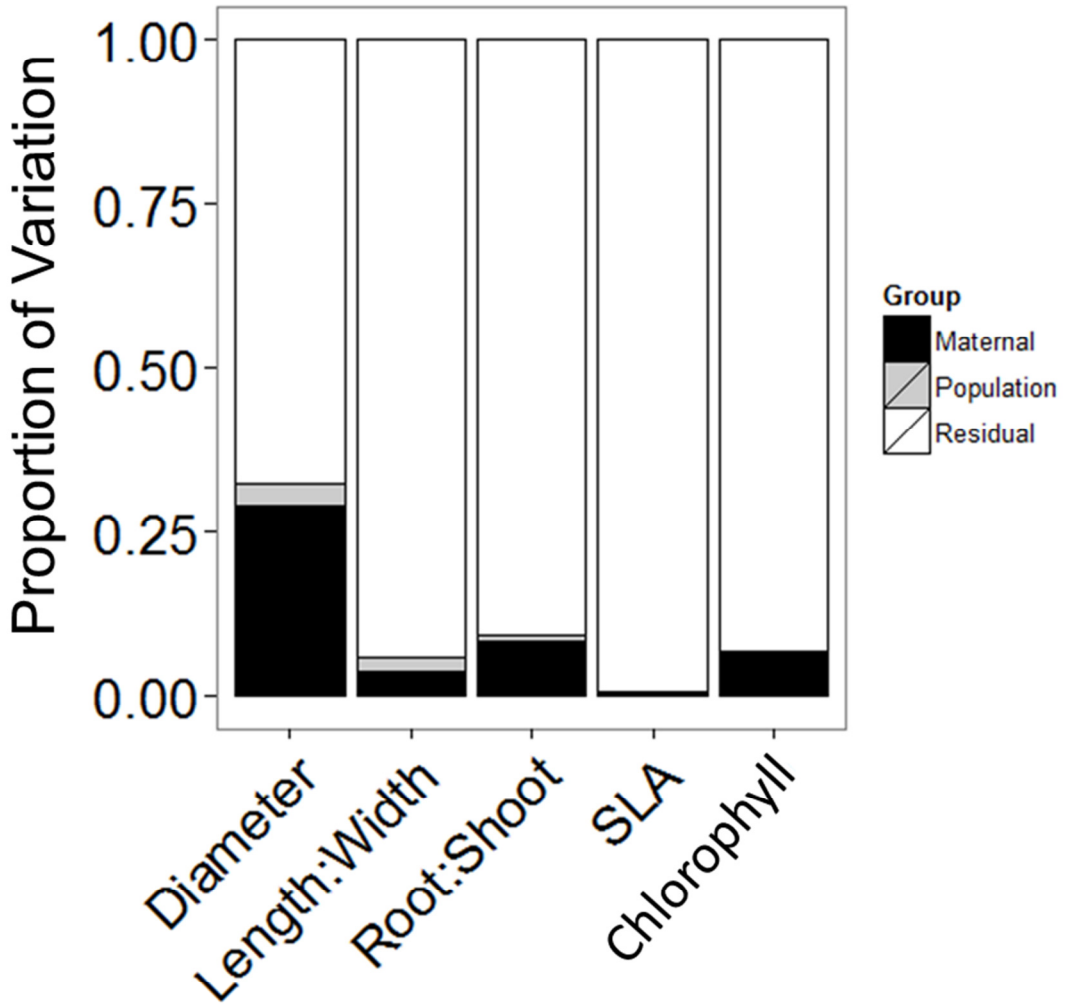

Figure 5.4 Proportion of total random effects variance explained by population of origin, maternal line, and residual variance (representing variance between individuals) for the five measured traits.

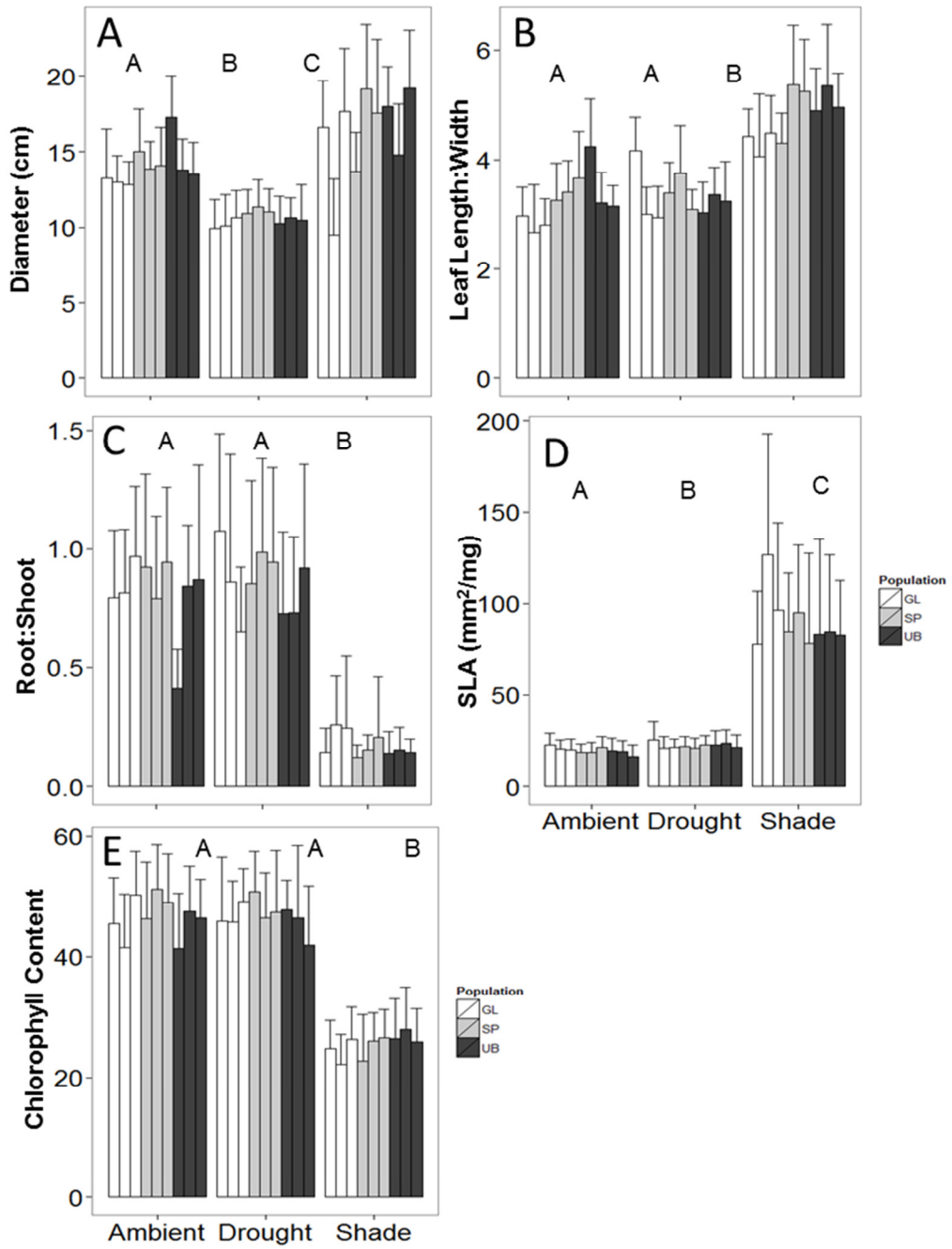

Figure 5.5 Mean (+ SD) values of A) final diameter, B) leaf length: width, C) root:shoot ratio, D) SLA, and E) final relative chlorophyll content for each maternal line (individual bar, arranged from low to high along based on scores from PC 1) in each population (unique color; defined in Table 5.1) for each treatment. Significant treatment differences ($\alpha = 0.05$) are indicated by different letters.

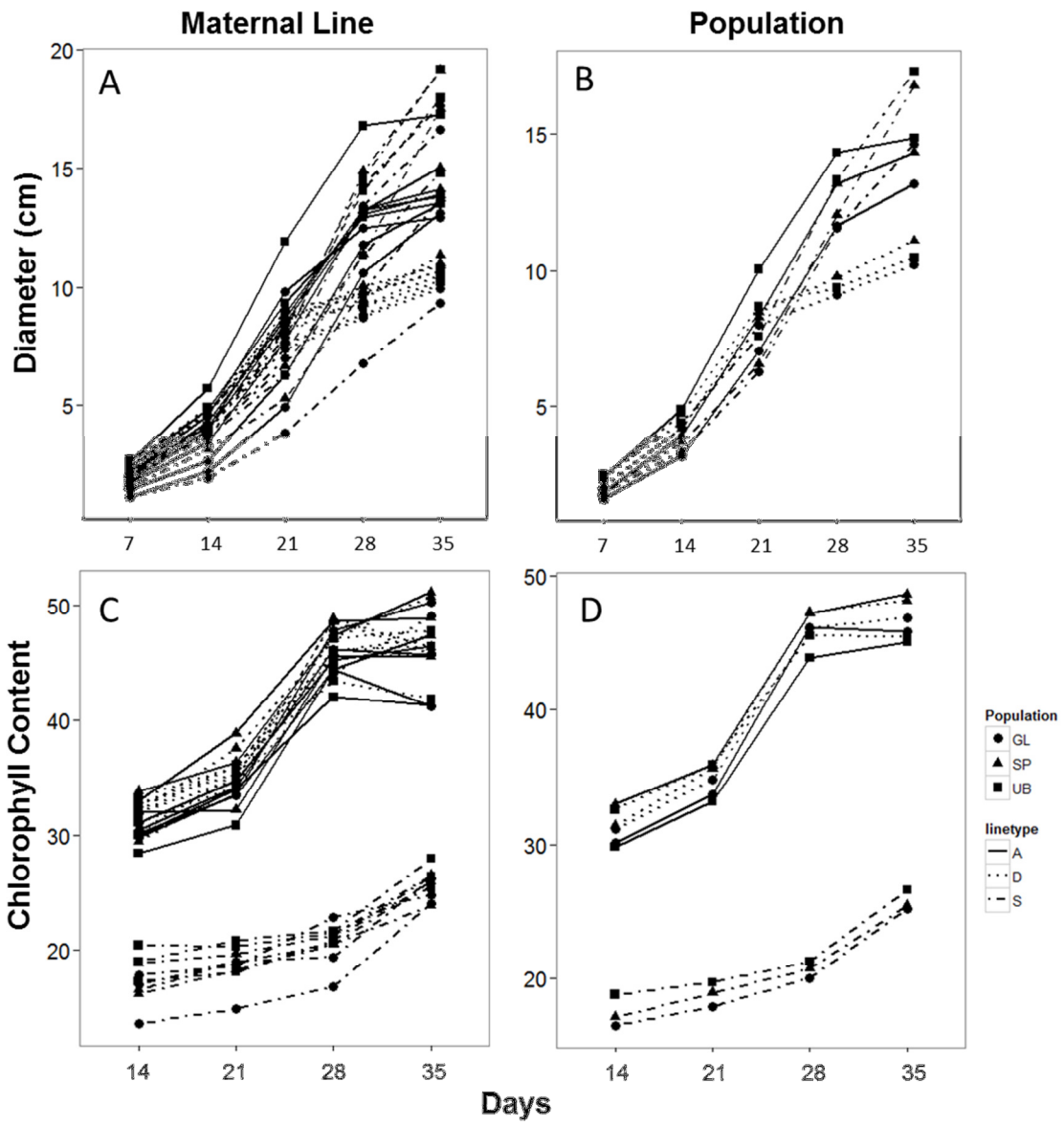

Figure 5.6 Change in average trait values for diameter (figures A and B) and relative chlorophyll content (Figures C and D) for individual maternal lines and populations. Variation among individuals within maternal lines and populations is not shown to maintain clarity.

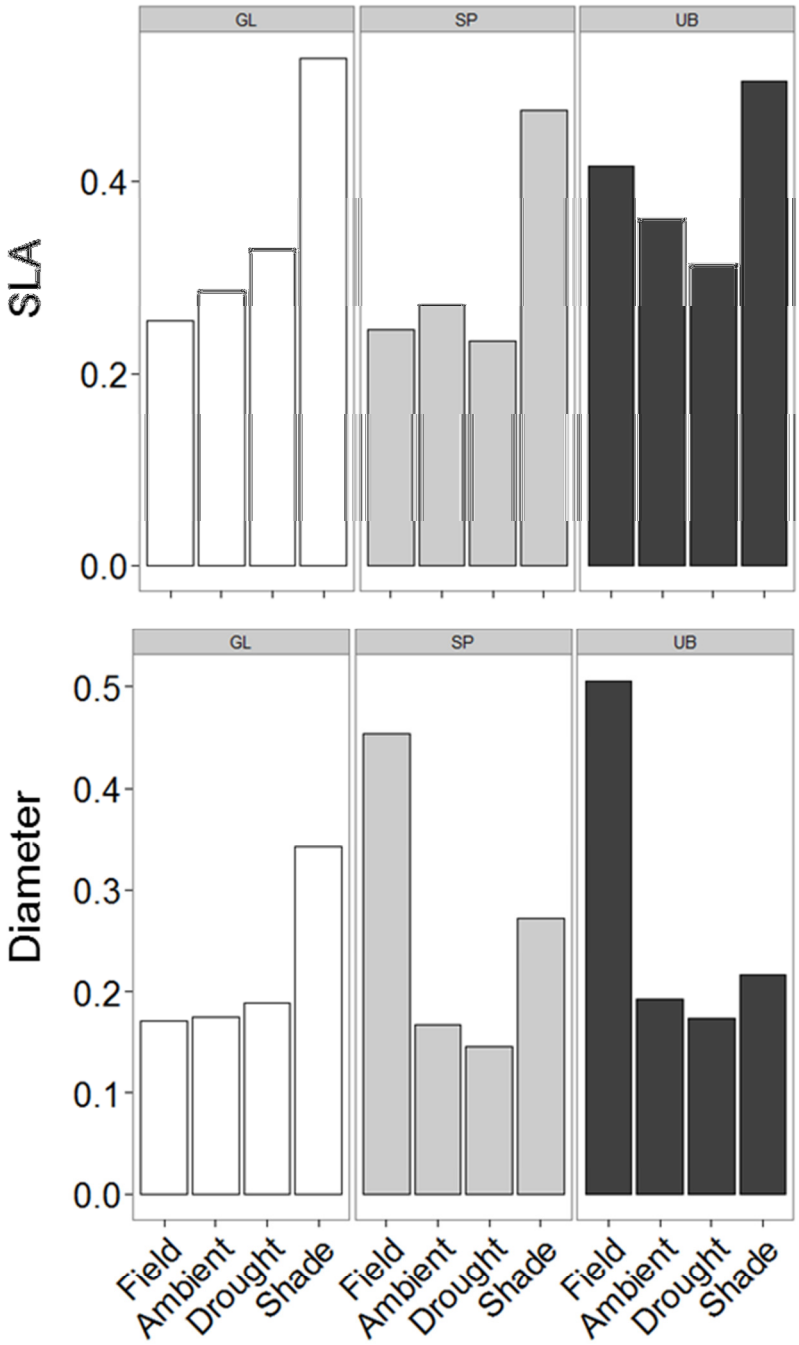

Figure 5.7 Coefficients of Variation for SLA and plant diameter measured in the field and under various abiotic conditions (ambient, 50% drought, 80% shade) in the greenhouse for each population of origin.

Chapter 6: Synthesis

While researching the extent, drivers and impacts of intraspecific functional trait variation, I found that although plant functional groups may respond predictably to alterations of soil C:N, and that these alterations may reduce cover of exotic grassland species, some exotic species do not follow a predictable pattern (Chapter 2). High variability in plant traits has been suggested as one reason why some species are able to persist across a wide range of environmental condition and filters (Diaz et al. 1998). The findings of chapter 2 support the idea that some species possess unique traits that allowed them to escape the filter imposed by altered soil C:N.

Previous research using plant functional traits has often assumed that traits varied minimally within species, and that species exhibited an "average" trait value applicable to all populations. I found that, for at least one species that was particularly resilient to alterations to soil C:N, there was very high intraspecific variation both within and across populations, and that published trait averages were a poor fit for several populations (Chapter 3). My findings support earlier studies demonstrating that intraspecific variation in plant traits can be high, and is worthy of consideration in a broader ecological context (Albert et al. 2010b, 2010a, 2011, Violle et al. 2012a). Indeed, high intraspecific variation may be more widespread than previously assumed, as I found that current methods for quantifying and comparing intraspecific variation often eliminate or minimize differences between individuals through transformation and commonly applied statistical techniques (Chapter 3). Using a Bayesian approach allows ecologists to directly quantify and compare variation in plant functional traits in a nuanced way not demonstrated by other statistical approaches. Furthermore, Bayesian models can be updated

with new information, and changes in plant functional traits at multiple scales can be quantified using these approaches.

Intraspecific variability in plant functional traits may allow species to shift their average trait value and/or the variance around that value in response to competition. This shifting may contribute to the maintenance of biodiversity in ecosystems, despite increased competitive pressures and functional redundancy. I found that species do shift the distribution of their functional traits in response to changes in the biotic community in a way that leads to less trait overlap between species, although this response was not directional (Chapter 4). Species shifted idiosyncratically: some shifted their means, other their variance, others both, and some did not shift at all. These results indicate that not only are traits not fixed around a species "average", but that species can dynamically alter them in response to biotic conditions during growth. This shifting may allow species that are functionally similar to coexist in the face of limiting resources, a process that may contribute to the maintenance of biodiversity.

Although plants can display high intraspecific variation in functional traits, what drives this observed variation is less studied. I found that, for *H. radicata*, trait variation was predominantly driven by plastic responses to abiotic conditions (Chapter 5). It is unclear whether this high trait plasticity can be generalized to other species however, as *H. radicata* is globally widespread and invasive. Previous research has indicated that such species may display greater plasticity or more genetic variability that non-invasive species (Gitzendanner and Soltis 2000, Sultan et al. 2009). In addition, I found that plant traits varied over the ontogeny of an individual, a fact that is often unaccounted for in trait collections. This temporal variability may be one driver of the variation recorded in the field, and may serve to reduce competition between species through temporal niche partitioning. Finally, I found that the amount of intraspecific

variation in a trait was not itself a functional trait (i.e. variation measured in the field was not representative of variation measured under greenhouse conditions). These results support the idea that some plant traits are highly plastic and largely a response to prevailing abiotic conditions.

These findings, taken together, demonstrate that intraspecific trait variation is widespread and is affected by both biotic and abiotic conditions. Care must be taken in how intraspecific variation is quantified, and further research should be conducted to assess the generality of these findings. Future research should examine how multiple abiotic filters can interact to effect trait expression, as species are simultaneously experiencing multiple filters in field conditions. When and how traits shift in response to the biotic community should also be examined in greater detail, using the entire plant community, rather than a suite of species. Such a study would demonstrate whether species consistently shift traits to reduce intraspecific competition, and to limit similarity. Finally, future research should examine whether the trait plasticity expressed by *H. radicata* is generalizable, unique to that species, or a characteristic of widespread and weedy species.

References

Al, R., H. Khaled, M. Duru, J. P. Theau, S. Plantureux, A. H. Khaled, and J. Pierre. 2005. Variation in leaf traits through seasons and N-availability levels and its consequences for ranking grassland species. Journal of Vegetation Science 16:391–398.

Albert, C. H., F. de Bello, I. Boulangeat, G. Pellet, S. Lavorel, and W. Thuiller. 2012. On the importance of intraspecific variability for the quantification of functional diversity. Oikos 121:116–126. doi: 10.1111/j.1600-0706.2011.19672.x.

Albert, C. H., F. Grassein, F. M. Schurr, G. Vieilledent, and C. Violle. 2011. When and how should intraspecific variability be considered in trait-based plant ecology? Perspectives in Plant Ecology, Evolution and Systematics 13:217–225. doi: 10.1016/j.ppees.2011.04.003.

Albert, C. H., W. Thuiller, N. G. Yoccoz, R. Douzet, S. Aubert, and S. Lavorel. 2010a. A multi-trait approach reveals the structure and the relative importance of intra- vs. interspecific variability in plant traits. Functional Ecology 24:1192–1201. doi: 10.1111/j.1365-2435.2010.01727.x.

Albert, C. H., W. Thuiller, N. G. Yoccoz, A. Soudant, F. Boucher, P. Saccone, and S. Lavorel. 2010b. Intraspecific functional variability: extent, structure and sources of variation. Journal of Ecology 98:604–613. doi: 10.1111/j.1365-2745.2010.01651.x.

Anderson, M. 2004. PERMDISP: a FORTRAN computer program for permutational analysis of multivariate dispersions (for any two-factor ANOVA design) using permutation. Department of Statistics, University of Auckland, Auckland, New Zealand.

Anderson, M. J. 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecology 26:32–46. Wiley Online Library. doi: 10.1046/j.1442-9993.2001.01070.x.

Auld, J. R., A. a Agrawal, and R. a Relyea. 2010. Re-evaluating the costs and limits of adaptive phenotypic plasticity. Proceedings of the Royal Society B. 277:503–11. doi: 10.1098/rspb.2009.1355.

Baruch, Z. 2011. Leaf trait variation of a dominant neotropical savanna tree across rainfall and fertility gradients. Acta Oecologica 37:455–461. Elsevier Masson SAS. doi: 10.1016/j.actao.2011.05.014.

Begg, G. S., J. Wishart, M. W. Young, G. R. Squire, and P. P. M. Iannetta. 2012. Genetic structure among arable populations of *Capsella bursa-pastoris* is linked to functional traits and in-field conditions. Ecography 35:446–457. doi: 10.1111/j.1600-0587.2011.07030.x.

Bilton, M. C., R. Whitlock, J. P. Grime, G. Marion, and R. J. Pakeman. 2010. Intraspecific trait variation in grassland plant species reveals fine-scale strategy trade-offs and size differentiation that underpins performance in ecological communities. Botany 88:939–952. doi: 10.1139/B10-065.

Bolker, B. M., M. E. Brooks, C. J. Clark, S. W. Geange, J. R. Poulsen, M. H. H. Stevens, and J.-S. S. White. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. Trends in Ecology & Evolution 24:127–35. doi: 10.1016/j.tree.2008.10.008.

Bolnick, D. I., P. Amarasekare, M. S. Araújo, R. Bürger, J. M. Levine, M. Novak, V. H. W. Rudolf, S. J. Schreiber, M. C. Urban, and D. a Vasseur. 2011. Why intraspecific trait variation matters in community ecology. Trends in Ecology & Evolution 26:183–92. doi: 10.1016/j.tree.2011.01.009.

Bonser, S. P., B. Ladd, K. Monro, M. D. Hall, and M. a Forster. 2010. The adaptive value of functional and life-history traits across fertility treatments in an annual plant. Annals of botany 106:979–88. doi: 10.1093/aob/mcq195.

Burns, J., and S. Strauss. 2012. Effects of competition on phylogenetic signal and phenotypic plasticity in plant functional traits. Ecology 93:126–137.

Cadotte, M. W., K. Carscadden, and N. Mirotchnick. 2011. Beyond species: Functional diversity and the maintenance of ecological processes and services. Journal of Applied Ecology 48:1079–1087. doi: 10.1111/j.1365-2664.2011.02048.x.

Calow, P. 1987. Towards a definition of functional ecology. Functional Ecology 1:57–61.

Choy, S. L., R. O'Leary, and K. Mengersen. 2009. Elicitation by design in ecology: Using expert opinion to inform priors for Bayesian statistical models. Ecology 90:265–77.

Cianciaruso, M. V, M. a Batalha, K. J. Gaston, and O. L. Petchey. 2009. Including intraspecific variability in functional diversity. Ecology 90:81–9.

Clark, J. S. 2010a. Individuals and the variation needed for high species diversity in forest trees. Science (New York, N.Y.) 327:1129–32. doi: 10.1126/science.1183506.

Clark, J. S. 2010b. Individuals and the variation needed for high species diversity in forest trees. Science (New York, N.Y.) 327:1129–32. doi: 10.1126/science.1183506.

Clarke, K., and R. Gorley. 2006. Primer v6: User Manual/Tutorial. PRIMER-E, Plymouth.

Cordell, S., G. Goldstein, D. Mueller-Dombois, D. Webb, and P. M. Vitousek. 1998. Physiological and morphological variation in *Metrosideros polymorpha* , a dominant Hawaiian tree species, along an altitudinal gradient: the role of phenotypic plasticity. Oecologia 113:188–196. doi: 10.1007/s004420050367.

Cornelissen, A. J. H. C., B. Cerabolini, J. P. Puyravaud, M. Maestro, M. J. A. Werger, and R. Aerts. 2003a. Functional traits of woody plants : correspondence of species rankings between field adults and laboratory-grown seedlings ? Functional traits of woody plants : correspondence of species rankings between field adults and laboratory-grown seedlings ? Journal of Vegetation Science 14:311–322.

Cornelissen, J., S. Lavorel, E. Garnier, S. Diaz, N. Buchmann, D. Gurvich, P. B. Reich, H. Ter Steege, H. Morgan, M. Van Der Heijden, J. Pausas, and H. Poorter. 2003b. A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. Australian Journal of Botany 51:335–380. CSIRO.

Crutsinger, G. M., L. Souza, and N. J. Sanders. 2008. Intraspecific diversity and dominant genotypes resist plant invasions. Ecology letters 11:16–23. doi: 10.1111/j.1461-0248.2007.01118.x.

Crutsinger, G. M., S. Y. Strauss, and J. A. Rudgers. 2010. Genetic variation within a dominant shrub species determines plant species colonization in a coastal dune ecosystem. Ecology 91:1237–43.

Dainese, M., and T. Sitzia. 2013. Assessing the influence of environmental gradients on seed mass variation in mountain grasslands using a spatial phylogenetic filtering approach. Perspectives in Plant Ecology, Evolution and Systematics 15:12–19. Elsevier GmbH. doi: 10.1016/j.ppees.2012.10.005.

Davidson, A. M., M. Jennions, and A. B. Nicotra. 2011. Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. Ecology Letters 14:419–31. doi: 10.1111/j.1461-0248.2011.01596.x.

Díaz, S., and M. Cabido. 2001. Vive la différence : plant functional diversity matters to ecosystem processes 16:646–655.

Diaz, S., M. Cabido, and F. Casanoves. 1998. Plant functional traits and environmental filters at a regional scale. Journal of Vegetation Science 9:113–122.

Diaz, S., J. G. Hodgson, K. Thompson, M. Cabido, J. H. C. Cornelissen, A. Jalili, G. Montserrat-Martí, J. P. Grime, F. Zarrinkamar, Y. Asri, S. R. Band, S. Basconcelo, P. Castro-Díez, G. Funes, B. Hamzehee, M. Khoshnevi, N. Pérez-Harguindeguy, M. C. Pérez-Rontomé, F. A. Shirvany, F. Vendramini, S. Yazdani, R. Abbas-Azimi, A. Bogaard, S. Boustani, M. Charles, M. Dehghan, L. Torres-Espuny, V. Falczuk, J. Guerrero-Campo, A. Hynd, G. Jones, E. Kowsary, F. Kazemi-Saeed, M. Maestro-Martínez, A. Romo-Díez, S. Shaw, B. Siavash, P. Villar-Salvador, and M. R. Zak. 2004. The plant traits that drive ecosystems: Evidence from three continents. Journal of Vegetation Science 15:295–304. doi: 10.1111/j.1654-1103.2004.tb02266.x.

Díaz, S., S. Lavorel, S. McIntyre, V. Falczuk, F. Casanoves, D. G. Milchunas, C. Skarpe, G. Rusch, M. Sternberg, I. Noy-Meir, J. Landsberg, W. Zhang, H. Clark, and B. D. Campbell. 2007. Plant trait responses to grazing: a global synthesis. Global Change Biology 13:313–341. doi: 10.1111/j.1365-2486.2006.01288.x.

Dimitrakopoulos, P. G., and B. Schmid. 2004. Biodiversity effects increase linearly with biotope space. Ecology Letters 7:574–583. doi: 10.1111/j.1461-0248.2004.00607.x.

Ellison, A. M. 2004. Bayesian inference in ecology. Ecology Letters 7:509–520. doi: 10.1111/j.1461-0248.2004.00603.x.

Fajardo, A., and F. I. Piper. 2011. Intraspecific trait variation and covariation in a widespread tree species (Nothofagus pumilio) in southern Chile. The New Phytologist 189:259–71. doi: 10.1111/j.1469-8137.2010.03468.x.

Fargione, J., and D. Tilman. 2005. Niche differences in phenology and rooting depth promote coexistence with a dominant C4 bunchgrass. Oecologia 143:598–606. doi: 10.1007/s00442-005-0010-y.

Firn, J., S. M. Prober, and Y. M. Buckley. 2012. Plastic traits of an exotic grass contribute to its abundance but are not always favourable. PloS one 7:e35870. doi: 10.1371/journal.pone.0035870.

Fonseca, C., J. McC. Overton, B. Collins, and M. Westoby. 2000. Shifts in Trait-Combinations along Rainfall and Phosphorus Gradients. Journal of Ecology 88:964–977.

Frenne, P. De, B. Graae, and A. Kolb. 2011. An intraspecific application of the leaf- height-seed ecology strategy scheme to forest herbs along a latitudinal gradient. Ecography 34:132–140. doi: 10.1111/j.1600-0587.2010.06399.x.

Gelman, A., J. B. Carlin, H. S. Stern, and D. B. Rubin. 2004. Bayesian Data Analysis. (C. Chatfield, M. Tanner, and J. Zidek, Eds.), 2nd edition. Chapman and Hall/CRC, Boca Raton.

Gitzendanner, M., and P. Soltis. 2000. PATTERNS OF GENETIC VARIATION IN RARE AND WIDESPREAD PLANT CONGENERS. American Journal of Botany 87:783–792.

Grassein, F., I. Till-Bottraud, and S. Lavorel. 2010. Plant resource-use strategies: the importance of phenotypic plasticity in response to a productivity gradient for two subalpine species. Annals of botany 106:637–45. doi: 10.1093/aob/mcq154.

Gubsch, M., N. Buchmann, B. Schmid, E.-D. Schulze, A. Lipowsky, and C. Roscher. 2011. Differential effects of plant diversity on functional trait variation of grass species. Annals of Botany 107:157–69. doi: 10.1093/aob/mcq220.

Hansen, C., M. B. Garcia, and B. K. Ehlers. 2013. Water availability and population origin affect the expression of the tradeoff between reproduction and growth in Plantago coronopus. Journal of Evolutionary … 26:1–10. doi: 10.1111/jeb.12114.

Hendrix, S. D., and I.-F. Sun. 1989. Inter- and intraspecific variation in seed mass in seven species of urabellifer. New Phytologist:445–451.

Hudson, J. M. G., G. H. R. Henry, and W. K. Cornwell. 2011. Taller and larger: shifts in Arctic tundra leaf traits after 16 years of experimental warming. Global Change Biology 17:1013–1021. doi: 10.1111/j.1365-2486.2010.02294.x.

Hulshof, C. M., and N. G. Swenson. 2010. Variation in leaf functional trait values within and across individuals and species: an example from a Costa Rican dry forest. Functional Ecology 24:217–223. doi: 10.1111/j.1365-2435.2009.01614.x.

Joshi, J., B. Schmid, M. C. Caldeira, P. G. Dimitrakopoulos, J. Good, R. Harris, a. Hector, K. Huss-Danell, a. Jumpponen, a. Minns, C. P. H. Mulder, J. S. Pereira, a. Prinz, M. Scherer-Lorenzen, a.-S. D. Siamantziouras, a. C. Terry, a. Y. Troumbis, and J. H. Lawton. 2001. Local adaptation enhances performance of common plant species. Ecology Letters 4:536–544. doi: 10.1046/j.1461-0248.2001.00262.x.

Jung, V., C. Violle, C. Mondy, L. Hoffmann, and S. Muller. 2010a. Intraspecific variability and trait-based community assembly. Journal of Ecology 98:1134–1140. Blackwell Publishing Ltd. doi: 10.1111/j.1365-2745.2010.01687.x.

Jung, V., C. Violle, C. Mondy, L. Hoffmann, and S. Muller. 2010b. Intraspecific variability and trait-based community assembly. Journal of Ecology 98:1134–1140. doi: 10.1111/j.1365-2745.2010.01687.x.

Kabouw, P., W. H. Putten, N. M. Dam, and A. Biere. 2010. Effects of intraspecific variation in white cabbage (Brassica oleracea var. capitata) on soil organisms. Plant and Soil 336:509–518. doi: 10.1007/s11104-010-0507-y.

Karley, A., and C. Hawes. 2008. Intraspecific variation in *Capsella bursa- pastoris* in plant quality traits for insect herbivores. Weed research 48:147–156. Wiley Online Library.

Keddy, P. A. 1992. Assembly and response rules : two goals for predictive community ecology. Journal of Vegetation Science:157–164.

Kennedy, T. A., S. Naeem, K. M. Howe, J. M. H. Knops, D. Tilman, and P. Reich. 2002. Biodiversity as a barrier to ecological invasion. Nature 417:636–638.

Kleyer, M., R. M. Bekker, I. C. Knevel, J. P. Bakker, K. Thompson, M. Sonnenschein, P. Poschlod, J. M. van Groenendael, L. Klimeš, J. Klimešová, S. Klotz, G. M. Rusch, M. Hermy, D. Adriaens, G. Boedeltje, B. Bossuyt, A. Dannemann, P. Endels, L. Götzenberger, J. G. Hodgson, A.-K. Jackel, I. Kühn, D. Kunzmann, W. A. Ozinga, C. Römermann, M. Stadler, J. Schlegelmilch, H. J. Steendam, O. Tackenberg, B. Wilmann, J. H. C. Cornelissen, O. Eriksson, E. Garnier, and B. Peco. 2008. The LEDA Traitbase: a database of life-history traits of the Northwest European flora. Journal of Ecology 96:1266–1274. doi: 10.1111/j.1365-2745.2008.01430.x.

Knops, J. M., and K. Reinhart. 2000. Specific leaf area along a nitrogen fertilization gradient. The American Midland Naturalist 144:265–272. doi: 10.1674/0003-0031(2000)144[0265:SLAAAN]2.0.CO;2.

Kuhnert, P. M., T. G. Martin, and S. P. Griffiths. 2010. A guide to eliciting and using expert knowledge in Bayesian ecological models. Ecology Letters 13:900–14. doi: 10.1111/j.1461-0248.2010.01477.x.

Kyle, G., and M. R. Leishman. 2009. Functional trait differences between extant exotic, native and extinct native plants in the Hunter River, NSW: a potential tool in riparian rehabilitation. River Research and Applications 25:892–903. Wiley Online Library. doi: 10.1002/rra.

Lankau, R. a. 2011. Intraspecific variation in allelochemistry determines an invasive species' impact on soil microbial communities. Oecologia 165:453–63. doi: 10.1007/s00442-010-1736-8.

Laughlin, D. C. 2011. Nitrification is linked to dominant leaf traits rather than functional diversity. Journal of Ecology 99:1091–1099. doi: 10.1111/j.1365-2745.2011.01856.x.

Laughlin, D. C., P. Z. Fulé, D. W. Huffman, J. Crouse, and E. Laliberté. 2011. Climatic constraints on trait-based forest assembly. Journal of Ecology 99:1489–1499. doi: 10.1111/j.1365-2745.2011.01885.x.

Laughlin, D. C., C. Joshi, P. M. van Bodegom, Z. a Bastow, P. Z. Fulé, and T. Fukami. 2012. A predictive model of community assembly that incorporates intraspecific trait variation. Ecology Letters 15:1291–9. doi: 10.1111/j.1461-0248.2012.01852.x.

Laughlin, D., J. Leppert, M. Moore, and C. Sieg. 2010. A multi-trait test of the leaf-height-seed plant strategy scheme with 133 species from a pine forest flora. Functional Ecology 24:493–501. doi: 10.1111/j.1365-2435.2009.01672.x.

Lavorel, S., K. Grigulis, P. Lamarque, M.-P. Colace, D. Garden, J. Girel, G. Pellet, and R. Douzet. 2011. Using plant functional traits to understand the landscape distribution of multiple ecosystem services. Journal of Ecology 99:135–147. doi: 10.1111/j.1365-2745.2010.01753.x.

Lavorel, S., S. McIntyre, J. Landsberg, and T. D. A. Forbes. 1997. Plant functional classifications: from general groups to specific groups based on response to disturbance. Trends in Ecology & Evolution 12:474–478.

Lecerf, A., and E. Chauvet. 2008. Intraspecific variability in leaf traits strongly affects alder leaf decomposition in a stream. Basic and Applied Ecology 9:598–605. doi: 10.1016/j.baae.2007.11.003.

Leger, E. A., E. K. Espeland, K. R. Merrill, and S. E. Meyer. 2009. Genetic variation and local adaptation at a cheatgrass (*Bromus tectorum*) invasion edge in western Nevada. Molecular Ecology 18:4366–79. doi: 10.1111/j.1365-294X.2009.04357.x.

Lele, S. R., and B. Dennis. 2009. Bayesian methods for hierarchical models: are ecologists making a Faustian bargain? Ecological Applications 19:581–584.

Lepš, J., F. de Bello, P. Šmilauer, and J. Doležal. 2011. Community trait response to environment: disentangling species turnover vs intraspecific trait variability effects. Ecography:no–no. doi: 10.1111/j.1600-0587.2010.06904.x.

Long, W., R. Zang, B. S. Schamp, and Y. Ding. 2011. Within- and among-species variation in specific leaf area drive community assembly in a tropical cloud forest. Oecologia 167:1103–1113. doi: 10.1007/s00442-011-2050-9.

López, R., J. Climent, and L. Gil. 2009. Intraspecific variation and plasticity in growth and foliar morphology along a climate gradient in the Canary Island pine. Trees 24:343–350. doi: 10.1007/s00468-009-0404-2.

Lunn, D., D. Spiegelhalter, A. Thomas, and N. Best. 2009. The BUGS project: Evolution, critique and future directions. Statistics in Medicine 28:3049–3067. doi: 10.1002/sim.

Maharjan, S., L. Poorter, and M. Holmgren. 2011. Plant functional traits and the distribution of West African rain forest trees along the rainfall gradient. Biotropica 43:552–561.

Mason, N., D. Mouillot, W. Lee, and J. Wilson. 2005. Functional richness, functional evenness and functional divergence: the primary components of functional diversity. Oikos 1:112–118.

Mason, N. W. H., F. de Bello, J. Doležal, and J. Lepš. 2011. Niche overlap reveals the effects of competition, disturbance and contrasting assembly processes in experimental grassland communities. Journal of Ecology 99:788–796. doi: 10.1111/j.1365-2745.2011.01801.x.

McGill, B. J., B. J. Enquist, E. Weiher, and M. Westoby. 2006. Rebuilding community ecology from functional traits. Trends in Ecology & Evolution 21:178–85. doi: 10.1016/j.tree.2006.02.002.

Messier, J., B. J. McGill, and M. J. Lechowicz. 2010. How do traits vary across ecological scales? A case for trait-based ecology. Ecology letters 13:838–48. doi: 10.1111/j.1461-0248.2010.01476.x.

Meziane, D., and B. Shipley. 1999. Interacting determinants of specific leaf area in 22 herbaceous species: effects of irradiance and nutrient availability. Plant, Cell & Environment 22:447–459.

Michaels, H., B. Benner, and A. Hartgerink. 1988. Seed size variation: magnitude, distribution, and ecological correlates. Evolutionary Ecology:157–166.

Mitchell, R. M., and J. D. Bakker. (n.d.). Quantifying and compariting intraspecific functional trait variablity: A case study with Hypochaeris radicata. In Review.

Mitchell, R. M., and J. D. Bakker. 2011. Carbon Addition as a Technique for Controlling Exotic Species in Pacific Northwest Prairies. Northwest Science 85:247–254.

Mix, C., P. F. P. Arens, R. Rengelink, M. J. M. Smulders, J. M. Van Groenendael, and N. J. Ouborg. 2006. Regional gene flow and population structure of the wind-dispersed plant species *Hypochaeris radicata* (Asteraceae) in an agricultural landscape. Molecular Ecology 15:1749–58. doi: 10.1111/j.1365-294X.2006.02887.x.

Moles, A. T., D. I. Warton, L. Warman, N. G. Swenson, S. W. Laffan, A. E. Zanne, A. Pitman, F. a. Hemmings, and M. R. Leishman. 2009. Global patterns in plant height. Journal of Ecology 97:923–932. doi: 10.1111/j.1365-2745.2009.01526.x.

Moles, A. T., and M. Westoby. 2003. Latitude, seed predation and seed mass. Journal of Biogeography 30:105–128. doi: 10.1046/j.1365-2699.2003.00781.x.

Moreira, B., C. Tavsanoglu, and J. G. Pausas. 2012. Local versus regional intraspecific variability in regeneration traits. Oecologia 168:671–7. doi: 10.1007/s00442-011-2127-5.

Myers, J. a., J. M. Chase, I. Jiménez, P. M. Jørgensen, A. Araujo-Murakami, N. Paniagua-Zambrana, and R. Seidel. 2012. Beta-diversity in temperate and tropical forests reflects dissimilar mechanisms of community assembly. Ecology Letters:n/a–n/a. doi: 10.1111/ele.12021.

Nakamura, I., Y. Onoda, N. Matsushima, J. Yokoyama, M. Kawata, and K. Hikosaka. 2011. Phenotypic and genetic differences in a perennial herb across a natural gradient of CO2 concentration. Oecologia 165:809–18. doi: 10.1007/s00442-010-1900-1.

Nouvellon, Y., J.-P. Laclau, D. Epron, A. Kinana, A. Mabiala, O. Roupsard, J.-M. Bonnefond, G. le Maire, C. Marsden, and J.-D. Bontemps. 2010. Within-stand and seasonal variations of specific leaf area in a clonal Eucalyptus plantation in the Republic of Congo. Forest Ecology and Management 259:1796–1807. doi: 10.1016/j.foreco.2009.05.023.

Ortiz, M. A., S. Talavera, J. L. Garcia-Castano, K. Tremetsberger, T. Stuessy, F. Balao, and R. Casimiro-Soriguer. 2006. Self-Incompatibility and Floral Parameters in Hypochaers sec. Hypocaheris (Asteraceae). American Journal of Botany 93:234–244.

Petchey, O. L., and K. J. Gaston. 2002. Functional diversity (FD), species richness and community composition. Ecology Letters 5:402–411. doi: 10.1046/j.1461-0248.2002.00339.x.

Pezzani, F., and C. Montaña. 2006. Inter- and intraspecific variation in the germination response to light quality and scarification in grasses growing in two-phase mosaics of the Chihuahuan Desert. Annals of Botany 97:1063–71. doi: 10.1093/aob/mcl053.

Pico, F., N. J. Ouborg, and J. M. Van Groenendael. 2004. Influence of selfing and maternal effects on life- cycle traits and dispersal ability in the herb *Hypochaeris radicata* (Asteraceae). Botanical Journal of the Linnean Society 146:163–170.

Pontes, L. D. S., J. F. Soussana, F. Louault, D. Andueza, and P. Carrère. 2007. Leaf traits affect the above-ground productivity and quality of pasture grasses. Functional Ecology 21:844–853. doi: 10.1111/j.1365-2435.2007.01316.x.

Poorter, H. 1990. Leaf area ratio and net assimilation rate of 24 wild species differing in relative growth rate. Oecologia 83:553–559.

Poorter, H. 1998. Photosynthetic nitrogen-use efficiency of species that differ inherently in specific leaf area. Oecologia 116:26–37.

Prior, L., D. Bowman, and D. Eamus. 2005. Intra-specific variation in leaf attributes of four savanna tree species across a rainfall gradient in tropical Australia. Australian Journal of Botany 53:323–335.

Pruitt, J., and M. Ferrari. 2011. Intraspecific trait variants determine the nature of interspecific interactions in a habitat-forming species. Ecology 92:1902–1908.

Qian, S. S., and Z. Shen. 2007. Ecological applications of multilevel analysis of variance. Ecology 88:2489–95.

R Development Core Team. 2011. R: A language and environment for statistical computing. R Foundation for Statitical Computing, Vienna, Austria.

Ramirez-Valiente, J. A., D. Sanchez-Gomez, I. Aranda, and F. Valladares. (n.d.). Phenotypic plasticity and local adaptation in leaf ecophysiological traits of 13 contrasting cork oak populations under different water availabilities. Tree Physiology 30:618–627.

Ramírez-Valiente, J. A., D. Sánchez-Gómez, I. Aranda, and F. Valladares. 2010. Phenotypic plasticity and local adaptation in leaf ecophysiological traits of 13 contrasting cork oak populations under different water availabilities. Tree physiology 30:618–27. doi: 10.1093/treephys/tpq013.

Reich, P. B., M. B. Walters, and D. Ellsworth. 1992. Leaf life-span in relation to leaf, plant and stand characteristics among diverse ecosystems. Ecological Monographs 62:365–392.

Roach, D., and R. Wulff. 1987. Maternal effects in plants. Annual Review of Ecology and Systematics 18:209–235.

Robson, T. M., D. Sánchez-Gómez, F. J. Cano, and I. Aranda. 2012. Variation in functional leaf traits among beech provenances during a Spanish summer reflects the differences in their origin. Tree Genetics & Genomes 8:1111–1121. doi: 10.1007/s11295-012-0496-5.

Roscher, C., B. Schmid, N. Buchmann, A. Weigelt, and E.-D. Schulze. 2011. Legume species differ in the responses of their functional traits to plant diversity. Oecologia 165:437–52. doi: 10.1007/s00442-010-1735-9.

Ruhnke, H., M. Schädler, S. Klotz, D. Matthies, and R. Brandl. 2009. Variability in leaf traits, insect herbivory and herbivore performance within and among individuals of four broad-leaved tree species. Basic and Applied Ecology 10:726–736. doi: 10.1016/j.baae.2009.06.006.

Scheepens, J., E. Frei, and J. Stöcklin. 2010. Genotypic and environmental variation in specific leaf area in a widespread Alpine plant after transplantation to different altitudes. Oecologia 164:141–150. Springer Berlin / Heidelberg. doi: 10.1007/s00442-010-1650-0.

Scheffer, M., and E. H. van Nes. 2006. Self-organized similarity, the evolutionary emergence of groups of similar species. Proceedings of the National Academy of Sciences of the United States of America 103:6230–5. doi: 10.1073/pnas.0508024103.

Schlichting, C. 1986. The evolution of phenotypic plasticity in plants. Annual Review of Ecology and Systematics 17:667–693.

Schöb, C., B. J. Butterfield, and F. I. Pugnaire. 2012. Foundation species influence trait-based community assembly. The New phytologist 196:824–34. doi: 10.1111/j.1469-8137.2012.04306.x.

Sharma, G. P., and K. J. Esler. 2008. Phenotypic plasticity among *Echium plantagineum* populations in different habitats of Western Cape, South Africa. South African Journal of Botany 74:746–749. doi: 10.1016/j.sajb.2008.04.006.

Shipley, B., D. Vile, and E. Garnier. 2006. From plant traits to plant communities: a statistical mechanistic approach to biodiversity. Science 314:812–4. doi: 10.1126/science.1131344.

Siefert, A. 2012. Incorporating intraspecific variation in tests of trait-based community assembly. Oecologia 170:767–75. doi: 10.1007/s00442-012-2351-7.

Da Silveira Pontes, L., F. Louault, P. Carrère, V. Maire, D. Andueza, and J.-F. Soussana. 2010. The role of plant traits and their plasticity in the response of pasture grasses to nutrients and cutting frequency. Annals of Botany 105:957–965. Oxford University Press.

Smith, E. A., S. B. Collette, T. A. Boynton, T. Lillrose, M. R. Stevens, M. F. Bekker, D. Eggett, and S. B. St Clair. 2011. Developmental contributions to phenotypic variation in functional leaf traits within quaking aspen clones. Tree physiology 31:68–77. doi: 10.1093/treephys/tpq100.

Sultan, S. E., K. Barton, and A. M. Wilczek. 2009. Contrasting patterns of transgenerational plasticity in ecologically distinct congeners. Ecology 90:1831–1839. doi: doi:10.1890/08-1064.1.

Swenson, N. G., P. Anglada-Cordero, and J. a Barone. 2011. Deterministic tropical tree community turnover: evidence from patterns of functional beta diversity along an elevational gradient. Proceedings. Biological sciences / The Royal Society 278:877–84. doi: 10.1098/rspb.2010.1369.

Swenson, N. G., and M. D. Weiser. 2010. Plant geography upon the basis of functional traits: an example from eastern North American trees. Ecology 91:2234–41.

Thuiller, W., S. Lavorel, G. Midgley, and S. Lavergne. 2004. Relating plant traits and species distributions along bioclimatic gradients for 88 Leucadendron taxa. Ecology 85:1688–1699.

Tilman, D., D. Wedin, and J. Knops. 1996. Productivity and sustainability influenced by biodiversity in grassland ecosystems. Nature 379:718–20.

Violle, C., H. Castro, J. Richarte, and M.-L. Navas. 2009. Intraspecific seed trait variations and competition: passive or adaptive response? Functional Ecology 23:612–620. doi: 10.1111/j.1365-2435.2009.01539.x.

Violle, C., B. J. Enquist, B. J. McGill, L. Jiang, C. H. Albert, C. Hulshof, V. Jung, and J. Messier. 2012a. The return of the variance: intraspecific variability in community ecology. Trends in Ecology & Evolution 27:244–252. doi: 10.1016/j.tree.2011.11.014.

Violle, C., B. J. Enquist, B. J. McGill, L. Jiang, C. H. Albert, C. Hulshof, V. Jung, and J. Messier. 2012b. Viva la variance! A reply to Nakagawa & Schielzeth. Trends in Ecology & Evolution 27:475–476. doi: 10.1016/j.tree.2012.06.004.

Weiher, E., and P. A. Keddy. 1995. Assembly rules, null models and trait dispersion: New questions from old patterns. Oikos 74:159–164.

Westoby, M. 1998. A leaf-height-seed (LHS) plant ecology strategy scheme. Plant and Soil 199:213–227.

William, K. C., H. C. C. Johannes, A. Kathryn, D. Ellen, T. E. Valerie, G. Oscar, E. H. Sarah, H. Bart, K. Hiroko, Pè.-H. Natalia, M. Q. Helen, S. S. Louis, A. W. David, J. W. Ian, A. Rien, D. A. Steven, B. Peter van, B. Victor, C. Alex, V. C. Terry, Dì. Sandra, G. Eric, E. G. Diego, K. Elena, A. K. Julia, R. Jenny, B. R. Peter, A. S. Nadejda, M. V. Vaieretti, and W. Mark. 2008. Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. Ecology Letters 11:1065–1071.

Wilson, P., K. Thompson, and J. Hodgson. 1999. Specific leaf area and leaf dry matter content as alternative predictors of plant strategies. New Phytologist 143:155–162.

Wright, I. J., P. B. Reich, J. H. C. Cornelissen, D. S. Falster, E. Garnier, K. Hikosaka, B. B. Lamont, W. Lee, J. Oleksyn, N. Osada, H. Poorter, R. Villar, D. I. Warton, and M. Westoby. 2005. Assessing the generality of global leaf trait relationships. New Phytologist 166:485–96. doi: 10.1111/j.1469-8137.2005.01349.x.

Wurst, S., N. M. Van Dam, F. Monroy, A. Biere, and W. H. Van der Putten. 2008. Intraspecific variation in plant defense alters effects of root herbivores on leaf chemistry and aboveground herbivore damage. Journal of chemical ecology 34:1360–7. doi: 10.1007/s10886-008-9537-9.

Zuur, A., E. Ieno, N. Walker, A. Savelieve, and G. Smith. 2009. Mixed Effects Models and Extensions in Ecology with R. Springer.

Appendix A: **Supplementary Materials for Chapter 3**

A Brief Summary of Bayesian ANOVA Methods

Bayesian techniques are powerful but relatively uncommon, so we report analysis methods in detail here. Bayesian parameter estimation allows direct estimation of probability distributions of parameters of interest from measured or simulated datasets (for further reading on the general use of Bayesian techniques and their applications to ecology, see Gelman *et al.* 2004; Ellison 2004; Qian & Shen 2007; Choy, O'Leary, & Mengersen 2009; and Kuhnert, Martin, & Griffiths 2010). In its most basic form, Bayesian statistics can be summarized as:

$$\text{Posterior distribution } \alpha \text{ Likelihood} \times \text{Prior distribution} \quad \textbf{(eqn 1)}$$

From the posterior distribution, point estimates and distributions of common moments, such as the mean, median, and standard deviation can be quantified. The distributions of the parameters of interest can be directly compared with a variety of techniques.

We tested for differences among populations in means and standard deviations using hierarchical random effects Bayesian ANOVAs (Gelman *et al.* 2004) with non-homogenous variance and diffuse, non-informative priors. Markov Chain Monte Carlo simulation techniques with Gibb's sampling implemented in OpenBUGS (version 3.2.2, Lunn *et al.* 2009) through R (version 2.14.2, R Development Core Team 2011). Three chains were run for 5000 iterations and, after visual inspection for convergence, the first 500 iterations were discarded as burn-in. Differences within and across populations were evaluated by comparing effect size and credible intervals (CIs) for both estimations of the mean and standard deviation parameters. Effect size was calculated as the differences between posterior parameter estimates for the mean for each

population and the estimated grand-mean for the regional population. Credible intervals are the 95% likelihood of the posterior distribution for a given parameter (i.e. there is a 95% probability that the parameter lies within that interval). Population effect was considered significant if the 95% CI did not bound zero, which represents no difference from the grand mean. Means for populations were considered significantly different if their 95% CIs did not overlap.

Significant differences in trait variation were indicated by a lack of overlap in 95% CIs. Population-level trait distributions were constructed using hyper-parameters estimated in hierarchical models.

Models

For the *i*th individual in the *j*th population, we assume SLA is log-normally distributed with a population level mean (θ_j) and population precision term ($\tau_{[j]}$) (where $\tau = 1/\sigma^2$):

$$SLA_{i,j} \sim ln\ N\ (\theta_j, \tau_{[j]})\ i=1,\ldots,n_j;\ j=1,\ldots,10 \quad \textbf{(eqn 1)}$$

Next, we assume that the population level mean is lognormally distributed with a species-level mean (μ) and population to population level precision term ($\tau_{[j]}$):

$$\theta_j \sim ln\ N(\mu, \tau_{[j]})\ j=1,\ldots,10 \quad \textbf{(eqn 2)}$$

We gave our species level mean μ an uninformative prior:

$$\mu \sim N(0,1) \quad \textbf{(eqn 3)}$$

Finally, we gave our population precision terms uninformative priors:

$$\tau_{[j]} \sim \Gamma(\alpha, \beta) \quad \textbf{(eqn 4)}$$

where:

$$\alpha \sim U(0,10) \quad \textbf{(eqn 7)}$$

$$\beta \sim U(0,10) \quad \textbf{(eqn 8)}$$

For the *i*th individual in the *j*th population, we assume diameter was normally distributed with a population level mean (θj) and population precision term (τ_j)

$$Diameter_{i,j} \sim N(\theta j, \tau_{[j]})\ i=1,\ldots,n_j;\ j=1,\ldots,10 \quad \textbf{(eqn 9)}$$

Next, we assume that the population level mean is normally distributed with a species-level mean (μ) and population level precision term ($\tau_{[j]}$):

$$\theta_j \sim N(\mu, \tau_{[j]})\ j=1,\ldots,10 \quad \textbf{(eqn 10)}$$

We gave our species level mean μ uniformative priors:

$$\mu \sim N(0,100) \quad \textbf{(eqn 11)}$$

And our population precision term $\tau_{[j]}$ uniformative priors:

$$\tau_{[j]} \sim Gamma(\alpha, \beta) \quad \textbf{(eqn 12)}$$

where:

$$\alpha \sim U(0.1, 0.1) \quad \textbf{(eqn 15)}$$

$$\beta \sim U(0.1, 0.1) \quad \textbf{(eqn 16)}$$

Table S.1 Collection site name, map location (Fig. 3), identifying code and total number of samples collected.

Site Name	Collection Site	Code	Number of Samples
Ebey's Landing	A	1	25
Smith Prairie	A	2	10
Bothell	B	3	25
Golden Gardens	B	4	25
Wedgwood	B	5	25
Union Bay Natural Area	B	6	25
Greenhouse	B	7	41
Glacial Heritage Preserve	C	8	25
West Rocky Prairie	C	9	10
Corvallis	D	10	30

Sites are referenced by their identifying code throughout the article.

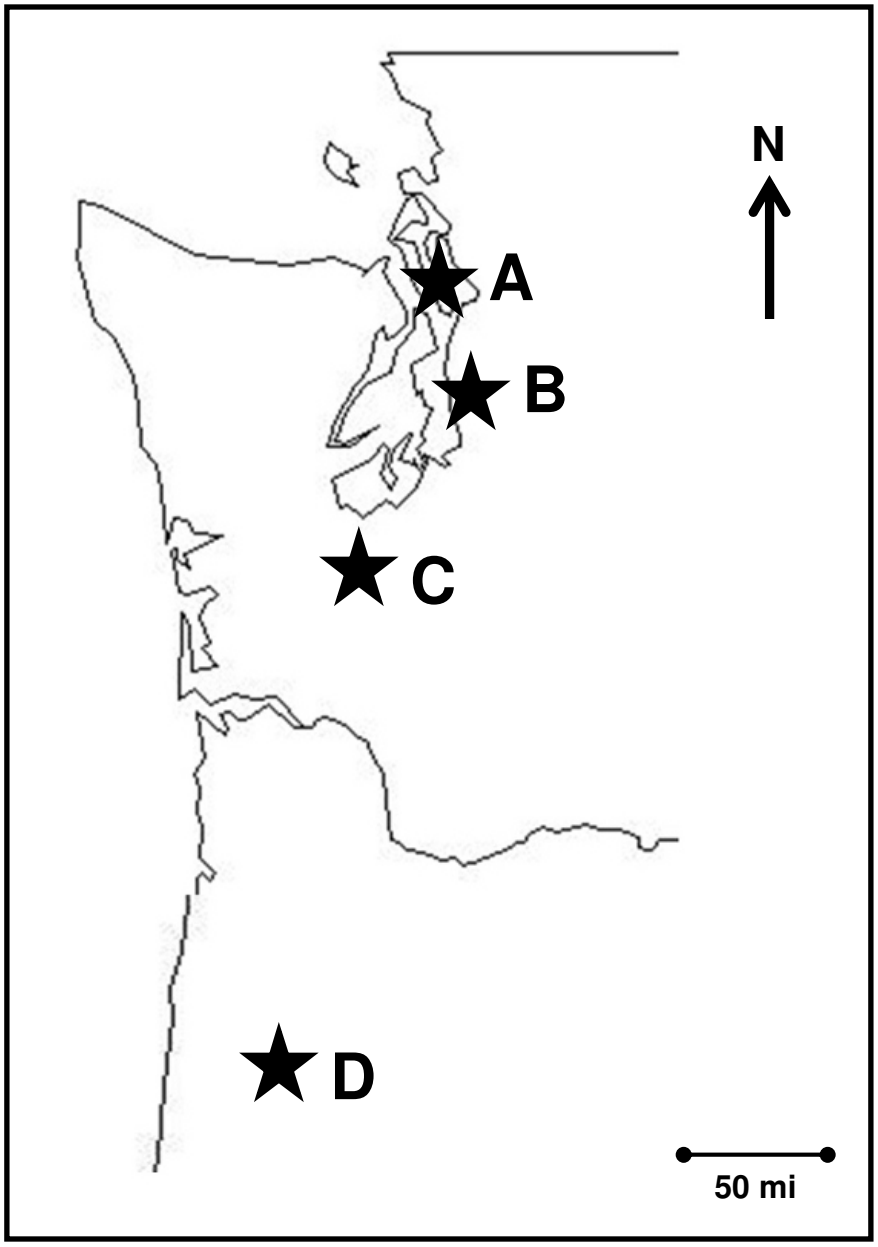

Figure S.1 Map depicting collection sites for the 10 populations. Population details are provided in Table S.1.

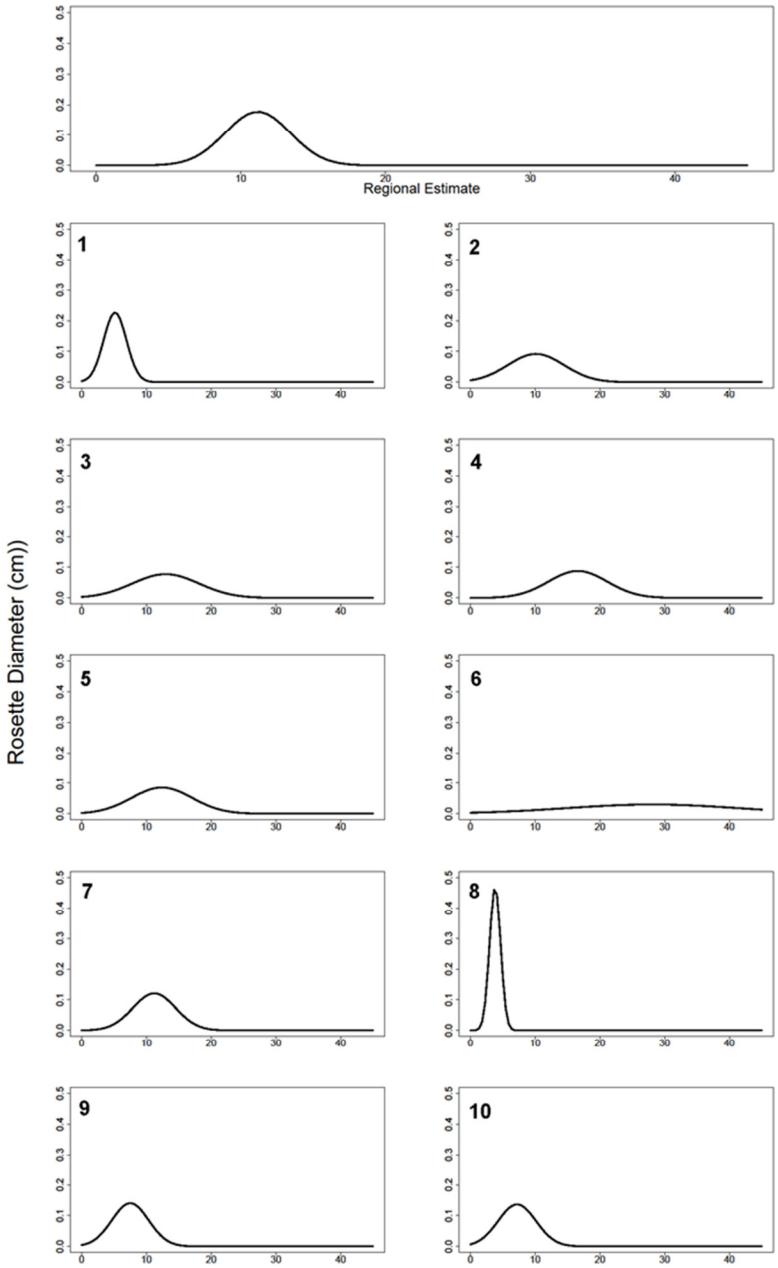

Figure S.2 Posterior distributions of rosette diameter for the regional population (top panel) and 10 populations of *Hypochaeris radicata.*

VITA

Rachel Mitchell was born in Phoenix, Arizona and currently resides in Seattle, Washington. She graduated with a Bachelor of Science in Veterinary Sciences from the University of Arizona in 2006. In 2013, she earned a Doctor of Philosophy at the University of Washington in Forest and Environmental Sciences.