ABSTRACT

Annual manipulations of temperature and rainfall have been maintained in intact calcareous grassland since 1993 at the Buxton Climate Change Impacts Laboratory (BCCIL) in northern England (UK). Here I investigated the role of local adaptation as mechanism of the apparent resistance of species' to long-term climate manipulations at BCCIL using a common forb, *Plantago lanceolata*. *Plantago lanceolata* is a rosette-forming, perennial herb of wide-ranging distribution, and one of the more common forbs in calcareous grasslands, including BCCIL. In the first study I used a common garden approach to test for evidence of selection for different suites of functional traits in *P. lanceolata* populations exposed to chronic summer drought at BCCIL. Results suggest that avoidance strategies associated with high reproductive allocation were more common in populations exposed to long term experimental drought versus populations from controls and that soil depth moderated treatment effects. In the second study I revealed significant treatment based genetic differentiation in *P. lanceolata* populations using molecular markers (AFLPs: Amplified Fragment Length Polymorphisms) that suggests a genetic basis for the functional differentiation evident in the common garden study. Finally I expanded environmental monitoring and trait analyses of *P. lanceolata* to calcareous grassland systems in the landscape surrounding BCCIL in an effort to relate the extent and spatial structure of nested landscape gradients corresponding to soil water supply and demand and the spatial structure of variation in six functional traits that reflect the main axes of functional differentiation found in the common garden study. Abiotic gradients associated with soil water dynamics had distinct spatial structures which in turn promoted the hierarchical partitioning of intraspecific functional diversity in five of the six functional traits measured. Taken together my results suggest a genetic basis for local intraspecific functional differentiation in *P. lanceolata* which in turn has allowed this species to adapt *in situ* to experimental climate manipulations. Furthermore, local and landscape scale gradients in factors related to climate change (e.g., soil moisture) promote functional trait variation at associated scales which may buffer this species from future climatic change.

Local adaptation to climate change in a calcareous grassland system

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Chapter 1

INTRODUCTION

Overview

Global mean temperatures are projected to increase by 1.8-4° C by the end of the 21st century (IPCC 2007). The magnitude and direction of changes in precipitation are much more uncertain and region specific (IPCC 2007), but projections consistently predict more extreme intra-annual precipitation regimes with longer periods of intermittent drought (Easterling et al. 2000, IPCC 2007). Higher temperatures and altered precipitation regimes could potentially expose plants to novel climatic conditions, with no modern analog (Williams et al. 2007). Given that warming stimulates evapotranspiration and reduces soil water availability (e.g., Harte & Shaw, 1995, Rustad et al., 2001; Niu et al., 2008), increased temperatures could exacerbate drought conditions and cause large carbon losses (Angert et al., 2005, Breshears et al., 2005, Ciais et al., 2005) with feedbacks that could exacerbate the pace of ongoing climatic change (Heimann & Reichstein, 2008).

Evidence that climatic changes are affecting ecosystem processes is accumulating rapidly (e.g., Rustad 2008, Wu et al. 2011). Ecosystem process rates such as photosynthesis and respiration both generally increase with temperature (Rustad et al., 2001), and their respective rates dictate the resulting carbon balance. Warming tends to increase plant productivity but ecosystems vary in the magnitude of their response (Rustad et al., 2001, Lin et al., 2010, Wu et al., 2011). Altered precipitation regimes influence community- and ecosystem-level responses to warming (Dukes et al., 2005, Wu et al.,2011). In a recent meta-analysis of 85 studies of ecosystem level responses to warming and precipitation manipulations, warmer temperatures generally increased rates of carbon cycling and temperature based responses were generally stimulated with increased precipitation and reduced under drought conditions (Wu et al. 2011).

Migration and adaptation

Climatic conditions have long been known to be a primary determinant of species' geographic ranges (Schimper 1903), due to the demonstrated influence of temperature and precipitation on plant survival and reproductive success (Woodward 1987, Woodward & Williams 1987). Projected climatic changes, including increased temperatures and more variable precipitation regimes, are expected to impose strong directional selection pressures on plant populations (Davis and Shaw, 2001, Davis et al., 2005, Jump and Penuelas, 2005, Reusch and Wood 2007, Jump et al. 2009a, Anderson et al. 2012). Evidence suggests that significant poleward shifts and upslope migrations have already occurred in a wide array of taxa (Parmesan & Yohe 2003, Parmesan 2006, Walther 2003, 2010). Although these studies suggest that species have the potential to migrate, dispersal limitation (Davis et al. 1986) will likely reduce the ability of species to track the climate to which they are adapted (Davis & Shaw 2001).

While many studies have examined the need and capacity of species to migrate poleward as the climate shifts (e.g., Davis and Shaw 2001, McLachlan *et al.* 2005, Neilson *et al.* 2005, Pearson 2006), the potential for local adaptation remains understudied despite the recognized potential for genetic diversity to buffer species from climate-induced local extinction (Jump and Peñuelas 2005, Jump et al. 2009a). Selection on extant genetic variation is considered to be a primary mechanism by which populations could adapt in-situ to rapid environmental changes (Jump and Penuelas

2009a, Anderson et al. 2012). It is the ‘option value’ of genetic diversity (sensu, Jump and Penuelas 2009a), that makes it such an important component of species potential resistance to climate change (Reusch et al. 2005, Jump and Penuelas 2005, Reusch and Wood 2007, Gienapp et al. 2008, Hoffmann and Willi 2008, Jump et al. 2009a).

Spatial and temporal variation in selection pressures alter the relative fitness of genotypes through space and over time, and thus can be a mechanism by which genetic diversity is maintained in natural populations (Linhart and Grant 1996, Jump et al., 2006, Jump et al. 2009a). Spatial and temporal abiotic heterogeneity combined with biotic factors including competition (Aarssen and Turkington 1985, Taylor and Aarssen 1990, Fridley et al. 2007, Whitlock et al. 2010, 2011), may alternate selection pressures such that effective selective neutrality is maintained over longer timescales at the population level (Jump et al. 2006, Jump et al. 2009b).

Climate linked genetic variation can provide a means for populations to respond to selection on a timescale relevant to the fast rates of change projected under contemporary climate change (IPCC 2007, Bradshaw and Holzapfel 2006, Hoffman and Willi 2008). Adaptation was generally considered to have played only a minor role in mitigating plant responses to past climate changes (Bradshaw 1991, Huntley 1991). However, evidence of both local adaptation and rapid evolution in response to contemporary climate change (Reusch et al. 2005, Bradshaw and Holzapfel 2006, Jump et al. 2006, Jump et al. 2008, Jump et. 2009), and population differentiation along climate gradients (Jump et al. 2006, 2008, 2009b), demonstrate that local climate gradients exert strong selection pressure on plant populations (Linhart & Grant 1996) and suggest that the role of genetic variation in species' response to past climatic changes may well have been underestimated (Davis & Shaw 2001).

Study System

The Buxton Climate Change Impacts Laboratory (BCCIL) in northern England (UK) is an experimental manipulation of climate factors (winter warming, summer drought, enhanced summer rainfall, warming/drought and warming/rainfall) in intact limestone grassland. Annual manipulations of temperature and rainfall have been maintained at BCCIL since 1993 and community composition has remained relatively stable in experimental treatments (Grime *et al.* 2000, 2008, Fridley *et al.* 2011). Such resistance is rare, most studies that manipulate climate on extant terrestrial ecosystems report fairly rapid species-level changes, suggesting that local adaptation is not significant (*e.g.*, Harte and Shaw 1995, Arft et al. 1999, Grime *et al.* 2000, Zavaleta *et al.* 2003, Evans et al. 2011). Resistance is attributed to the relatively stress tolerant flora typical of these infertile calcareous grasslands, which may be more resistant to climatic changes than more productive grassland communities of fertile soils (Grime et al. 2000, 2008, Matesanz et al. 2009). Similar relationships between resistance and fertility have been found in serpentine systems (reviewed in Damschen et al. 2012), where resistance is also attributed to reduced productivity and selection for stress tolerant functional traits (Fernandez-Going 2012).

Although compositional shifts have been relatively minor at BCCIL overall, there were rapid and persistent effects of climate manipulations on species composition which are attributed to differences in soil moisture status among experimental treatments (Grime et al. 2008). These minor compositional shifts are consistent with known species associations corresponding to topographic orientation and associated soil moisture dynamics (Perring 1960, Bennie et al. 2008). Fine-scale migration of individuals along a soil depth gradient may also be part of the mechanism maintaining species stability in experimental treatments at BCCIL (Fridley et al., 2011).

Genetic restructuring has been hypothesized as one potential mechanism in the apparent resistance of this grassland community to long-term climate manipulations (Grime et al. 2008). Compared to migration, the potential for local adaptation is likely to be highest under circumstances where: a) there is sufficient local genetic variation that underlies quantitative traits relevant to climatic shifts; b) the environmental shift is more than can be accommodated by phenotypic plasticity; and c) species-level changes are restricted (e.g., from dispersal limitation) or occur more slowly than population-level change (Moser *et al.* 2011). Most species common to this calcareous grassland system are obligate outcrossers with high local phenotypic variation that has a genetic basis (Booth and Grime 2003, Fridley *et al.* 2007, 2010, Bilton *et al.* 2010, Whitlock et al. 2007, 2010).

Research overview

My dissertation focuses on one of the more common forbs at BCCIL, *Plantago lanceolata P. lanceolata* is a rosette-forming, perennial herb with a wide-ranging distribution (Sagar and Harper 1964). *Plantago lanceolata* is self-incompatible and wind pollinated but displays substantial local genetic differentiation (Bos *et al.* 1986) and has distinct genetically determined phenotypes both at regional and fine scales (Primack and Antonovics 1982, Teramura 1983, van Tienderen 1992, Tonsor and Goodnight 1997, Wolf and van Delden 1987, 1989). Recent work documents genetic differentiation in *P.*

lanceolata populations exposed to elevated ozone and suggests high local diversity allowed populations to respond to abiotic perturbations by genetic restructuring (Kölliker et al. 2008).

Chapter II: Local adaptation to long-term climate change in a calcareous grassland

In this chapter I examined whether there was evidence of functional differentiation in *P. lanceolata* populations exposed to nearly two decades of summer drought at BCCIL. Individuals were harvested from drought and control treatments across a range of soil depth classes, propagated, and grown in a common greenhouse environment. I measured 24 functional traits that reflect primary axes of interspecific functional co-variation, corresponding to drought tolerance, drought avoidance, and competitive strategies (Grime et al. 1977). Traits consistent with a competitive strategy (e.g., high specific leaf area (SLA), high photosynthetic capacity, large vegetative allocation, rapid vegetative expansion, more erect growth habit) confer a fast rate of growth at the cost of resource retention (high respiration, high leaf turnover) (Grime et al. 1997, Reich *et al.* 2003). Tolerance syndromes are characterized by low rates of biomass turnover and growth but high water- and resource-use efficiency (e.g, low respiration, low SLA, low total biomass, low maximum photosynthetic rate, thicker leaves) (Grime 1997, Diaz *et al.* 2004, Wright 2004). A drought avoidance strategy is characterized by an earlier growth and flowering phenology, before the onset of drought (Geber and Dawson 1990, Heschel and Riginos 2005).

Increased water stress in drought treatments was expected to favor drought avoidance or tolerance strategies. If chronic summer drought creates ephemeral patches suitable for colonization and growth, then an avoidance strategy that promotes rapid growth and regeneration before the next drought would be favored. In contrast, selection for more competitive strategies was expected in control treatments and in deeper soils. The main axis of functional variation reflected a tradeoff between reproductive and vegetative allocation, consistent with drought avoidance and competitive strategies, respectively. Avoidance strategies were more prominent in drought populations whereas competitive strategies were more prominent in populations from control treatments, but only in deeper soils. Results suggest that population-level shifts can be a mechanism of resistance to local climate-induced extinction and that local edaphic heterogeneity fosters high genetic diversity, which provides a range of local phenotypes upon which drought-based selection may act. Such a result is consistent with adaptation as a mechanism contributing to community-level resistance to climate change at BCCIL (Grime *et al.* 2008).

Chapter III: Genetic response to long-term climate manipulations and fine-scale abiotic heterogeneity in a common herb

Evidence of both local adaptation and rapid evolution in response to contemporary climate change suggests that local genetic diversity can be an important mechanism of species resistance to climate change (Reusch et al. 2005, Bradshaw and Holzapfel 2006, Jump et al. 2006, Jump et al. 2008, Jump et. 2009a). In the third chapter I used molecular techniques (AFLPs: amplified fragment length polymorphisms) to determine whether there is evidence of genetic differentiation among populations in different climate treatments. The replicated block design of the experimental treatments at BCCIL offered a powerful means to distinguish between population structure generated by stochastic processes (e.g., drift and differential gene flow) versus structure generated by selection (Bonin et al. 2007, Nosil et al. 2008). Differentiation associated with drift would generate strong but stochastic spatial genetic structure whereas treatment based selection would generate parallel divergence patterns among control-treatment pairs in separate experimental blocks.

I detected minor, but significant amount of treatment-based genetic structure, which supports the hypothesis that *P. lanceolata* populations have adapted to long term experimental manipulations at BCCIL through local genetic restructuring. Evidence of treatment based selection was most pronounced in precipitation manipulation treatments (drought and increased rainfall treatments, including factorial combinations with heating), which suggests that genetic differentiation in *P. lanceolata* corresponds to gradients in soil water dynamics determined by treatment based differences in soil water supply (precipitation manipulations), which in turn can be modulated by increased demand in increased temperature manipulations. Taken together this suggests that *P. lanceolata* is particularly sensitive to gradients in water availability. Parallel divergence patterns in replicated pairwise control-treatment contrasts strongly suggest local adaptation in response to climate treatments and such a pattern would be unlikely to arise due to type I error or genetic drift (Campbell and Bernatchez 2004, Bonin et al. 2006, 2007, Nosil et al. 2008).

Chapter IV: Intraspecific plant trait variation in a heterogeneous landscape: population response to fine-scale soil moisture gradients

Environmental conditions imposed by experimental treatments and modified by fine-scale edaphic heterogeneity ('microsite') in the experimental plots at BCCIL are representative of gradients in temperature and water availability, known to exert strong selective pressure on plant populations (Delcourt and Delcourt 1988, Dunning et al. 1992, Levin 1992). Populations may be able to adapt in-situ to future climatic shifts through functional restructuring via selection for gentoypes based on their relative fitness and through individual phenotypic plasticity. A better understanding of the spatial structure of intraspecific adaptive functional variation could improve our ability to predict species' range changes under climate change that generally assume no variation within species (Jump and Peñuelas, 2005).

In the fourth chapter, I expanded environmental monitoring and trait analyses of *P. lanceolata* to calcareous grassland systems in the landscape surrounding BCCIL. I measured six functional traits that reflect major tradeoffs and strategies demonstrated at the species level (Grime et al. 1997, Diaz et al. 2004, Wright et al. 2004) which are also reflected in the main axes of local intraspecific variation found in *P. lanceolata* populations at BCCIL (Chapter 2). My objectives were to characterize the spatial structure of gradients in soil moisture that occur at nested spatial scales in this calcareous grassland; 2) determine the extent and spatial structure of functional trait variation in six traits that correspond to well-known functional tradeoffs and strategies demonstrated at species level; 3) to relate the extent and spatial structure of abiotic gradients and functional variation.

Abiotic gradients associated with soil water dynamics had distinct spatial structures which in turn promoted the hierarchical partitioning of intraspecific functional diversity in five of the six functional traits measured. Trait-environment relationships were particularly pronounced for SLA. Given that landscape scale gradients in soil moisture dynamics emulate a range of conditions expected under future climate change, evidence of associated functional structuring suggests that extant populations may be able to adjust to climatic shifts through individual plasticity or genetic restructuring. Such a result suggests that local and landscape scale intraspecific functional trait variation may buffer this species from future climate change.

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Local adaptation to long-term climate change in a calcareous grassland

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Running headline: Adaptation to climate change

SUMMARY

1. Populations of the common perennial herb *Plantago lanceolata* have been exposed to nearly two decades of summer drought at the Buxton Climate Change Experiment (BCCIL), a controlled manipulation of climate factors in a species-rich limestone grassland in northern England.

2. We used a common garden approach to test for evidence of selection for different suites of functional traits in *P. lanceolata* populations exposed to chronic summer drought and across a soil depth gradient.

3. The main axis of functional variation reflected a tradeoff between reproductive and vegetative allocation, consistent with drought avoidance and competitive strategies, respectively. Avoidance strategies were more prominent in droughted populations whereas competitive strategies were more prominent in populations from control treatments. Treatment differences were more pronounced in shallower soils. Deeper soils in both control and drought treatments promoted functional differentiation associated with competitive strategies suggesting that selective pressures imposed by different climate treatments are modified by fine scale edaphic heterogeneity.

4. Synthesis: Results suggest that population-level shifts can be a mechanism of resistance to local climate-induced extinction. Trait differentiation with respect to fine-scale variation in soil-depth suggests that edaphic heterogeneity fosters high local genetic diversity, which provides a range of local phenotypes upon which drought-based selection may act.

Key-words: Adaptation, climate change, drought, functional traits, grassland, *Plantago lanceolata*

INTRODUCTION

The need and capacity of species to migrate poleward as the climate shifts has been well documented (Davis and Shaw 2001, McLachlan et al. 2005, Neilson et al. 2005). The potential for local genetic diversity to buffer species from climate-induced extinction, however, remains understudied (Jump and Peñuelas 2005). Compared to migration, the potential for an evolutionary response to climate change is likely to be highest under circumstances where: a) there is sufficient local genetic variation underlying quantitative traits relevant to climatic shifts; b) the environmental shift is more than can be accommodated by phenotypic plasticity; and c) species-level changes are restricted (e.g., from dispersal limitation; Davis et al. 1986, Davis and Shaw 2001) or occur more slowly than population-level change (Moser et al. 2011). Most experimental studies of climate change in terrestrial systems have led to fairly rapid species-level changes, suggesting that compositional shifts preclude evolutionary responses and thus population-level responses are at best a weak component of community climate responses (Chapin et al. 1995, Harte and Shaw 1995, Grime et al. 2000, Zavaleta et al. 2003, Klein et al. 2007, Mikkelson et al. 2008).

At the Buxton Climate Change Impacts Laboratory (BCCIL) in northern England (UK), annual manipulations of temperature and rainfall have been maintained since 1993 and most species have not experienced large changes in abundance in response to experimental treatments (Grime et al. 2000, 2008, Fridley et al. 2011). Most species common to this calcareous grassland system are obligate outcrossers with high local phenotypic variation that has a genetic basis (Booth and Grime 2003, Fridley et al. 2007, Bilton et al. 2010, Whitlock et al. 2010). Adaptation to experimental treatments has been hypothesized as one potential mechanism of species persistence (Grime et al. 2008), but it is unclear whether selection or plasticity underlies species' stability. In a recent genetic study of one of the most common BCCIL forbs, *Plantago lanceolata* L., Ravenscroft et al. (in prep.) detected significant treatment-based genetic structure (3%) and parallel divergence patterns in droughted plots across experimental blocks. This supports the hypothesis that extant populations have resisted long term experimental manipulations at BCCIL through local genetic restructuring. However, it remains unclear how populations responding to chronic drought have differentiated from control populations, and whether such variation stems from natural polymorphisms maintained locally in response to spatial heterogeneity in environmental conditions such as water availability (Grime et al. 2008, Fridley et al. 2011).

Differentiation across gradients of temperature and water availability has been reported for several grassland species (Hamrick andAllard 1972, Hamrick and Holden 1979, Owuor et al. 1997, Li et al. 1999) and local differentiation mirrors patterns found at range-wide scales (Hamrick and Allard 1972, Hamrick and Holden 1979). Interannual variation in temperature promotes fine-scale genetic differentiation in *Betula pendula* (European white birch) (Kelly et al. 2003) and rising temperatures have cause rapid adaptive differentiation in *Fagus sylvatica* (Jump et al. 2006). Moisture stress is considered to be the primary driver of fine-scale adaptive differentiation in several conifer species(Jump et al. 2005). Drought has led to the rapid evolution of drought avoidance strategy in the annual plant *Brassica rapa* (Franks et al. 2011). Further, significant local (<500 m^2) population differentiation has been reported in *Fumana thymifolia* (Jump et al. 2009b), which may have contributed to the ability of this species to undergo rapid genetic change following 5 years of experimental drought and temperature manipulations (Jump et al. 2008).

P. lanceolata is a self-incompatible, perennial herb with a wide-ranging distribution (Sagar and Harper 1964) and remains present in all experimental treatments at BCCIL at moderate to high abundance. The species is wind pollinated but displays substantial local genetic differentiation (mean gene transport distance per generation: 0.2-1.4 m; Bos et al. 1986) and has distinct genetically determined phenotypes both at regional and fine scales (Primack and Antonovics 1982, Teramura 1983, van Tienderen 1992, Tonsor and Goodnight 1997, Wolf and van Delden 1987). Annual simulated short turf grazing in all experimental plots at BCCIL has increased *P. lanceolata* abundance since the onset of the experiment, but increases occurred at different times in different climate treatments (Fig. 1a). Abundance increased steadily in control plots in the first ten years of the experiment, whereas in drought plots abundance was consistently low until 2004, suggesting mortality imposed by summer drought and a population recovery time of about nine years (Fig. 1a). Abundance was similar in control and drought plots in 2008 but differences between treatments were still apparent in deep soil microsites, where abundance in drought plots was lower than controls (Fig.1b). Reduced abundance in deep soils is surprising given that deeper soils could ameliorate the effects of the drought treatment, and suggests population dynamics are also sensitive to biotic processes, including competition, that vary locally along a soil depth gradient (Fridley et al. 2011).

Here we report a common garden experiment to test for functional trait divergence in *P. lanceolata* populations exposed to long-term experimental summer drought and as modified by fine-scale edaphic heterogeneity. We grew individuals of control and droughted populations in a common greenhouse environment and measured 24 traits that reflect primary axes of interspecific functional variation, corresponding to drought tolerance, drought avoidance, and competitive strategies (Grime 1977) (Table 1). Traits consistent with a competitive strategy (e.g., high specific leaf area (SLA), high photosynthetic capacity, large vegetative allocation, rapid vegetative expansion, more erect growth habit) confer a fast rate of growth at the cost of resource retention (high respiration, high leaf turnover) (Grime 1977, Reich et al. 2003). Tolerance syndromes are characterized by low rates of biomass turnover and growth but high water- and resource-use efficiency (e.g, low respiration, low SLA, low total biomass, low maximum photosynthetic rate, thicker leaves) (Grime 1997, Reich et al. 2003, Diaz et al. 2004). An avoidance strategy associated with high reproductive allocation has been associated with an earlier growth and flowering phenology in other drought-avoiding species (Geber and Dawson 1990, Heschel and Riginos 2005).

Our objective was to quantify differences in plant attributes that, as expressed in a standardized environment, point to divergences in how individuals from control and drought populations use resources. We expected increased water stress in drought treatments to favor drought avoidance or tolerance strategies that may be mutually exclusive (Geber and Dawson 1997, McKay et al. 2003, Heschel and Riginos 2005, Franks 2011). Because competitiveness is negatively associated with traits that confer resistance to or avoidance of drought stress (Grime 1977, Reich et al. 2003, Diaz et al. 2004) we expected competitive strategies in populations from control treatments and in deeper soils. Functional trait divergence in populations exposed to chronic summer drought as expressed in a common environment would be consistent with genetic restructuring as a mechanism contributing to the resistance of this species to experimental drought at BCCIL (Grime et al. 2008).

METHODS

Study site

The BCCIL study is an experimental manipulation of climate, including winter (Nov-Apr) warming, summer (Jul-Aug) drought, enhanced summer (Jun-Sep) rainfall, and temperature-rainfall interactions, in a species-rich limestone grassland in Harpur Hill, Derbyshire, UK. Temperature and rainfall manipulations reflect early GCM predictions for northern England of winter warming of approximately 3° C and decreased summer rainfall. Temperature treatments fall within the range of more recent climate projections, which predict an increase in global mean temperature of 1.8-4° C by the end of the 21st century (IPCC 2007). The magnitude and direction of changes in precipitation are more uncertain and region specific (IPCC 2007) but more extreme intra-annual precipitation regimes with longer periods of intermittent drought are widely expected (Easterling et al. 2000, IPCC 2007). Intensification of the hydrologic cycle due to temperature increases has already caused more extreme precipitation events and droughts in temperate regions (Dai et al. 1998).

Treatments in plots of 3 x 3 m were established in 1993 and include summer drought manipulations accomplished via automated rainout shelters. Treatments are fully randomized within five replicate blocks. Each year in October vegetation is cut and removed from plots at a height of 50 mm to simulate sheep grazing. Within each plot are eight 10 x 10 cm permanent quadrats (‘microsites’), two in each of four soil-depth classes (0-7, 8-12, 13-20, and 21+cm) which capture natural fine scale edaphic heterogeneity (<100 cm^2), characteristic of the poorly developed soils of calcareous grasslands (Balme 1953, Pigott 1962). Local substrate heterogeneity mediates species’ response to experimental treatments and thus contributes to compositional stability in this grassland community despite long term climate manipulations (Fridley et al. 2011). For further details on microsite properties and other aspects of the experimental design at BCCIL see Grime et al. (2008) and Fridley et al. (2011).

Common garden trait measurements

Mature individuals of *P. lanceolata* were harvested from drought and control treatments at BCCIL in September 2008. A total of 72 genetically distinct individuals were collected from three experimental blocks at BCCIL, including 36 from each experimental treatment and 12 in three soil depth classes (0-7cm, 8-12cm, and 13+cm). We sampled mature individuals rather than seeds because mature individuals are the product of successful establishment and growth in experimental treatments, two important stages of selective filtering that would not be captured by collecting seed. Sampling mature individuals also reduced any potential influence of parental effects on offspring phenotype, which are generally more prevalent in juvenile life-history stages relative to adult life history stages (Roach and Wulff 1987, Weiner et al. 1997). This is consistent in studies of *P. lanceolata* where parental temperature influences germination, growth and reproduction of newly germinated seeds but not survival or reproduction of offspring after one year (Lacey 1996, Lacey and Herr 2000).To further reduce any potential influence of parental environment on offspring phenotype sampled individuals were grown in separate pots in watered and fertilized medium in a greenhouse at Syracuse University, New York, USA, for 6 months before propagation.

After this sixth month acclimation period, individuals were propagated vegetatively by cutting a standard size off the crown of the main plant. Six clones of each experimental individual were propagated and grown in individual pots for one week. The initial survival rate averaged over all individuals was 87%. Three clones of each individual were selected at random for the common garden experiment, conducted from March-June of 2009. Each clone was transferred to a 14 cm^2 pot filled with 1:1 mixture of sand and pro-mix compost mixed with Osmocote-Plus controlled release fertilizer pellets (Scotts-Sierra Horticultural Products Company, N:15%, P: 9%: K: 12% plus 9 micronutrients, 2g/L). Greenhouse conditions reflected average summer temperatures at the BCCIL (day temp 18-30° C, night temperature 8.8-11.67 °C, 16 hr days). All experimental individuals were given an ample supply of water throughout the entire experimental period and nutrient levels were considerably higher than the nutrient deficient soils typical in these calcareous grasslands.

The following traits were recorded prior to harvest (91 days post planting): angle of the youngest fully expanded leaf, length of longest flowering stem (scape), and number of rosettes. The number of scapes was recorded weekly during the experiment. Leaf emergence rate was estimated by marking the newest leaf on each rosette in the third week of the experiment and counting the number of new leaves that subsequently emerged over a two week period. Chlorophyll content was measured three times (35, 70, 95 days post planting) on the same leaf (older leaf one position away from marked leaf) using a handheld chlorophyll meter (Opti-Sciences CCM-200). Chlorophyll readings were taken at five leaf positions and averaged. Photosynthesis was measured on each individual via gas exchange (LI-COR 6400, LI-COR Biosciences, Lincoln, NE; 400 μmol CO_2 mol^{-1}, $700 \cdot \mu mol \cdot s^{-1}$ flow rate, 20 °C, light intensities of 800, 300, 100, and 50 μmol $photon \cdot m^{-2} \cdot s^{-1}$). Apparent quantum yield (*AQY),* maximum photosynthetic rate (A_{max}), and dark respiration (R_d) were estimated from each light curve using a nonrectangular hyperbolic light curve function as described in Fridley (2012).

Individuals were harvested after 98 days and this time period was sufficient to capture the production of mature seed before significant leaf senescence. Reproductive and vegetative tissue were separated at harvest and weighed after drying to constant weight. Five leaves were removed from each individual before biomass was harvested. Leaf fresh weight was recorded immediately after leaves were removed from intact plants. Leaf area and length were calculated with ImageJ software (Abramoff et al. 2004) using scanned images (300 dpi) of individual leaves. Leaves were dried until they reached a constant dry weight. Three leaves were ground to a fine powder and analyzed for carbon and nitrogen concentration on a NC 2100 Soil Analyzer (CE Instruments, Lakewood, NJ, USA).

Analysis

We conducted two multivariate analyses, principal components analysis (PCA) and multivariate analysis of variance (MANOVA) on a rank ordered trait matrix (trait values converted to non-parametric rank order) that included all 24 traits measured and all experimental individuals. Rank order standardization is a common means of transforming attributes to a uniform scale (Grime et al. 1997), as PCA and MANOVA are both sensitive to the relative scaling of the original variables (Jongman et al. 1987). Missing data (1.2% of overall dataset) were assigned the average trait average rank so as not to influence axis loadings (Grime et al. 1997).

We used PCA to determine whether the main axes of functional variation in *P. lanceolata* reflect anticipated avoidance, tolerance, and competitive functional strategies. We extracted the trait loadings on the first three components (PC1-3), and used ANOVA to test for significant differences in individual axis scores with respect to treatment, soil depth, experimental block at BCCIL, and greenhouse block. MANOVA was used to test for significant effects of climate treatment, soil depth, BCCIL block and greenhouse block on the 24 traits measured. We used ANOVA to test for significant differences in individual traits with respect to treatment, soil depth, BCCIL block and greenhouse block using unstandardized trait data. In both ANOVA analyses P-values were adjusted for multiple comparisons using Hochberg's (1988) test. Experimental block (BCCIL and greenhouse) was never significant. All analyses were conducted in R (R Development Core Team, 2011).

RESULTS

Main components of trait variation across individuals

The first three principal components explained 43.5% of the variation among individuals (17%, 16.5%, and 10% for components 1-3, respectively). The total variance explained and the distribution of variance across axes is consistent with similar analyses of functional differentiation among species (Grime et al. 1997, Diaz et al. 2004) and within species (Whitlock et al. 2010). The first principal component (PC1) reflected a tradeoff between vegetative and reproductive investment (Fig. 2a). Reproductive allocation was associated with longer scapes, higher chlorophyll content and later flowering phenology (Fig. 2a, Table 2). Vegetative allocation (negative PC1 scores) and associated traits, such as number of rosettes, total biomass, vegetative biomass, erect growth habit, and plant diameter, are consistent with a competitive strategy (Fig. 2a, Table 2). PC2 represented a tradeoff between traits that minimize resource loss (low PC2 scores: CN, LDMC, low SLA) and those associated with high growth rates (high PC2 scores: SLA, foliar N, A_{max}, chlorophyll content) (Table 2, Fig. 2b). Traits associated with reproductive allocation on PC1 loaded negatively on PC2 (Table 2). Competitive traits (SLA, rosette number) loaded negatively on PC3 and traits associated with reproductive allocation (chlorophyll content, longer scapes) loaded positively (Table 2).

Individuals from experimental drought populations allocated more to reproduction (higher PC1 scores) and those from control allocated more to vegetative growth (lower PC1 scores) (Fig. 2a). However, treatment differences were only significant in shallow and mid soil depth classes ($P<0.01$, Fig. 2a). There were no significant differences in PC2 scores with respect to treatment, soil-depth and their interaction (Fig. 2b). PC3 scores were significantly negatively correlated with soil depth ($P<0.01$, Fig. 2c). Plants in shallow microsites had higher PC3 scores in both control and drought treatments, whereas plants in mid and deep soil classes had lower PC3 scores in control plots relative to drought (Fig.2c).

Variation in individual traits

Although the 24 traits showed significant differences across soil depth classes (MANOVA, $P<0.05$, $F=1.62$ on 24, 169 DF), climate treatment and the climate x depth interaction term were not significant ($P=0.24$, $F=1.21$ on 24, 169 DF and $P=0.22$, $F=1.24$ on 24, 169 DF, respectively) and the overall effects of drought were less apparent when examined on a trait-by-trait basis (Table 1). Before post-hoc correction for multiple comparisons, ANOVAs on individual traits revealed significant soil depth effects for total biomass, reproductive biomass, diameter, LDMC, maximum scape length, and respiration (Table 1). Trait values for these seven traits were lower in deeper sites with the exception of respiration, which was highest in deep soils. The only significant difference with respect to climate treatment was the number of scapes, which was higher in the drought treatment (Table 1). Individuals from the drought treatment also had higher reproductive allocation, although the difference was not significant (Table 1). There were no significant effects of climate treatment, soil depth, or their interaction when P values were adjusted for multiple comparisons when using Hochberg post-hoc test (Hochberg 1988).

DISCUSSION

The primary tradeoff in functional strategies for P. lanceolata involved vegetative versus reproductive allocation. High vegetative allocation (low PC1 scores) was associated with number of rosettes, erect growth habit, and plant size, consistent with a competitive strategy of maximizing vertical and lateral spread (Grime 1977). Individuals that allocated more to reproduction (high PC1 scores) had less total biomass, higher chlorophyll content, longer scapes and a later flowering phenology, consistent with a drought avoidance strategy. Similar to results reported by Lacey et al. (2003) we found differences in flowering phenology associated with competitive and avoidance strategies which could increase the potential for within-population assortative mating and thus contribute to the maintenance of functional diversity within these local populations of P. lanceolata. The second axis of functional variation explained an equivalent amount of trait variation in *P. lanceolata* and reflected leaf-level tradeoffs associated with resource acquisition (high SLA, leaf nitrogen, A_{max}) and resource conservation (LDMC, leaf C:N) (Grime 1977, Reich et al. 2003, Diaz et al. 2004). The third axis of functional variation reflected tradeoffs associated with competitive functional strategies (high SLA, rosette number) and avoidance strategies (e.g., chlorophyll content, scape length), although variation along this axis was not associated with biomass allocation.

Populations from drought treatments allocated significantly more to reproduction relative to control populations. Although depth was not associated with allocation per se, treatment-based functional differentiation along PC1 was only significant in populations from shallow and intermediate soil depth classes. Populations from deeper soils in both treatments had more centralized scores on PC1, suggesting intermediate allocation to both growth and reproduction. Depth effects were significant with respect to PC3 scores where deeper soils also promoted traits associated with competitive functional strategies (high SLA, rosette number), whereas populations from shallow soils shared suites of functional traits associated with an avoidance strategy (e.g., chlorophyll content, scape length).

Although results suggest that competitive strategies are favored in both treatments in deeper soils, *P. lanceolata* abundance is much lower in deep soils of drought treatments (Fig. 1b). Reduced abundance in deep soils is surprising given that deeper soils could ameliorate the effects of the drought treatment, and suggests population dynamics are also sensitive to biotic processes, including competition, that vary locally along a soil depth gradient (Fridley et al. 2011). Increased mortality in the deep sites of droughted plots suggests that species level shifts may preclude adaptive responses in more productive microsites, similar to results from experimental climate manipulations in more productive communities where species turnover occurs rapidly (Chapin et al. 1995, Harte and Shaw 1995, Grime et al. 2000, Zavaleta et al. 2003, Klein et al. 2007, Mikkelson et al. 2008).

Evidence of functional differentiation associated with soil depth suggests that fine-scale substrate variation may be important in the maintenance of local functional diversity. Similar local population differentiation has been reported in *Fumana thymifolia* (Jump et al. 2009b), which may have contributed to the ability of this species to undergo rapid genetic change following 5 years of experimental drought and temperature manipulations (Jump et al. 2008). The existence of fine-scale heterogeneity in the BCCIL grassland may ultimate drive community stability in the face of climate change in two ways, by 1) allowing species-level shifts along the soil depth gradient as edaphic conditions like soil moisture change (Fridley et al. 2011), and 2) fostering local genetic diversity in some populations that in turn allows for population restructuring in response to new moisture conditions.

Functional differentiation expressed when individuals from each population were grown in the non-stressed conditions of the greenhouse could be due to plasticity, in that differentiation expressed in the common greenhouse does not necessarily correspond to phenotypic differences among treatments at the BCCIL. If true, we would expect a plastic response would cause all individuals to develop more competitive phenotypes. Instead, drought avoidance strategies were maintained in the common garden despite adequate water and nutrient supply. This suggests a genetic basis for functional differentiation which is further supported by a related study using molecular markers (Ravenscroft et al., in preparation).

Although not measured in this study, estimates of narrow-sense heritability in *P. lanceolata* for suites of traits measured here suggest low heritability of physiological traits ($h^2<0.03$ for photosynthetic capacity, transpiration, and water use efficiency) but high additive genetic variability for leaf traits (average $h^2=0.45$ for leaf length, width, area, and specific leaf weight; Wolff and Van Delden 1987, Tonsor and Goodnight 1997). Traits associated with plant size and resource allocation (diameter, number of rosettes, number of leaves, reproductive allocation) also show high heritability (average $h^2= 0.41$; Tonsor and Goodnight 1997, Wolf and van Delden 1987). Furthermore, Wolff and Van Delden (1989) demonstrated rapid divergence in leaf angle in *P. lanceolata* following four generations of bidirectional artificial selection and found significant genetic correlations between angle and other functional traits including leaf morphology and biomass allocation.

Taken together, our observations of differential population responses to drought compared to controls and past studies of heritability in *P. lanceolata* support the conclusion that the phenotypic differences expressed in the present common garden study are due to changes in gene frequencies in populations exposed to nearly two decades of chronic summer drought. Treatments at the BCCIL were of long duration in the context of the lifespan of *P. lanceolata* (lifespan 2-5 years, Cavers et al. 1980, Antonovics and Primacks 1982, Lacey and Herr 2000, Roach 2003), spanning 3- to 8 generations of *P. lanceolata*. The most parsimonious explanation for functional differentiation expressed in this common environment is that avoidance and competitive strategies offer different fitness benefits in contrasting climatic conditions as integrated over two decades of experimental climate manipulations. For example, competitiveness is negatively associated with traits that confer avoidance of drought stress (Grime 1977, Reich et al. 2003, Diaz et al. 2004) and thus more ‘competitive’ individuals should have higher fitness in more productive conditions (e.g, control treatments and deeper depths).

Given that populations expressed functional trait differences consistent with 'competitive' and 'drought avoidance' strategies recorded in the literature at the species level, and these differences could not be accounted for by plasticity because plants were grown under identical conditions, we find this result to be consistent with a mechanism of local adaptation as demonstrated in other species in response to temperature and moisture stress (Jump et al. 2006, Franks et al. 2011). Nonetheless, we have no proof that these specific traits convey superior fitness under summer drought, nor can we entirely rule out the possibility of maternal effects, despite sampling mature individuals in the field and propagating crown tissue for 6 months in the greenhouse before re-propagating at the start of our study. Thus, although we find the process of local adaptation to be the most parsimonious explanation for the results reported here, we cannot completely falsify alternative hypotheses in our study. A critical nest step in this research would be to follow the success of reciprocal transplants of *P. lanceolata* across climate treatments at the BCCIL.

CONCLUSION

In one of the first studies to investigate the importance of adaptive responses to climate change in a long-term climate manipulation, we show evidence of selection for different functional strategies in populations exposed to chronic summer drought expressed when plants of each population were grown under the same non-stressed conditions. Furthermore, we show evidence of differentiation with respect to a soil depth gradient which suggests that abiotic heterogeneity can promote genetic diversity of functional relevance in the context of future climate change. The trait syndromes represented in this population reflect functional strategies demonstrated at the species level (Grime 2002), and thus show similarities between the main axes of inter- and intra- specific functional variation. Although results suggest some populations may have the capacity to adapt to climate change and thus resist climate-induced local extirpation given adequate levels of genetic variation, this does not mean that these systems are inherently stable. In particular, barriers to dispersal limit the invasion of southerly species, which may over longer time scales cause species-level shifts even if extant populations are able to adapt to new environmental regimes (Moser et al. 2011). The likelihood of long-term population persistence in the face of new species immigration over the coming decades remains a key unresolved area in global change research.

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Table 1: The 24 traits measured in the experiment with the expectation of their relative values under competitive, drought resistance, and drought avoidance plant strategies. Statistics are for each individual trait (mean, maximum, minimum), calculated using all experimental individuals. In all cases the adjusted P value (Hochberg post-hoc test) was greater than 0.1. Responses to treatment, soil depth, and their interaction on unadjusted values are denoted ** P <0.05, * P <0.1. Experimental block effects (BCCIL and greenhouse) were not significant. Leaf Angle (1: <10°, 2: 10-25°, 3: 25-45°, 4: >45°); AQY: Apparent Quantum Yield; A_{max}: Maximum Photosynthetic capacity

	Competitive	Tolerance	Avoidance	Mean	Min	Max	
Biomass							
Total Biomass (g)	High	Low	Low	13.85	2.74	21.90	depth**
Biomass - veg. (g)	High	Low	Low	6.18	1.52	15.10	treat*
Biomass - rep. (g)	Low	Low	High	7.67	0.14	13.42	depth**
Rep. allocation (rep/tb)	Low	Low	High	0.56	0.01	0.78	treat *, treat x depth *
Veg. allocation (veg/tb)	High	High	Low	0.44	0.22	0.99	treat *, treat x depth *
Morphological							
Diameter (cm)	High	Low	Low	29.80	12.00	41.00	depth **
Leaf angle	High	Low	Low	1.52	1.00	4.00	treat *
Leaf emergence	High	Low	High	0.55	0.00	1.50	
Number of Rosettes	High		Low	2.18	1.00	6.00	treat *
Number of Scapes	Low	Low	High	34.04	1.00	80.00	treat **

Scape length (cm)	Low	Low	High	29.44	2.50	57.50	depth **
Phenology							
Week of first flower	Late	Late	Early	1.78	1.00	5.00	
Leaf traits							
SLA (cm^2/gram)	High	Low		128.38	74.45	262.53	
LDMC (mg/g)	Low	High	Low	162.95	107.26	214.74	depth**
Leaf length (mm)	High	Low		10.33	7.47	14.43	
Leaf area (cm^2)	High	Low		8.10	3.58	16.21	depth *
Chlorophyll/cm^2	High	Low	High	66.64	17.60	163.37	
Chlorophyll/gm	High	Low	High	8459.26	2162.13	20360.50	
Carbon:Nitrogen	Low	High	Low	19.25	9.88	29.69	
Nitrogen	High	Low	High	2.25	1.39	5.08	treat x depth *
Photosynthetic							
Dark Respiration	High	Low	Medium	3.38	0.29	18.84	depth **
A_{max}	High	Low	High	12.06	3.12	22.00	
AQY	High	Low	High	0.05	0.01	0.29	
Water Use Efficiency	Low	High	Low	2.03	0.52	5.06	

Table 2: Trait loadings on first three principal components

	PC1		PC2		PC3
Biomass - vegetative (g)	-0.44	Carbon:Nitrogen	-0.36	Number of Scapes	-0.22
Vegetative allocation	-0.42	Biomass- reproductive (g)	-0.35	SLA (cm^2/gram)	-0.20
Number of Rosettes	-0.34	LDMC (mg/g)	-0.30	Number of Rosettes	-0.17
angle	-0.29	Total Biomass (g)	-0.25	angle	-0.07
Total Biomass (g)	-0.29	Maximum scape length	-0.24	Leaf emergence (# leaves/day)	-0.05
Diameter (cm)	-0.17	Number of Scapes	-0.19	Dark Respiration	-0.03
Leaf length (mm)	-0.14	Reproductive allocation (rep/tb)	-0.18	Vegetative allocation (veg/tb)	-0.01
LDMC (mg/g)	-0.14	Week of first flower	-0.13	Reproductive allocation (rep/tb)	0.01
Carbon:Nitrogen	-0.08	Diameter (cm)	-0.09	Carbon:Nitrogen	0.01
SLA (cm^2/gram)	-0.07	Biomass - vegetative (g)	-0.08	N	0.02
Water Use Efficiency	-0.07	Number of Rosettes	-0.02	Week of first flower	0.04
Leaf area (cm^2)	-0.07	Leaf area (cm^2)	0.02	Apparent Quantum Yield	0.06
Number of Scapes	-0.02	Leaf emergence (# leaves/day)	0.03	LDMC (mg/g)	0.09
Apparent Quantum Yield	0.01	angle	0.04	Biomass - vegetative (g)	0.10
Dark Respiration	0.02	Dark Respiration	0.04	Biomass- reproductive (g)	0.10

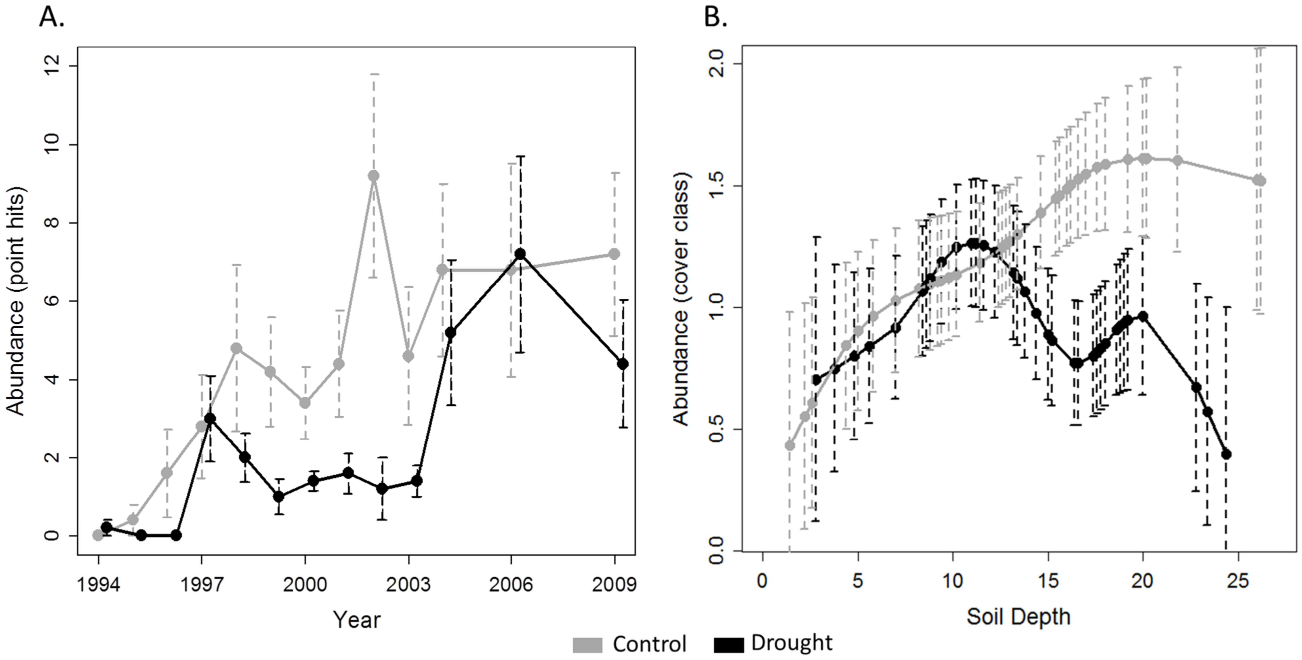
A_{max}	0.04	Leaf length (mm)	0.11	Water Use Efficiency	0.11
N	0.05	Chlorophyll/cm^2	0.11	Amax	0.13
Biomass- reproductive (g)	0.06	Water Use Efficiency	0.17	Total Biomass (g)	0.13
Leaf emergence (# leaves/day)	0.07	Vegetative allocation (veg/tb)	0.18	Maximum scape length	0.27
Maximum scape length	0.11	Amax	0.20	Chlorophyll/gm	0.32
Chlorophyll/gm	0.12	Apparent Quantum Yield	0.21	Leaf length (mm)	0.37
Week of first flower	0.13	Chlorophyll/gm	0.24	Leaf area (cm^2)	0.38
Chlorophyll/cm^2	0.15	SLA (cm^2/gram)	0.30	Diameter (cm)	0.40
Reproductive allocation	0.42	N	0.33	Chlorophyll/cm^2	0.41

FIGURE LEGENDS

Fig. 1. Abundance of *Plantago lanceolata* in drought (black) and control (grey) treatments at BCCIL from 1994-2009. A) Point hits of *P. lanceolata* in 9 m^2 plots (five replicate blocks per a climate treatment). Dashed lines represent ± standard error. B) *P. lanceolata* abundance across a soil-depth gradient in 2008. Fitted line and standard error from generalized additive model (GAM) of *P. lanceolata* abundance (cover class) with respect to soil depth in control and drought treatments.

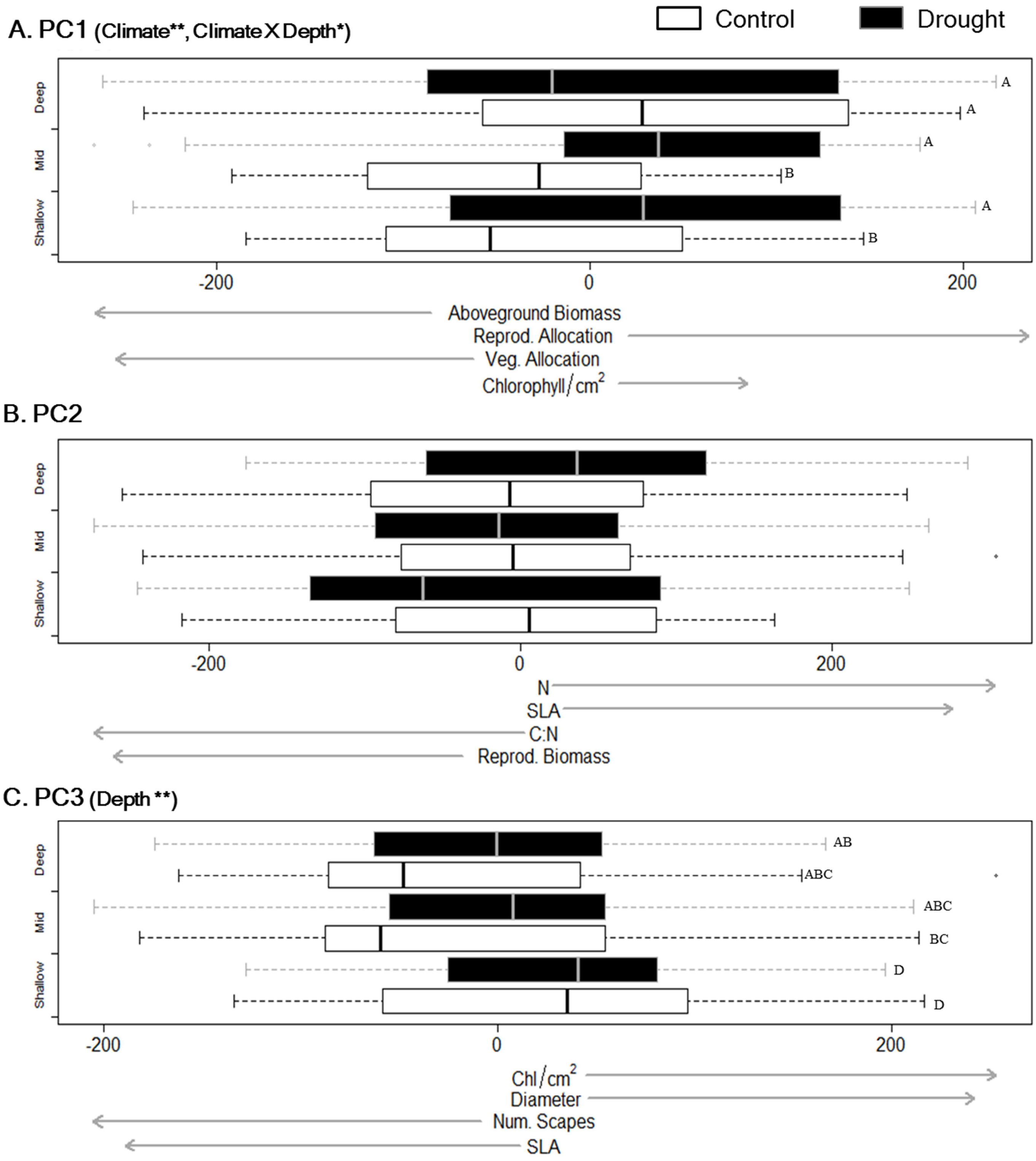
Fig 2. Principal component scores (axes 1-3) with respect to the interaction of climate treatments and soil depth class. Four traits with the highest loadings (both positive and negative) are listed below the associated graph of component scores, with the highest eigenvector score placed closest to the axis. Arrows are scaled to the absolute difference between eigenvector scores and length reflects the relative weight of each trait loading. Eigenvector scores for all traits are in Table 2. Significant differences with respect to climate and soil depth (ANOVA) are listed in the title of each panel (** $P<0.05$, * $P<0.1$). Experimental block was not significant. Results from multiple ANOVA comparisons are summarized by letters on the graph, different letters indicate a significant difference ($P <0.05$).

Fig. 1



A.
Abundance (point hits)
0
2
4
6
8
10
12
1994
1997
2000
2003
2006
2009
Year
B.
Abundance (cover class)
0.0
0.5
1.0
1.5
2.0
0
5
10
15
20
25
Soil Depth
Control
Drought

Fig. 2


Control
Drought
A. PC1 (Climate**, Climate X Depth*)
Deep
Mid
Shallow
A
A
A
B
A
B
-200
0
200
Aboveground Biomass
Reprod. Allocation
Veg. Allocation
Chlorophyll/cm^2
B. PC2
Deep
Mid
Shallow
-200
0
200
N
SLA
C:N
Reprod. Biomass
C. PC3 (Depth **)
Deep
Mid
Shallow
AB
ABC
ABC
BC
D
D
-200
0
200
Chl/cm^2
Diameter
Num. Scapes
SLA

Chapter 3

Genetic response in a common herb to long-term climate manipulations and fine-scale abiotic heterogeneity.

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Keywords: Climate change, adaptation, AFLPs, genetic differentiation, grassland, *Plantago lanceolata*.

Running headline: Genetic response to climate change

ABSTRACT

Evidence of both local adaptation and rapid evolution in response to contemporary climate change suggests that local genetic diversity can be an important mechanism of species resistance to climate change. Here we used amplified fragment length polymorphisms (AFLPs) to detect signatures of selection in populations of *Plantago lanceolata* subjected to long-term experimental climate manipulations (warming, summer drought, increased precipitation, and factorial combinations of heating with both drought and increased precipitation) in an intact calcareous grassland system. Climate treatments were replicated five times in a randomized block design. The replicated design offered a powerful means of distinguishing between population structure generated by stochastic processes (e.g., drift and differential gene flow), versus treatment based selection that would generate parallel divergence patterns in replicate control-treatment contrasts. We detected minor, but significant amounts of treatment-based genetic structure (3%), supporting the hypothesis that *P. lanceolata* populations have adapted to long term experimental manipulations through local genetic restructuring. Evidence of treatment based selection was most pronounced in precipitation manipulation treatments (drought and increased rainfall, including factorial combinations with heating), suggesting that genetic differentiation in *P. lanceolata* was associated with treatment based differences in soil water supply. Parallel divergence patterns in replicated pairwise control-treatment contrasts strongly suggest local adaptation in response to treatment based differences and such a pattern would be unlikely to arise due stochastic processes such as genetic drift.

INTRODUCTION

Climate change is expected to impose strong directional selection pressures on plant populations (Davis and Shaw, 2001, Davis et al., 2005, Jump and Penuelas, 2005, Bradshaw and Holzapfel 2006, Reusch and Wood 2007, Anderson et al. 2012). Evidence of fine-scale adaptive differentiation despite high levels of gene flow has been reported for many plant species (e.g., Owuor et al. 1997, Li et al. 1999, Skøt et al. 2002, Jump and Peñuelas 2005, Kölliker et al. 2008, Jump et al. 2009b, Manel et al. 2010, Parisod and Christin 2008, Vega-Vela 2012). Selection on extant genetic variation is considered to be a primary mechanism by which populations adapt in-situ to rapid environmental changes (Hoffman and Willi 2008, Jump and Penuelas 2009a). It is the 'option value' of genetic diversity (Jump and Penuelas 2009b) that makes it such an important component of species potential resistance to climate change (Reusch et al. 2005, Jump and Penuelas 2005, Reusch and Wood 2007, Gienapp et al. 2008, Hoffmann and Willi 2008, Jump et al. 2009b).

Gradients in variables known to be selective agents for plant species, such as temperature and water availability, occur at nested spatial and temporal scales (Delcourt and Delcourt 1988) and thus could promote hierarchical partitioning of genetic diversity. Differentiation across gradients of temperature and water availability over landscape scales (<10 m - 1 km) have been reported for several species of grasses (Hamrick & Allard 1972, Hamrick & Holden 1979, Owuor et al. 1997, Li et al. 1999) and local differentiation mirrors patterns found at range wide scales (Hamrick & Allard 1972, Hamrick & Holden 1979). Local genetic diversity can be promoted and maintained by strong selection pressures that occur over very fine spatial scales (Linhart and Grant, 1996). Interannual variation in temperature promotes microgeographical genetic differentiation in *Betula pendula* (European white birch) (Kelly et al. 2003) and rising temperatures have cause rapid adaptive differentiation in *Fagus sylvatica* (Jump et al. 2006). Moisture stress is considered to be the primary driver promoting microgeographical adaptive differentiation in several conifer species with local patterns repeated at range wide scales (reviewed in Jump et al. 2005). Drought has led to the rapid evolution of drought avoidance strategy in the annual plant *Brassica rapa* (Franks et al. 2011). Recent evidence suggests significant local (<500 m^2) population differentiation in *Fumana thymifolia* (Jump et al. 2009b). Such fine scale genetic structure may have contributed to the ability of this species to undergo rapid genetic change following 5 years of experimental drought and temperature manipulations (Jump et al. 2008).

The Buxton Climate Change Impacts (BCCIL) study is an experimental manipulation of temperature and precipitation (Figure 1) in an intact limestone grassland community in northern England. Established in 1992, it is among the longest running experimental climate manipulations in the world. Community composition has remained relatively stable in all experimental treatments (Grime et al. 2000, 2008). Such stability is rare; the majority of experimental climate manipulations studies report rapid community and ecosystem responses (e.g., Harte and Shaw 1995, Grime et al. 2000, Zavaleta et al. 2003, Evans et al. 2011). Genetic restructuring has been hypothesized as one potential mechanism in the apparent resistance of this grassland community to long-term climate manipulations (Grime et al. 2008).

The potential for local adaptation to buffer species from climate change is likely to be highest under circumstances where there is high local genetic diversity and species-level changes are restricted due to dispersal limitation (Davis et al. 1986). The invasion of southerly species, considered to be “pre-adapted” to warmer and drier conditions in experimental treatments at BCCIL, is limited by barriers to dispersal (Moser et al. 2011). Furthermore, high local (<10m) phenotypic variation with a genetic basis has been documented in several species common in this system (Booth and Grime 2003, Fridley et al. 2007, Whitlock et al. 2007, Bilton et al. 2010, Whitlock et al. 2010). A recent common garden study on one of the most common herbaceous species at BCCIL, *Plantago lanceolata,* suggests a genetic basis for functional differentiation in experimental drought treatments (Ravenscroft et al. 2013) and differentiation with respect to fine-scale variation in the edaphic environment

In this study we used amplified fragment length polymorphisms (AFLPs) to test for genetic differentiation in populations of *P. lanceolata* exposed to over 15 years of experimental climate manipulations (warming, summer drought, increased precipitation and factorial combinations of heating with both drought and increased precipitation) at BCCIL (Figure 1). AFLPs are a PCR-based technique for detecting polymorphisms in DNA that offer several advantages over other molecular techniques, including their relatively low cost and ability to analyze many loci scattered throughout the genome (Vos et al. 1995, Meudt and Clarke 2007). AFLPs are particularly useful for non-model species because the method does not require prior knowledge of a species' genome. Therefore, AFLPs are commonly utilized in investigations of the genotypic structure in plant populations (*e.g.* Escaravage et al.1998, Suyama et al. 2000, Douhovnikoff et al. 2004, Jump et al. 2006, 2008, 2009b, Dlugosh et al. 2007, Avolio et al. 2011, 2012).

Population genetic structure is shaped simultaneously by gene flow, drift, and selection (Lenormand, 2002, Latta, 2003). The replicated block design of the experimental treatments at BCCIL (Figure 1) offers a powerful means of analyzing the relative contribution of stochastic processes (e.g., drift and differential gene flow) versus treatment based selection on population genetic structure (Nosil et al. 2008). We used multivariate methods to test for evidence of parallel divergence in treatment-control contrasts across the five experimental blocks at BCCIL (Figure 1). Differentiation associated with stochastic processes such as drift should generate strong but stochastic spatial structure among experimental blocks. Parallel divergence across multiple treatment pairs that are separated spatially would suggest selection imposed by climate treatment and such a pattern is unlikely to arise due to type I error or genetic drift (Bonin et al. 2007, Nosil et al. 2008).

Focal species

Plantago lanceolata is a rosette-forming, perennial herb of wide-ranging distribution, and one of the more common forbs at BCCIL. *P. lanceolata* is self-incompatible and wind pollinated, displays substantial local genetic differentiation (Bos et al. 1986), and has distinct genetically determined phenotypes both at regional and fine scales (Primack and Antonovics 1982, Teramura 1983, van Tienderen 1992, Tonsor and Goodnight 1997, Wolf and van Delden 1987, 1989). Recent work documents genetic differentiation in *P. lanceolata* populations exposed to elevated ozone and suggests high local diversity allowed populations to respond to abiotic perturbations by genetic restructuring (Kölliker et al. 2008).

Annual simulated short turf grazing in all plots at BCCIL has increased *P. lanceolata* abundance in all experimental treatments (Figure 2a). *P. lanceolata* abundance decreased below controls in all treatments following the onset of the experiment and abundance in drought and watered plots was consistently lower than controls from 1998-2003, suggesting a selection event imposed by changes in precipitation regimes and a population recovery time of about 11-13 years (Fig. 2a). Treatment differences are more pronounced at deeper soil depths (Figure 2b). *P. lanceolata* has generally responded favorably to warming treatments (Figure 2a), and its abundance is positively correlated with soil depth, except in both water addition treatments where there is a negative relationship between abundance and soil depth (Fridley et al. 2011), which is driven mainly by the decrease in *P. lanceolata* abundance in deeper soils in these two treatments (Figure 2b).

METHODS

The Buxton Climate Change Impacts (BCCIL) study is composed of six treatments: 1) Winter warming where soil surface is maintained at 3° C above ambient annually from November-April; 2) drought treatments where rainfall in July and August is intercepted by automated rainout shelters; 3) water addition where water is added to experimental plots from June-September at a rate of 20% above the long-term average; 4) warming and drought (heated/drought); 5) warming and increased precipitation (heated/watered); 6) Control. Experimental plots (3 x 3 m) are replicated five times in a randomized block design (Figure 1). For more details on the experimental design at BCCIL see Grime et al. 2000, 2008.

Tissue samples of *P. lanceolata* were collected in 30 experimental plots in the six climate treatments (Control, Drought, Heated, Watered, Heated x Watered, Heated x Drought) at BCCIL in June of 2009. In each plot sampling was stratified to include three individuals from four soil-depth classes, (0-3 cm, 6-12 cm, 13-20 cm, >21 cm), hereafter called ‘microsites’. A total of 12 samples per plot, 60 samples per climate treatment and 15 samples per microsite treatment combination were collected. For each individual a leaf tissue sample was stored in silica gel for genetic analysis. Replicate samples were collected from 40 randomly selected individuals for estimates of genotyping error (see below).

Lab protocols for DNA extraction from leaf tissue samples and subsequent AFLP analysis followed that of Whitlock et al. (2008a) with the following modifications. We used a plate centrifuge for DNA elution steps 1-4 described in Whitlock et al. (2008a). Pre-selective

PCR amplification products were diluted to a factor of 1:12 and 1 μL of this diluted product was used as a template for selective amplification. No formamide was added to the selective amplification mixture and selective amplification products were diluted by a factor of 1:25. Fragment analysis was run on 1 μL of diluted PCR product and 9 μL of size standard mix which contained 5 μL ROX size standard (Applied Biosystems), 2 μL of ABI ROX 585 size standard (to increase read length) (Applied Biosystems), 1 ml ABI HiDi formamide (Applied Biosystems) using an ABI 3730 Genetic Analyzer. These modifications produced the most consistent and clear AFLP profiles based on test runs using 48 individuals. Eight primer combinations were selected from a test panel of 32 based on clarity of the AFLP profiles and evidence of polymorphic loci using this same random subset of 48 samples, with a target of 200-300 total polymorphic loci (Table 1). Sample positions were randomized across four 96-well plates, and each plate included two positive (leaf material from the same individual) and two negative (blank sample with no leaf material added). Replicate tissue samples (40) were run on a separate plate.

AFLP chromatograms were analyzed using the software GENEMAPPER version 3.0 (Applied Biosystems). Sizing quality was checked manually for each fingerprint and samples with size standards of insufficient quality were rejected from the analysis. Bins were created automatically in GENEMAPPER. Bin positions were checked manually to ensure positions had been assigned correctly. Bins that were off center were manually adjusted to center on peaks. Bins that included large discontinuous fragments and bins that included fragments that were continuous with other bins were rejected from the analysis because of potential size homoplasy. Weak fingerprints with low peak height or short read length were rejected. Fingerprints that contributed many unique peaks were rejected from the analysis as this could be a result of contamination. Peak height data for each individual were exported from GENEMAPPER and genotypes were scored using a semi-automated method of genotyping AFLPs that excludes loci that contribute to high rates of error (AFLP-Score, Whitlock et al. 2008b). Of the 406 original loci identified, 270 polymorphic loci (ranging from 50-585 bp in length) were retained. The error rate over all retained loci was 4.6%, well within the range of errors reported in other studies that used automatic scoring methods (Meudt and Clarke 2007). One primer combination (TGA-CCC) had an unusually high error rate (30%) and was excluded from analyses. Peak height data for the 270 polymorphic AFLP loci were scored to create a presence-absence matrix for all sampled individuals.

Analysis

Subsequent analyses were conducted on 249 loci that met a maximum allowable allele frequency threshold of 95% across all experimental individuals (21 loci removed). Allele frequencies were calculated in GenAlEx 6.4 (Peakall and Smouse 2012). We used three complementary multivariate approaches to test for significant evidence of selection in *P. lancoleata* populations exposed to experimental treatments. Multivariate statistical techniques have been widely applied in studies of local adaptation in natural environments (Storfer et al. 2010) and are particularly well suited for studies of population genetic structure because these techniques do not require any assumptions of genetic models (e.g., Hardy-Weinberg equilibrium) (Meudt and Clarke 2007, Parisod and Christin 2008).

Multivariate analyses were conducted on a genetic distance matrix using allele presence absence data for the 249 loci that met the maximum allele frequency threshold of 95%. We conducted multivariate analyses using jaccard and euclidean distance metrics (R packages: vegan 1.17-4, labdsv 2.13.2). Euclidean distance places more weight on shared presences than shared absences and the jaccard distance metric puts more weight on shared absences (Legendre and

Legendre, 1998), which are common in dominant markers such as AFLPs. Results were consistent using alternative distance measures so we present results using euclidean distance.

We used analysis of molecular variance (AMOVA) (Excoffier, Smouse, and Quattro, 1992) to partition total molecular variance (including all experimental individuals) among experimental blocks, among treatments, among soil depth classes, and within treatments using the program GenAlEx (Peakall and Smouse 2012). We tested for significant effects of experimental treatments using a permutational analysis of variance (Legendre and Anderson 1999), implemented in the R vegan library using the function ‘adonis’(R package vegan 1.17-4). Permutational analysis of variance is analogous to redundancy analysis (Legendre and Anderson 1999) and an extension of the AMOVA which permits analysis of nested factors by restricting permutational randomizations of individuals to different ‘strata’ (experimental blocks). We ran individual permutational AMOVAs for each pairwise control-treatment contrast. Finally, to visualize genetic relationships among individuals we used PCoA (Principal Coordinates Analysis), which identifies axes of variation that explain the most genetic dissimilarity among individuals. We extracted individual scores on the first two PCoA axes and used Analysis of Variance (ANOVA) to test for significant effects of block, treatment and their interaction. For each block we calculated mean scores on PCoA axis 1 and 2 for each treatment x block combination (i.e., 5 per treatment). We used these means to determine if there was evidence of consistent directional change in treatments relative to controls.

RESULTS

Results from the AMOVA including all experimental treatments indicate minor but significant genetic differentiation among climate treatments (3%) and among experimental blocks (5%) (Table 2). The majority of genetic variance was within individual plots (90%) and thus unstructured with respect to experimental treatments and experimental block (Table 2). A large amount of unstructured variance was expected given that loci in fingerprinting techniques such as AFLPs largely represent neutral regions of the genome (Bonin et al. 2007). Soil depth was not a significant predictor of genetic distance when all treatments were included in the analysis (Table 2). Individual pairwise treatment-control comparisons (stratified by experimental block, with 5 replicate comparisons per a treatment-control contrast) revealed significant genetic differentiation in drought, watered, heated/drought and heated/watered treatments relative to controls and no evidence of significant differentiation in heated-control contrasts (Table 3). We found evidence of genetic differentiation with respect to soil depth class only in heated/watered-control treatment comparisons (Table 3).

PCoA axis one (PC1) and PCoA axis two (PC2) scores explained 22% and 19% of the total genetic variation, respectively. We found significant differences in PC1 and PC2 scores with respect to treatment, block and their interaction when all experimental treatments were included in the analysis (Table 4). ANOVA results from pairwise treatment-control contrasts revealed significant differentiation between watered, heated/drought and heated/watered treatments relative to controls, although genetic differentiation between heated/watered and control treatments was marginally insignificant when p-values were corrected for multiple comparisons (Table 4). PC2 scores were significantly different in drought-control and heated/water-control comparisons, although adjusted p-values were marginally insignificant in drought-control contrasts (Table 4). The first major axis of genetic differentiation separated watered, heated/drought and heated treatments from experimental controls (Figure 3). Stochastic processes were primarily responsible for variation in on the first PCoA axis, where drift or recruitment in experimental C block caused genetic differentiation in heated, watered and heated/drought experimental treatments (Figure 3). Drought, control and heated/watered treatments show relatively minor variation along the first PCoA axis and are almost entirely differentiated on the second PCoA axis.

DISCUSSION

Our results indicate a small but significant amount of treatment-based genetic structure which supports the hypothesis that *P. lanceolata* populations have adapted to long term experimental manipulations at BCCIL through local genetic restructuring. Evidence of treatment based selection was most pronounced in precipitation manipulation treatments (drought and increased rainfall treatments, including factorial combinations of both with heating). Such a result suggests that *P. lanceolata* is particularly sensitive to gradients in soil water supply imposed by drought and increased precipitation manipulations, which in turn can be modulated by increased demand in temperature manipulations. Similar levels of treatment based local genetic structure have been reported in *P. lanceolata* populations exposed to experimental manipulations of elevated ozone (Kölliker et al. 2008), in populations of *Andropogon gerardii* exposed to variable precipitation regimes (Avolio et al. 2012) and in *Fumana thymifolia* populations exposed to six years of experimental drought and warming treatments (Jump et al. 2008). Our results show a genetic signature of reduced survival and recruitment in deeper soils in the heated/watered treatments (Fridley et al. 2011) (Figure 2b, Table 3).

To our knowledge this is the first study that investigates local genetic differentiation using replicated pairwise treatment contrasts. The block design offers a powerful means of distinguishing between population structure generated by stochastic processes (e.g., drift and differential gene flow) versus structure generated by selection (Bonin et al. 2007, Gienapp et al. 2008, Nosil et al. 2008). Differentiation in stochastic processes such a drift and gene flow generated strong but stochastic spatial genetic structure whereas treatment based selection generated parallel divergence patterns in replicate control-treatment pairwise contrasts (5 replicates). Although associations between genetic and environmental heterogeneity do not necessarily imply causality, parallel divergence patterns in replicated pairwise control-treatment contrasts strongly suggest local adaptation in response to treatment based differences in soil water supply and demand; such a pattern would be unlikely to arise due to type I error or genetic drift (Bonin et al. 2007, Nosil et al. 2008).

Results from the permutational analysis of variance suggest parallel divergence patterns in all experimental treatments except heating. However, inspection of the distribution of experimental block along the first two PCoA axes indicated large divergence in watered, heated/drought and heated plots relative to controls. These three experimental treatments occupy the largest amount of space on the first two PCoA axes, perhaps indicating higher genetic diversity in these treatments relative to control, drought and heated/watered. Treatment differences were also evident along the second PCoA axis, where heated/watered, control and drought treatments showed the most differentiation. Both experimental treatments (drought, heated/watered) occupy a relatively restricted amount of space, perhaps indicating reduced genetic diversity in these experimental treatments. In general, the primary axis of variation as detected by the PCoA seems to reflect stochastic processes such as random recruitment or drift that have caused differentiation in block C. Discrepancies between the PCoA results and those of the permutational analysis of variance are likely due to the fact that the first two PCoA axes explained only 41% of genetic variation. Therefore, it is possible that treatment based directionality is obscured by differentiation associated with stochastic processes such as gene flow and drift.

Population genetic structure is shaped simultaneously by gene flow, drift, and selection (Lenormand, 2002, Latta, 2003). Evidence of treatment based genetic structure was surprising given that individuals were sampled from a continuous population over very small extent (30 9 m^2 plots, or a total of 270 m^2) and gene flow was expected to homogenize the distribution of genetic variation, especially in a wind dispersed obligate outcrosser such as *P. lanceolata*. Divergent selection can drive the simultaneous evolution of reproductive isolation (Muller 1942, Mayr 1947, Schluter and Nagel 1995, Schluter 2000), which would reduce gene flow between populations from contrasting habitat types (Nosil et al. 2005). Divergent selection would be favored particularly in cases where regeneration success is lower in non-native environments (Nosil et al., 2005). Phenological divergence is considered to be the most straightforward mechanism by which local divergence can be maintained in continuous plant populations (Fox, 2003, Hendry & Day, 2005). Asynchronous flowering could restrict gene flow between individuals growing in contrasting climate conditions and thus may have contributed to the maintenance of local genetic structure in populations of *P. lanceolata* (Fox 2003, Hendry & Day, 2005). Flowering time divergence is important to the maintenance of locally adapted subpopulations in continuous population of *B. laevigata* (Parisod et al. 2008). Recent work suggests high local variance in regenerative traits in populations of *P. lanceolata (*Ravenscroft and Fridley 2013*)* which could be associated with different establishment strategies which are maintained by differences in flowering phenology in this species (Lacey et al.2003).

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Table 1: Selective primer combinations used in the AFLP analysis. The initial number of candidate loci output by Genemapper is given, followed by the median and mean fingerprint intensity for each primer combination. The number of retained loci per primer combination is listed, these loci were selected based on an error rate analysis in AFLP-SURV (Whitlock et al. 2008a). Note one primer combination TGA-CCC had a high error rate so this primer combination was excluded from further analyses. The error rate is shown for retained loci and was calculated on 40 replicate samples.

Primer Combination	Initial Number of loci	Median fingerprint intensity (rfu)	Mean fingerprint intensity (rfu)	Retained loci	Error %
TCC-CAA	57	34818	866	44	2.5
TCC-CAT	62	33076	949	47	3
TCC-CAG	42	19041	929	35	5
TCC-CCT	40	29313	1233	28	6
TGA-CGA	51	35406	1131	41	7
TGA-CGT	46	23727	965	39	2.5
TGA-CCA	51	49078	1410	36	7.5
TGA-CCC	57	49078	1410	0	(30)*
				Total	**270**
				Error:	**4.6% ***

* Error rate average excluding the last primer combination (TGA-CCC) as it was not included in further analyses because it had an error rate of 30%.

Table 2. Permutational Analysis of Molecular Variance (AMOVA) results including all experimental treatments (stratified by block).

All Treatments

	Df	SS	F	R2	% of total variance	Pr (> F)	
Block	4	0.64	2.60	0.05	5	<1.E-4	***
Treatment	5	0.47	1.56	0.04	4	<1.E-4	***
Depth	1	0.06	0.98	0.00	0	0.48	
Treat. x Depth	5	0.31	1.02	0.02	2	0.40	
Residuals	206	12.62		0.89	89		
Total	221	14.09		1	100		

Table 3. Results from permutational AMOVA using individual pairwise treatment contrasts (5 replicates: control versus treatment indicated). Original and adjusted p-values (bonferonni correction) are listed for each contrast.

	Df	SS	F	R2	Pr (> F)	P (adj.)
DROUGHT						
Treatment	1	0.09	1.60	0.02	<0.01**	(.03 *)
Depth	1	0.07	1.19	0.02	0.20	
Treatment x Depth	1	0.04	0.77	0.01	0.85	
Residuals	64	3.66		0.95		
Total	67	3.86		1		
HEATED						
Treatment	1	0.08	1.21	0.02	0.13	
Depth	1	0.06	0.97	0.01	0.47	
Treatment x Depth	1	0.07	1.11	0.02	0.30	
Residuals	68	4.24		0.95		
Total	71	4.44		1		
WATERED						
Treatment	1	0.11	1.67	0.02	<0.01**	(.02 *)
Depth	1	0.08	1.20	0.02	0.14	
Treatment x Depth	1	0.05	0.79	0.01	0.74	
Residuals	75	4.83		0.95		
Total	78	5.07		1		
HEATED/DROUGHT						
Treatment	1	0.11	1.74	0.02	<0.01**	(.02 *)
Depth	1	0.05	0.87	0.01	0.66	
Treatment x Depth	1	0.08	1.24	0.02	0.10	
Residuals	68	4.21	6	0.95		
Total	71	4.45		1		
HEATED/WATERED						
Treatment	1	0.10	1.67	0.02	<0.01**	(.02 *)
Depth	1	0.08	1.42	0.02	0.04	(0.17)
Treatment x Depth	1	0.05	0.92	0.01	0.57	
Residuals	71	4.21	4	0.95		
Total	74	4.45		1		

Table 4: ANOVA using individual scores on PCO axes 1 and 2 for pairwise treatment comparisons between control and treatment indicated. Original and adjusted p-values (bonferonni correction) are listed for each contrast.

		PCoA Axis 1 (22%)						**PCoA Axis 2 (19%)**					
	Df	SS	F	Pr(>F)		P.adj		SS	F	Pr(>F)		P(adj)	
A. DROUGHT													
Treatment	1	0.75	2.77	0.10		0.51		3.60	6.22	0.02	**	0.08	.
Block	4	1.80	1.66	0.17		0.86		4.58	1.98	0.11		0.55	
Treat x Block	4	2.57	2.36	0.06	.	0.32		11.20	4.84	0.00	**	0.01	**
Residuals	58	15.78						33.55					
B. WATERED													
Treatment	1	16.97	83.58	2.E-13	***	8.E-13	***	0.00	0.01	0.94		4.71	
Block	4	39.13	48.17	<2E -16	***	1.E-15	***	9.84	3.87	0.01	**	0.03	**
Treat x Block	4	39.19	48.24	<2E -16	***	1.E-15	***	12.43	4.88	0.00	**	0.01	**
Residuals	69	14.01						43.91					
C. HEATED													
Treatment	1	0.35	0.60	0.44		2.21		0.32	0.52	0.47		2.36	
Block	4	9.68	4.16	5.E-03	***	0.02	*	14.92	6.11	0.00	***	0.00	***
Treat x Block	4	15.61	6.71	1.E-04	***	7.E-04	***	8.11	3.32	0.02	*	0.08	*
Residuals	62	36.03						37.87					
D. HEAT/DROUGHT													
Treatment	1	6.94	8.20	0.01	*	0.03	*	0.10	0.11	0.74		3.68	
Block	4	22.67	6.70	2.E-04	***	8.E-04	***	7.45	2.06	0.01	*	0.05	.
Treat x Block	4	22.78	6.73	1.E-04	***	7.E-04	***	9.74	2.70	0.04	*	0.20	*
Residuals	62	52.44						55.94					
E. HEAT/WATERED													
Treatment	1	2.68	6.50	0.01	*	0.07	.	5.05	7.76	0.01	*	0.03	**
Block	4	1.19	0.72	0.58		2.91		1.69	0.65	0.63		3.15	
Treat x Block	4	1.72	1.04	0.39		1.96		19.43	7.47	5.E-05	***	0.00	***
Residuals	65	26.80						42.28					

Figure 1: Layout of experimental plots at BCCIL. Each climate treatment is replicated five times in a randomized block design. C: Control, D: Drought, H: Heating, W: Watered, HD: Heating with Drought, HW: Heating with water.

Figure 2: A) Abundance of *P. lanceolata* in all experimental treatments at BCCIL over time. Dashed lines represent 95% ± standard error. B) *P. lanceolata* abundance across a soil-depth gradient. Fitted line and standard error from generalized additive model (GAM) of *P. lanceolata* abundance (cover class) with respect to soil depth in experimental treatments (see Fridley et al. 2011 for analytical details).

Figure 3: Average scores by treatment and block on PCoA axes 1 and 2 for all experimental treatments. Letters a-e indicate average for each experimental block.

Figure 1

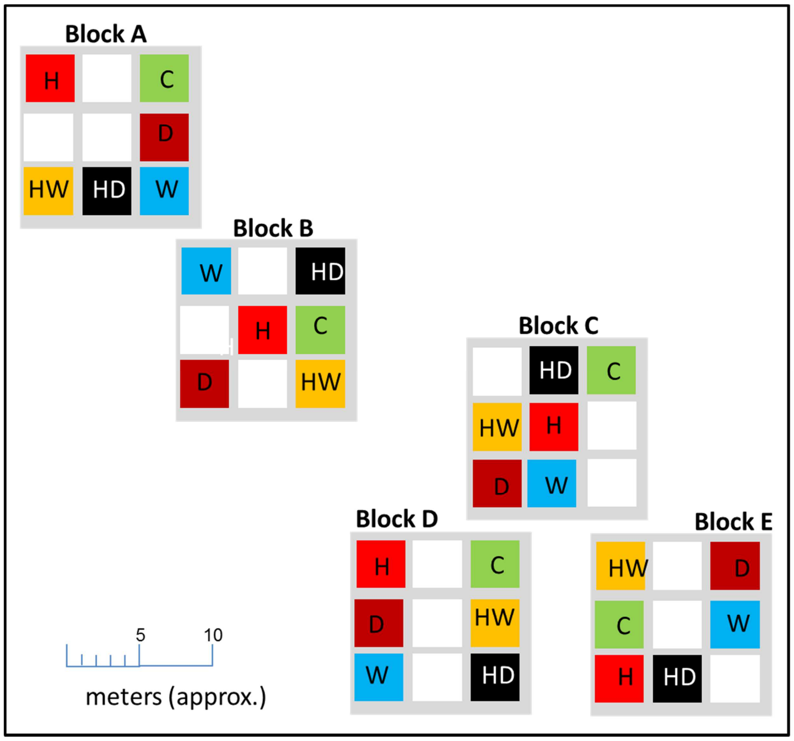

Block A
H
C
D
HW
HD
W
Block B
W
HD
H
C
D
HW
Block C
HD
C
HW
H
D
W
Block D
H
C
D
HW
W
HD
Block E
HW
D
C
W
H
HD
5
10
meters (approx.)

Figure 2

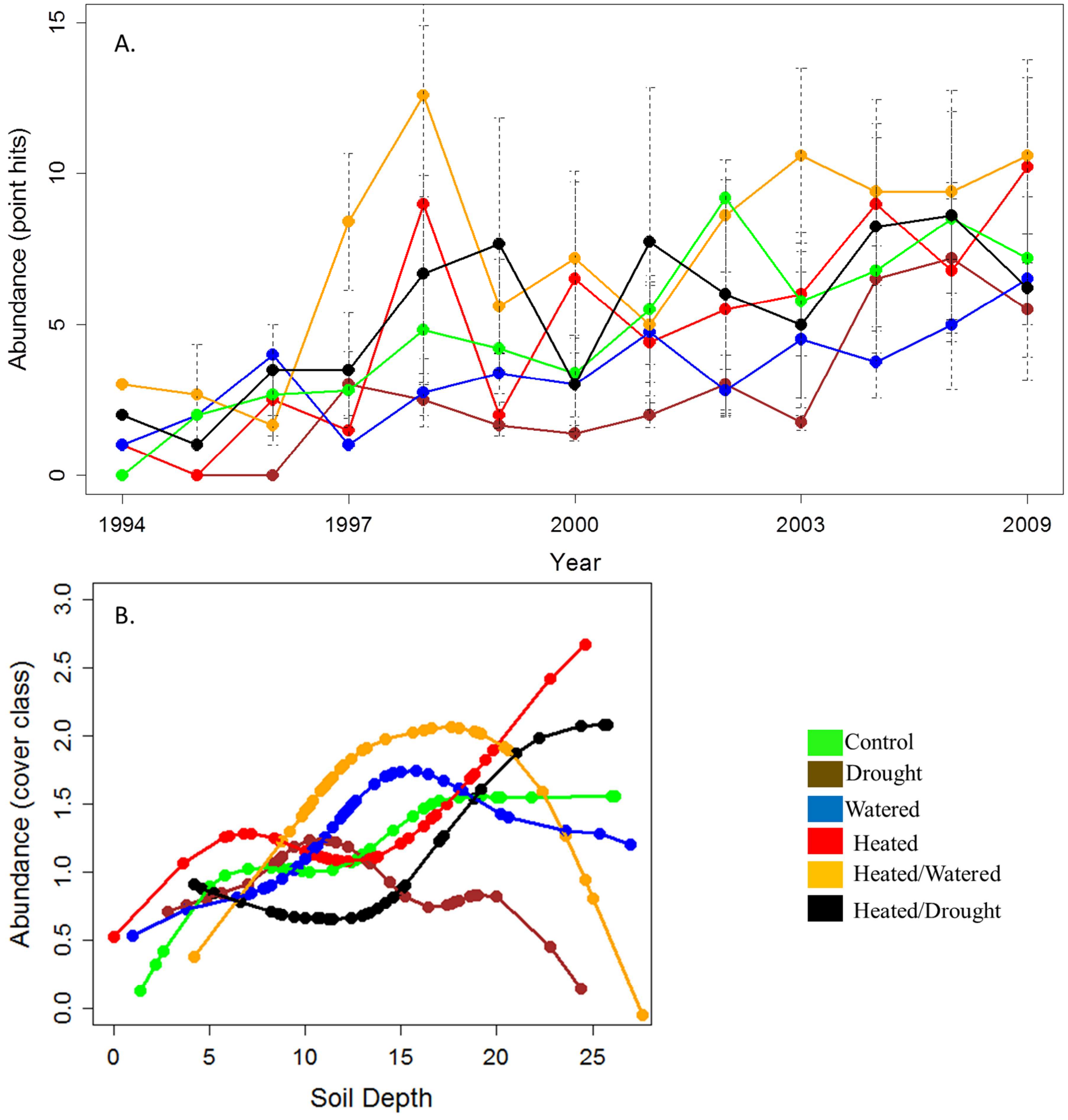
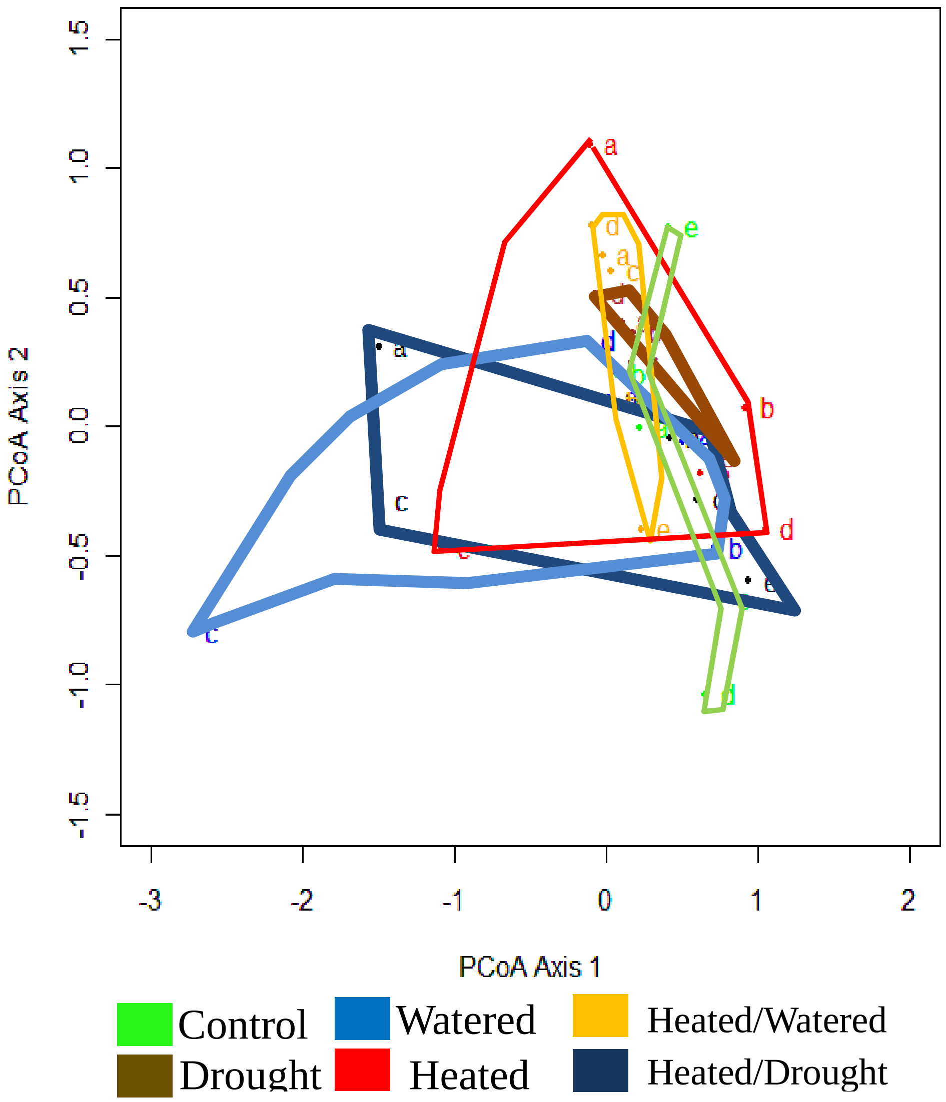

A.
Abundance (point hits)
0
5
10
15
1994
1997
2000
2003
2009
Year
B.
Abundance (cover class)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
0
5
10
15
20
25
Soil Depth
Control
Drought
Watered
Heated
Heated/Watered
Heated/Drought

Figure 3


PCoA Axis 2
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-3
-2
-1
0
1
2
PCoA Axis 1
a
b
c
d
e
🟩 Control
🟫 Drought
🟦 Watered
🟥 Heated
🟨 Heated/Watered
🟦 Heated/Drought

Chapter 4

Intraspecific plant trait variation in a heterogeneous landscape: population response to fine-scale soil moisture gradients

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ABSTRACT

Understanding the processes that determine the spatial distribution of intraspecific trait variation, and whether such variation is adaptive, would substantially improve predictions of vegetation response to environmental change. Landscape gradients of factors such as soil water availability vary at nested spatial scales and are thought to exert strong selection on plant trait variation. Here we measured functional trait variation in the common grassland forb *Plantago lanceolata* L. across nested abiotic gradients associated with soil moisture dynamics. Our objectives were to: 1) characterize the spatial structure of gradients in soil moisture from local (<10 m^2) to landscape (>10 km^2) scales; 2) determine the extent and spatial structure of variation in reproductive and vegetative traits that correspond to well-known plant functional tradeoffs demonstrated at species level; and 3) relate the extent and spatial structure of abiotic gradients to plant functional variation. Abiotic gradients associated with soil water dynamics had a distinct spatial structure that in turn promoted hierarchical partitioning of intraspecific functional variation in SLA, leaf length, number of rosettes, and number of scapes. Trait-environment relationships were particularly pronounced for specific leaf area (SLA) in association with soil water availability. Given that landscape-scale soil moisture dynamics reflect a range of conditions expected under future climate change, evidence of associated trait shifts in *P. lanceolata* suggests that extant populations may be able to adjust to climatic shifts in situ.

INTRODUCTION

Intraspecific functional variation is increasingly recognized as an important component of total community functional diversity (Cianciaruso et al. 2009, Albert et al. 2010a, Hulshof and Swenson 2010, Messier et al. 2010, Albert et al. 2012). Understanding the processes that determine the spatial distribution of intraspecific trait variation, and whether such variation is adaptive, could substantially improve predictions of species' response to climatic change. Recent evidence suggests that functional tradeoffs that define major axes of species level functional variation (e.g., resource use strategies, Grime et al. 1997, Diaz et al 2004, Wright et al 2004) are also evident within species, which suggests that intra- and inter- specific functional variation are driven by similar processes (Albert 2010b, Jung et al. 2010, Messier et al. 2010, Bolnick et al. 2011, Paine et al. 2011, but see Wright et al. 2012). Leaf level traits (e.g., SLA, nitrogen concentration (N)) associated with resource use strategies (Grime et al. 1997, Diaz et al. 2004, Wright et al. 2004) are favored in different climatic conditions (Wright et al. 2001, Wright et al. 2005). Furthermore, there is often strong correspondence between intra- and interspecific functional variation across climatic gradients associated with water availability and temperature (Sandquist and Ehleringer 1997, Boege and Dirzo 2010, Jung et al. 2010, Long et al. 2011, Moreira et al. 2012).

Gradients in temperature and water availability occur at nested spatial and temporal scales (Delcourt and Delcourt 1988, Dunning et al. 1992, Levin 1992) and thus could promote hierarchical partitioning of intraspecific functional trait variation. Temperature and water availability are modified at landscape scales due to differences in irradiance and moisture stress associated with topographic orientation (Perring 1960, Rorison et al. 1986, Bennie et al. 2008). At even finer scales, micro-topographic gradients can affect the moisture flow and redistribution following precipitation and thus alter soil water dynamics (Grime and Curtis 1976, Fridley et al. 2011). Genetic differentiation across gradients of temperature and water availability over landscape scales (<10 m - 1 km) have been reported for several species of grasses (Hamrick & Allard 1972, Hamrick & Holden 1979, Owuor et al. 1997, Li et al. 1999) and local differentiation mirrors patterns found at range wide scales (Hamrick & Allard 1972, Hamrick & Holden 1979). Moisture stress is considered to be the primary driver promoting fine scale genetic differentiation in several conifer species with local patterns repeated at range wide scales (reviewed in Jump et al. 2005). Rising temperatures have caused rapid adaptive differentiation in *Fagus sylvatica* (Jump et al. 2006) and drought has led to the rapid evolution of a drought avoidance strategy in the annual plant *Brassica rapa* (Franks et al. 2011). Recent evidence suggests significant local (<500 m^2) population differentiation in the Mediterranean shrub *Fumana thymifolia* (Jump et al. 2009a). Such fine scale genetic structure may have contributed to the ability of this species to undergo rapid genetic change following 5 years of experimental drought and temperature manipulations (Jump et al. 2008).

A recent study suggests a genetic basis for functional differentiation among *P. lanceolata* populations exposed to nearly two decades of ongoing experimental drought treatments in intact calcareous grassland at the Buxton Climate Change Experiment (BCCIL), Derbyshire, UK (Ravenscroft et al., 2013). Here we combine environmental monitoring and functional trait measurements in *P. lanceolata* in the landscape surrounding BCCIL, in order to: 1) characterize the spatial structure of abiotic gradients associated with soil moisture dynamics; 2) determine the extent and spatial structure of variation in vegetative and reproductive traits that correspond to well-known functional tradeoffs demonstrated at the species level; and 3) relate the extent and spatial structure of moisture gradients and functional variation to determine if nested abiotic gradients promote hierarchical partitioning of intraspecific functional diversity in *P. lanceolata*. We expected that functional traits would differ with respect to their predominant scales of variance and the ability of abiotic filters to filter different functional traits would depend on the trait and abiotic filter considered. Given that trait-based selection can only act at the scales where functional variance occurs, a traits' spatial structure will determine the extent to which populations can accommodate shifts in climate through functional restructuring of genotypes based on their relative fitness and through individual phenotypic plasticity.

METHODS

Study area

The study was conducted in summer 2010 on opposing slope facets at three locations within Peak District National Park in northern England (Derbyshire, UK). Sites were within 9 km of each other and 13 km from the experimental populations at BCCIL. Each site is topographically complex with steep valleys (limestone dales) formed by the erosion of fissures in carboniferous limestone bedrock (Pigott 1962). South slope facets intercept more solar radiation and tend to be both drier and warmer than north facets (Rorison et al. 1986, Bennie et al. 2006). Steeper slopes, more common towards the top of slope facets, often have a thinner mineral soil layer and more outcrops of exposed limestone. At even finer scales (<3 m^2), extreme variation in soil depth (>40 cm) can buffer temporal fluctuations in soil water availability in this system (Grime and Curtis 1976, Fridley et al. 2011). The dales are therefore an ideal study system for examining the landscape spatial structure of environmental conditions relating to gradients in soil water supply and demand (Balme 1953, Pigott 1962, Perring 1960, Rorison et al. 1986, Bennie 2006).

Focal species and selected traits

Plantago lanceolata L. is a self-incompatible, perennial herb of wide-ranging distribution and is an abundant forb species across the limestone grasslands of northern England, including BCCIL (Sagar and Harper 1964). The species is wind pollinated but displays substantial local genetic differentiation (Bos et al. 1986, Ravenscroft et al. 2013b) and exhibits distinct genetically determined phenotypes both at regional and fine scales (Primack and Antonovics 1982, Teramura 1983, van Tienderen 1992, Tonsor and Goodnight 1997, Wolf and van Delden 1987, 1989).

For individuals of *P. lanceolata* across three sites, we measured four vegetative traits, including SLA, leaf length, number of rosettes, and leaf angle, and two reproductive traits including number of flowering stems (scapes) and scape length. These traits are associated with individual fitness (Violle et al. 2007) and collectively reflect the main axes of functional differentiation found locally among *P. lanceolata* populations exposed to long term experimental drought at BCCIL (Ravenscroft et al., in prep.). Leaf length is strongly correlated with leaf dry matter content (LDMC) in *P. lanceolata* (Ravenscroft, unpublished data). LDMC reflects the average density of leaf tissue, tends to scale inversely with SLA (Cornelissen et al. 2003), and has been shown to be negatively correlated to relative growth rate (Weiher et al. 1999). Leaf-level traits such as SLA and LDMC are related to rates of resource acquisition and retention, a major axis of functional differentiation demonstrated among species (e.g., Grime et al. 1997, Diaz et al. 2004, Wright et al. 2004). Scape number and scape length relate to different regeneration strategies in *P. lanceolata* (Lacey and Herr 2000, Lacey et al. 2003) and these traits represent two distinct axes of functional variation found locally among *P. lanceolata* populations exposed to long term experimental drought at BCCIL (Ravenscroft et al. in prep.).

Sampling design

Plants were sampled over a five week period beginning June 8. Plots were established on opposing slope facets in Lathkill Dale, Cressbrookdale, and Millers Dale (Table 1). Three 10 m^2 (2 x 5 m) plots were established in up to three different slope positions (top, mid, bottom) for a maximum of nine plots per slope facet. The minimum distance between different slope positions was 50 m. Plots were established in two slope positions in the Cressbrookdale southeast facet and in one position in the Lathkill Dale northeast, Millers Dale southeast, and Millers Dale northwest facets (Table 1). The Lathkill Dale northeast facet was large enough to accommodate plots at two slope positions, but *P. lanceolata* was only found at mid-slope positions and so plots were only established there (Table 1).

Plots were positioned randomly at each slope position. For each plot we recorded its geocoordinates, the slope angle at five positions, and facet orientation (aspect). Aspect was transformed into a linear variable ranging from 0 (southwest) to 2 (northeast) using the Beers transformation (Beers et al. 1996). Each 2 x 5 m plot was divided into 40 0.5-m^2 quadrats. Soil depth measurements were taken at quadrat corners (55 measurements per plot). From the soil depth survey we randomly selected four shallow (soil depth: < 8cm), medium (soil depth: 8-14 cm), and deep (soil depth: >14 cm) quadrats for more extensive survey efforts (depth thresholds based on the analysis of Fridley et al. 2011). Within quadrats the presence of *P. lanceolata* was recorded in each of 100 10-cm^2 microsites and one occupied microsite was arbitrarily selected for trait measurements, stratified with respect to soil depth (4 individuals/depth class).

Number of rosettes, orientation angle of youngest most fully expanded leaf (degrees from horizontal), maximum scape length (cm), and number of scapes were measured in the field. We sampled two fully expanded leaves from each individual for measurement of SLA, leaf length, and leaf area. Leaves were laid flat, wrapped in moistened newspaper and stored in small cooler. Leaves were scanned the evening following sampling and stored at room temperature to a constant dry weight.

Environmental data

We monitored water potential (Decagon MPS-1 dialectric; Decagon Devices, Pullman, WA, USA) and volumetric water content (Decagon EC5) in shallow (top 7 cm) and deep microsites (15-20 cm) at half-hour intervals during the summer growing season (June 6 – August 29). Two sensors of each type were deployed in mid-slope positions on the south facets of Cressbrookdale and Lathkill Dale and on the southwest facet of Millers Dale. We used an additional Decagon EC5 sensor to measure soil water content in each plot up to two days before vegetation was sampled. Rainfall was measured at Cressbrookdale and Lathkill Dale using a HOBO tipping bucket rain gauge (Onset Computer Corporation, Bourne, MA, USA). Precipitation data from Cressbrookdale was used for Millers Dale as these two dales are less than 2 km apart.

Analysis

We fit water retention curves (Appendix 1) to translate measures of volumetric water content (collected in individual plots) into water potential for each dale. We also extracted dale-scale minimum water potential and volumetric water content to include as fixed effects in the modeling approach that follows.

We used mixed effects models (‘lme4’ library in R version 2.13.0; Bates et al. 2011) to partition trait variance across the five scales represented in the experimental design: dale (site), slope facet, slope position, between 10 m^2 plots of the same slope position ('between plot'), and within 10 m^2 plots ('within plot'). For each trait we constructed a null model of random effects reflecting the hierarchical (nested) structure of the sampling design, allowing us to partition trait variance among spatial extents. SLA, leaf length, scape length and leaf angle were modeled with Gaussian error. Scape and rosette number were modeled using Poisson-distributed error more appropriate for count data. We estimated the index of dispersion for models with Poisson-distributed error by calculating the sum of squared Pearson residuals and comparing it to the residual degrees of freedom (Venables and Ripley 2002). The index of dispersion was in both cases close to one.

After specifying nested random effects, we added fixed effects of soil moisture content, water potential, soil depth, slope angle, and precipitation (Table 2). We included both water content and water potential because these factors are non-linearly related (Appendix 1) and reflect different aspects of soil water dynamics in the rooting zone. For example, water potential varies more in dry conditions (Appendix 1) and thus is a better indicator of drought stress than volumetric content, whereas volumetric content is a better indicator of oxidative stress. Fixed effects for soil moisture for each plot were based on instantaneous volumetric water content taken in each plot. Plot level water potential was estimated by converting measures of water content to water potential using the water retention curves constructed for each dale (Appendix 1). To capture differences in minimum water potential and volumetric content over the course of the entire study period, we extracted minimum water potential and water content from continuous monitoring data collected in each dale. We included two measures of soil depth as fixed effects: soil depth of each sampled individual and mean plot soil depth. We felt two measures were appropriate as soil moisture might differ for individuals occupying a shallow soil depth class in an otherwise deep plot, compared to occupying a shallow depth class in an otherwise shallow plot.

Coefficients for fixed effects were fit by maximum likelihood and the significance of each fixed effect was assessed with log-likelihood tests based on the Akaike Information Criterion (AIC). We compared variance components from models with and without each fixed effect to determine the contribution of each predictor variable to trait variance at each spatial scale. We constructed a full model including all fixed effects for each individual trait. Following Bolker (2009), we started with a full (maximal) model which included all fixed effects and performed a backwards selection procedure with likelihood ratio tests. We used the same method to test whether random (spatial) effects were necessary in the final model. Finally, we calculated the total amount of functional variance explained by the full model.

We used mixed effects models to test for significant differences in leaf traits (SLA, leaf length, and area) among opposing slope facets and among sampling periods for each individual dale. Significant temporal variation would indicate plasticity, such that individual populations were able to adjust trait values in response to reduced water availability later in the summer.

RESULTS

Spatial distribution of abiotic factors

Mean plot depth, volumetric water content, and soil water potential showed considerable variance between plots within the same slope position (Fig. 1). Mean plot depth exhibited the most spatial structure, with variance partitioned across all spatial scales considered except among different slope positions (Fig. 1). Variance in mean plot depth between slope facets (35%) and between plots of the same slope position (34%) were both higher than the combined variance associated with slope position (17%) and dale (13%) (Fig. 1). All variance in plot level water potential was between plots within the same slope position. Plot level volumetric content also showed substantial variance between plots of the same slope position (83%) with the remaining variance associated with differences among slope facets (17%). Variance in slope angle was almost entirely associated with differences between slope positions (Fig. 1).

Spatial distribution of trait variation

The six traits measured had distinct spatial structures (Fig. 2). The majority of variance in leaf angle, scape length, and scape number was found within 10 m^2 plots (85%, 90% and 75%, respectively). The remaining variance in scape number was attributed to differences among slope facets (21%) and slope position (4%), whereas variance among slope positions and plots accounted for the remaining variance in scape length (3% and 7%, respectively, Fig. 2). Within plot variance in leaf length (60%) was over three times the variance found at any other scale and the remaining variance was attributed to differences among plots, slope position, and facets (18%, 14%, and 8%, respectively, Fig. 2). Almost all of the variance in number of rosettes was associated with differences between slope positions (79%), with the remaining 21% attributed to variance below the plot level. SLA exhibited the most spatial structure and was the only trait that showed significant variance at the largest spatial scale, between dales (11%).Variance in SLA was highest within plots (38%), among slope facets (29%), and between plots at the same slope position (21%) (Fig. 2).

Trait variation in response to environmental factors

Scape number was negatively correlated with slope angle and plot depth and including these factors as fixed effects in a mixed effect model reduced overall trait variation by 22% (Fig. 2). Including soil depth reduced the variance associated among facets whereas slope angle reduced variance associated with slope position (Appendix 2). We did not find any significant correlations between abiotic factors and scape length. Leaf angle was negatively correlated with aspect and slope angle and positively correlated with individual depth, but moisture and landscape factors explained only 6% of intraspecific variation in this trait due to substantial within-plot variance (Fig. 2). Slope angle was the only significant predictor of rosette number, explaining 19% of trait variation as individuals on steep slopes had fewer rosettes (Fig. 2). Leaf length was positively correlated with soil depth and negatively correlated with slope angle. Plot level volumetric content, plot depth and minimum seasonal water potential were significant predictors of SLA, and collectively explained 31% of the total variance in this trait (Fig. 2). Including seasonal WP reduced variation in SLA between dales, VC reduced variation in SLA among opposing slope facets, and soil depth reduced variation among plots in the same slope position (Fig. 2, Appendix 2).

Trait variation in individual dales

SLA was the only trait that had variation at the largest scale considered, among dales (Figure 2). SLA was significantly higher in Cressbrookdale than in Lathkill Dale or Millers Dale (Figure 3). SLA was highest in Cressbrookdale north facet and higher values in this particular slope facet contribute to the observed dale scale variation in SLA. SLA was significantly lower in south facets relative to north facets in Cressbrookdale and Lathkill dale (Figure 3). In Lathkill dale and Cressbrookedale there was also significant variation associated with sampling period, where SLA was generally higher in June relative to July in all slope facets except the second southwest facet of Lathkill Dale (Figure 3). We found no evidence of spatial or temporal variation in SLA in Millers Dale (Figure 3). Leaf area and length were higher in south facets relative to north facets in Cressbrookedale and Lathkill Dale (Figure 3). In Millers Dale, differences among opposing slope facets were less pronounced, but leaf length and area were higher in north versus south facets. Leaf length and area did not vary significantly with respect to sampling periods in any dale.

DISCUSSION

The spatial structure of a critical plant resource, soil moisture, is determined by abiotic factors controlling water supply (slope position), storage (soil depth), and evaporative demand (slope angle and orientation). Some, but not all, traits of a dominant forb express coordinated variation with soil moisture or other correlated environmental drivers (e.g., soil nitrogen availability). To our knowledge, this is the first study that explicitly connects the spatial structure of intraspecific functional variation to the spatial structure of nested abiotic gradients. For our focal species, *Plantago lanceolata*, the expression of functional traits such as SLA in part match environmental gradients consistent with similar studies in other systems (Albert 2010b, Jung et al. 2010, Messier 2010, Long et al. 2011, Paine et al. 2011). Other traits, and particularly reproductive traits, appear to exhibit greater fine-scale variation that are weakly if at all associated with gradients of water stress.

We found high local (within 10 m^2 plot) variance in reproductive traits (scape length and number per individual), whereas traits associated with vegetative growth (SLA, number of rosettes, leaf length) generally had more variance partitioned at larger spatial scales. High local variance in regenerative traits could be associated with different establishment strategies that are maintained by differences in flowering phenology in *P. lanceolata* (Lacey et al. 2003). Similar patterns are found in fire prone ecosystems, where the frequency, intensity, and extent of fires promotes local intraspecific functional differentiation in reproductive traits (Moreira et al. 2012), whereas traits related to vegetative growth are differentiated at broader spatial scales associated with regional climatic gradients (Farley and McNeilly 2000). These findings are also consistent with species-level predictions, where traits associated with vegetative growth are expected to be subject to stronger abiotic filtering, and thus local trait convergence is expected in vegetative traits and local trait divergence is expected in traits relevant to reproductive output (Grime 2006).

Leaf-level traits such as SLA and LDMC are related to rates of resource acquisition and retention, a major axis of functional differentiation demonstrated among species (e.g., Grime et al. 1997, Diaz et al. 2004, Wright et al. 2004). SLA in particular has been linked to moisture availability in several other studies of various spatial extent and taxonomic resolution (Jung et al. 2010, Long et al. 2010, Messier et al. 2010). Of the traits measured, SLA exhibited the most complex spatial structure, with variance distributed at all spatial scales except between slope positions. SLA also was the only trait that exhibited significant temporal variation. Individuals in northerly facets tended to have high SLA earlier in the summer, when conditions were cooler and wetter. Later in the summer variance in SLA associated with slope position was less evident due to a reduction in SLA in populations in northerly aspect. This suggests that individuals are able to adjust SLA seasonally in response to climatic conditions, in this case reducing SLA in response to drier conditions later in the summer. Since we found no associated temporal plasticity in leaf length or area, reductions in SLA are likely due to increased investment per unit area in later summer leaves. Leaf length and area also varied significantly among opposing slope facets and trait values in both cases were relatively fixed through time. Given that leaf length is strongly and positively correlated with LDMC in *P. lanceolata* (Ravenscroft, unpublished data), our finding suggests a drought resistance strategy (low SLA, high LDMC) may be more prominent in drier south facets.

Landscape scale gradients in soil moisture dynamics emulate a range of conditions expected under future climate change, evidence of associated functional structuring suggests that extant populations may be able to adjust to climatic shifts through individual plasticity and genetic restructuring. The relevance and importance of associated hierarchical spatial partitioning of plant trait variation depends on the extent to which such variation is the result of relatively fine-scale genetic differentiation (Linhart and Grant 1996). Recent work suggests a genetic basis for fine-scale functional differentiation among *P. lanceolata* populations exposed to long term climate manipulations at BCCIL (Ravenscroft et al. 2013a, 2013b). A strong genetic component to trait variation suggests populations of *P. lanceolata* harbor sufficient variation in functional traits within landscapes to be able to adapt to future drier climates *in situ*. The presence of extreme soil moisture gradients within the relatively small spatial extent of one dale may be the reason that *P. lanceolata* has been able to tolerate experimentally induced extreme summer drought in this grassland (Grime et al. 2000, Fridley et al. 2011). More generally, local and landscape scale climatic heterogeneity is considered to promote local diversity and thus may provide a buffering capacity of populations in the face of climatic shifts (Jump et al. 2005, Jump et al. 2009a, Jump et al. 2009b, Ashcroft et al. 2010).

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Table 1: Precipitation, soil volumetric content and water potential at each site. Slope orientation and the number of plots per slope position are listed for each site.

		Number of 10 m^2 plots			Precipitation (mm)			Volumetric content (m^3/m^3)			Water potential (kPa)		
	Slope Orientation	Top	Mid	Low	June	July	Aug.	mean	min	max	mean	min	max
Cressbrookdale	SE (140°)	3		3	237	320	224	**0.12**	0.01	0.30	**-132**	-503	-16
	NW (330°)	3	3	3									
Millers Dale	SW (205°)		3		237*	320*	224*	**0.15**	0.01	0.33	**-107**	-585	-10
	NE (26°)		3										
Lathkill Dale	SW (230°)	3	3	3	151	262	231	**0.13**	0.01	0.40	**-200**	-621	-12
	NE (35°)		3										
	SW (195°)	3	3	3									

* Data are from Cressbrookdale which is located < 2 km away.

Table 2: Traits measured.

	min	max	mean	sd
SLA (cm2/g)	18.6	272.6	94.0	37.3
Leaf length (cm)	2.6	31.7	13.2	5.8
Number of Rosettes	1	23	1.8	2.0
Angle*	1	4	3.0	0.8
Number of Scapes	0	12	1.0	1.5
Scape Length (cm)	0	55	12.4	14.8

* Categorical: 1: <10°, 2: 10-25°, 3: 25-45°, 4: >45°

Table 3: Predictor variables used in mixed-effect models.

Within Plot	**Units**	**Source/Description**
Individual depth	cm	Depth for each sample individual

Plot Level	**Units**	**Source/Description**
Mean plot depth	cm	55 depths per plot
Volumetric water content (mean)	m^3/m^3	6 per plot
Water Potential (mean)	kPa	Estimated from VC using retention curve
Slope Angle	°	Plot inclination
Aspect	NA	Beers (1966) linear transformation of aspect

Dale Scale	**Units**	**Source/Description**
Volumetric content (mean/min/max/var)	m^3/m^3	Recorded at half-hour intervals from June 6- August 29
Water Potential (mean/min/max/var)	kPa	Recorded at half-hour intervals from June 6- August 29
Precipitation	cm	Total June precipitation

Figure Legends

Figure 1. The distribution of variance at nested spatial scales for five abiotic factors. Individual depth, seasonal minimum water content and volumetric content are not included because variance partitioning was associated with experimental design. Seasonal minimum volumetric content and seasonal minimum water potential were measured at the site scale, with all variance expressed between dales. Similarly, since sampling was purposely stratified across soil-depth class, individual depth variance was limited to within plot variation only.

Figure 2. The distribution of trait variation across the nested spatial scales represented in the sampling design. Grey bars indicate the percentage of functional variance at each spatial scale under a null spatial model of random effects. Dashed black lines indicate the distribution of trait variation under the full model constructed via a backwards selection procedure with likelihood ratio tests, following the procedure set on in Bolker (2009). Percentage of the total variance explained by abiotic factors under the final model (total variance of the fixed effect model/total variance in the random model) is listed under each trait, followed by the full model with estimated values for each predictor in parentheses after each term. *** $P < .0001$, ** $P <0.01$, * $P <0.05$

Figure 3. Variation in leaf level traits across opposing slope facets and through time for the three dales considered. For each slope facet trait values for the first sampling period (June) are in black and for the second sampling period (July) values are in grey. Dales are separated by vertical grey lines. Cressbrookedale (NW, SW) on the left, Lathkill dale (NE, SW1, SW2) in the middle, and Millers Dale (NE, SW) on the right.

Figure 1

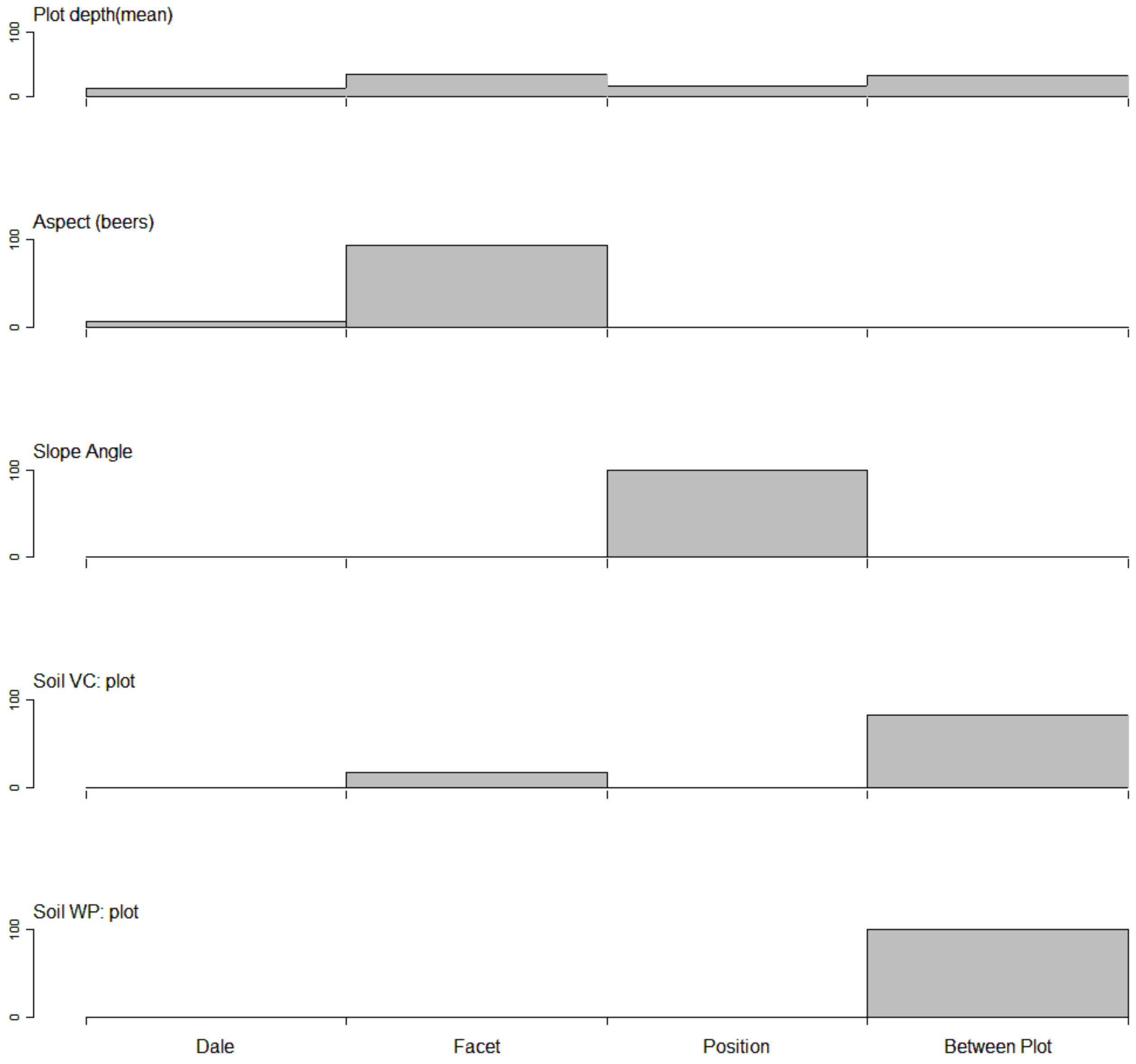

Plot depth(mean)
Aspect (beers)
Slope Angle
Soil VC: plot
Soil WP: plot
100
0
Dale
Facet
Position
Between Plot

Figure 2

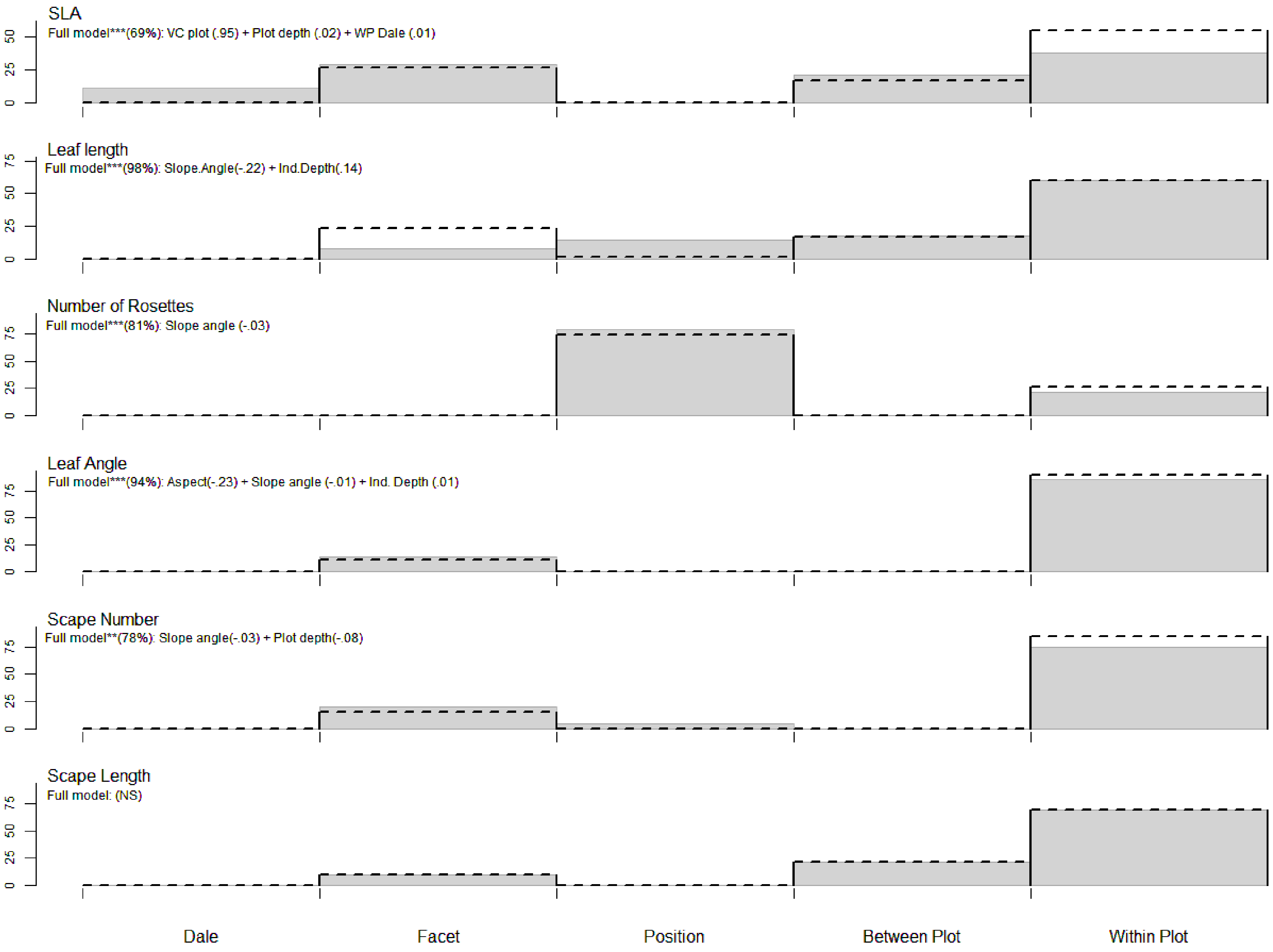
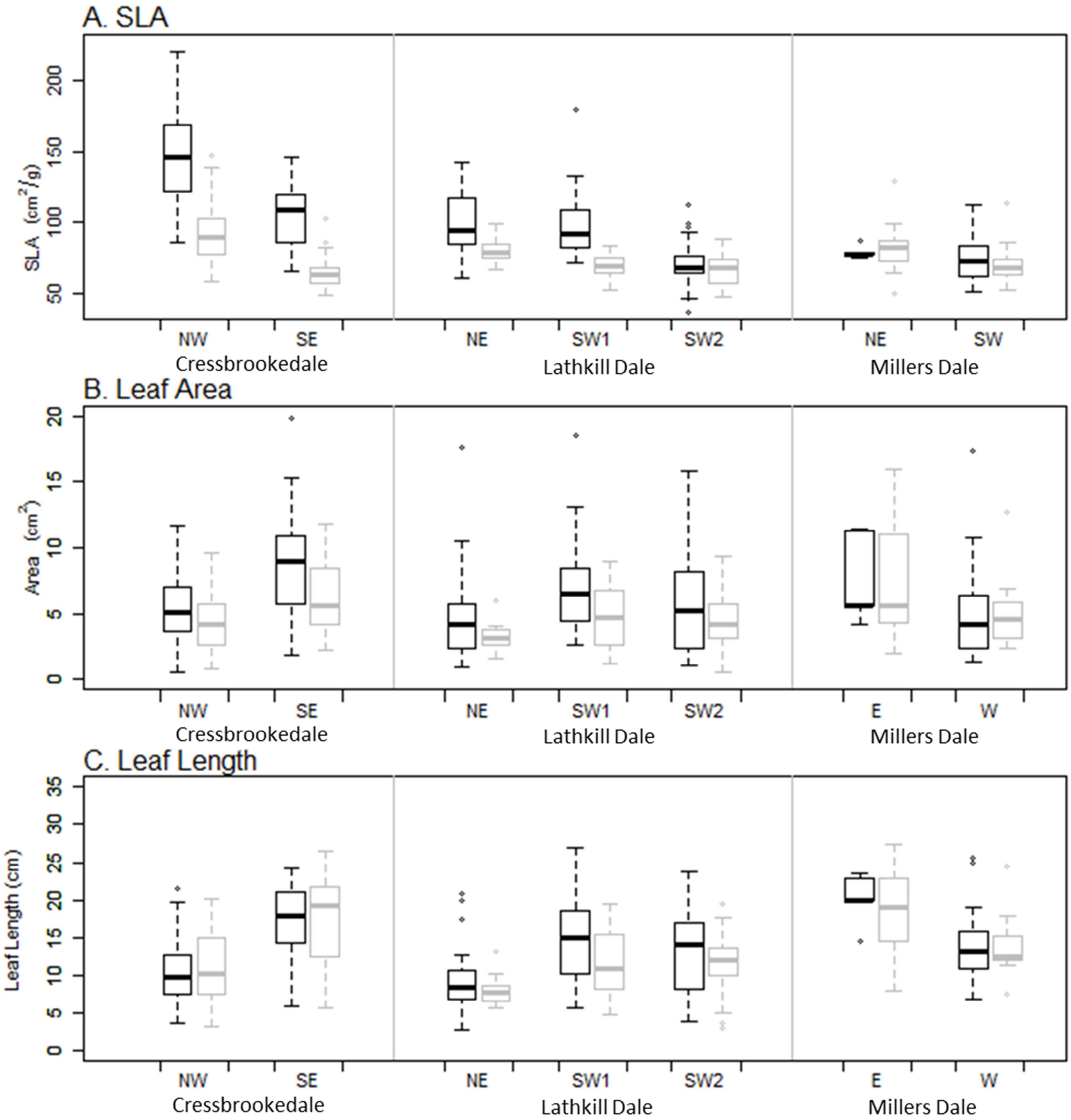
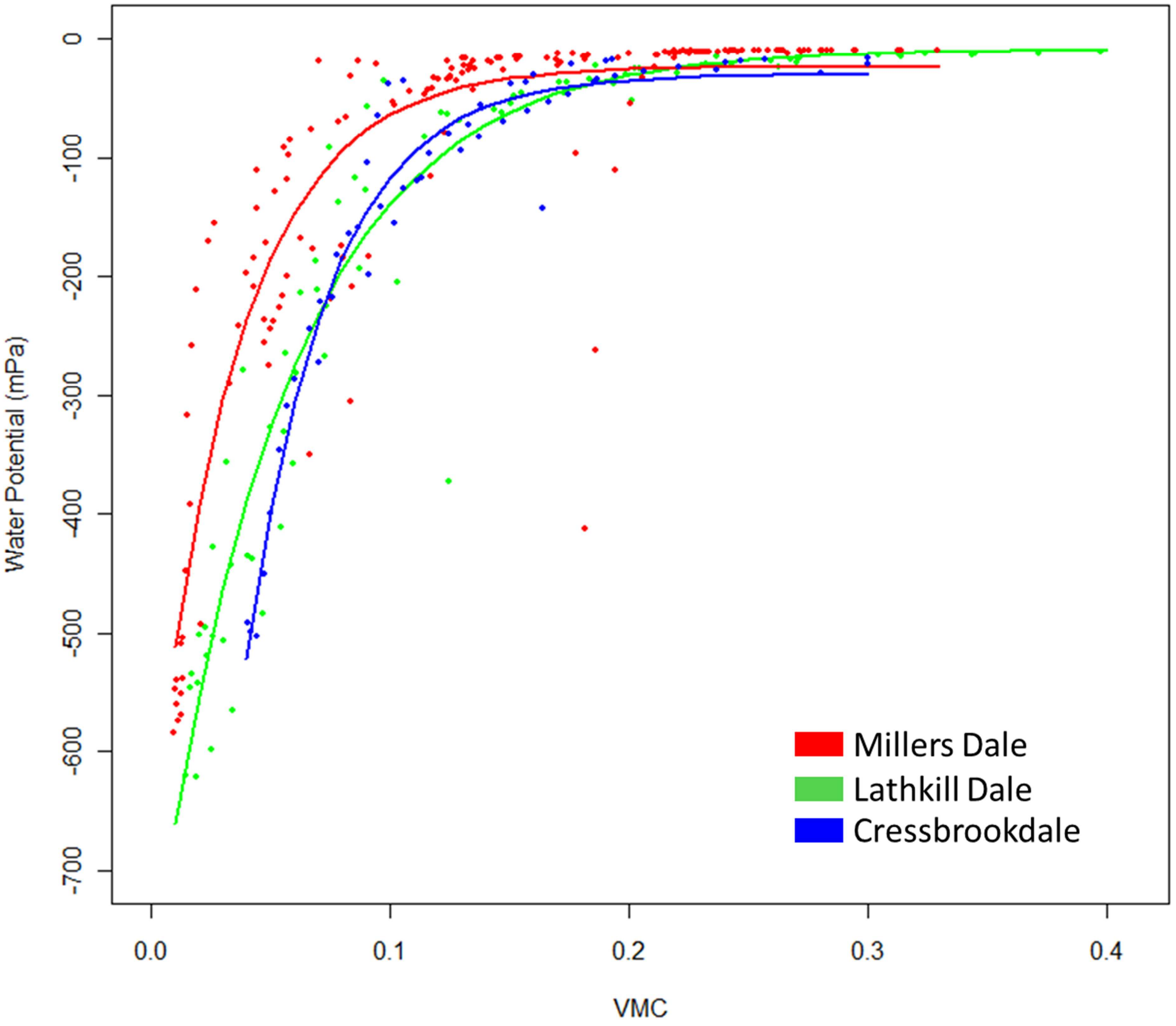

SLA
Full model***(69%): VC plot (.95) + Plot depth (.02) + WP Dale (.01)
0
25
50
Leaf length
Full model***(98%): Slope.Angle(-.22) + Ind.Depth(.14)
0
25
50
75
Number of Rosettes
Full model***(81%): Slope angle (-.03)
0
25
50
75
Leaf Angle
Full model***(94%): Aspect(-.23) + Slope angle (-.01) + Ind. Depth (.01)
0
25
50
75
Scape Number
Full model**(78%): Slope angle(-.03) + Plot depth(-.08)
0
25
50
75
Scape Length
Full model: (NS)
0
25
50
75
Dale
Facet
Position
Between Plot
Within Plot

Figure 3


A. SLA
SLA (cm^2/g)
50
100
150
200
NW
SE
Cressbrookedale
NE
SW1
SW2
Lathkill Dale
NE
SW
Millers Dale
B. Leaf Area
Area (cm^2)
0
5
10
15
20
NW
SE
Cressbrookedale
NE
SW1
SW2
Lathkill Dale
E
W
Millers Dale
C. Leaf Length
Leaf Length (cm)
0
5
10
15
20
25
30
35
NW
SE
Cressbrookedale
NE
SW1
SW2
Lathkill Dale
E
W
Millers Dale

Appendix 1: Water retention curves relating volumetric content (%) to water potential for each dale. The relationship between Volumetric Content and water potential was estimated using a 3-parameter asymptotic exponential modeled with a least-squares model in R (Bolker, 2009) using WP and VC daily averages collected in each dale. Final models: 1) Cressbrookdale: $-30 – 1563e^{-29 * VC}$; 2) Millers Dale: $-23 - 644e^{-28 * VC}$; 3) Lathkill Dale: $-9 - 781e^{-18 * VC}$



Appendix 2: Variance components for the six functional traits measured including the total variance and the percentage of this variance found at each spatial scale. % Random is the total variance of the fixed effect model divided by the total variance in the random model and thus represents the variance explained when individual predictors are included. P-values for individual terms reflect likelihood ratio tests using AIC values of the fixed effect and random model. Full models are listed after tables of individual fixed effects, and estimated values are in parentheses after each term. *** P < .0001, ** P <0.01, * P <0.05

A. SLA

Model	Fixed effect Value	Total Variance	% Random	Dale	Aspect	Position	Plot	Within Plot	P. Random vs. Fixed
Random		0.12		11	29	0	21	38	
Ind. depth	-4E-04	0.12	100	11	29	0	21	38	0.80
Plot depth	**0.03**	**0.11**	**91**	**5**	**36**	**3**	**15**	**42**	**1E-3 ****
Aspect (beers)	**0.14**	**0.11**	**91**	**7**	**27**	**0**	**24**	**42**	**>.05 ***
Slope angle	-0.01	0.11	95	13	24	0	23	40	0.08 .
VC plot	**1.17**	**0.10**	**84**	**16**	**20**	**0**	**19**	**46**	**2E-4 *****
WP plot	**6E-04**	**0.11**	**94**	**18**	**20**	**0**	**21**	**41**	**0.01 ***
WP Dale	**2E-03**	**0.10**	**87**	**0**	**31**	**0**	**25**	**44**	**0.03***
VC Dale	-0.39	0.13	113	21	26	0	19	34	1.00
Precipitation Dale	2E-03	0.12	102	12	29	0	21	38	0.44

Full Model: **VC plot (.95) + Plot depth (.02) + WP Dale (.01) ***** **69%**

B. Leaf Length

Model	Fixed effect Value	Total Variance	% Random	Dale	Aspect	Position	Plot	Within Plot	P. Random vs. Fixed
Random		34.82		0	8	14	18	60	
Ind. depth	**0.14**	**34.46**	**99**	**0**	**6**	**16**	**19**	**59**	**7E-06 *****
Plot depth	-0.01	35.21	101	0	8	14	19	59	1.00
Aspect (beers)	-0.96	35.27	101	1	8	14	18	59	0.50
Slope angle	**-0.22**	**34.36**	**99**	**0**	**22**	**0**	**16**	**61**	**0.02 ***
VC plot	-2.34	34.82	100	0	5	16	20	60	0.68
WP plot	5 E-05	35.11	101	0	8	13	19	60	1.00
WP Dale	0.01	35.69	103	1	9	13	18	59	1.00
VC Dale	-15.09	34.50	99	0	7	14	18	61	0.20
Precipitation Dale	0.03	34.82	100	0	8	14	18	60	0.28

Full Model: **Angle (-.22) + Ind. Depth (.14) ***** **98%**

C. Leaf Angle

Model	Fixed effect Value	Total Variance	% Random	Dale	Aspect	Position	Plot	Within Plot	P. Random vs. Fixed
RANDOM		0.65		0	14	0	1	85	
Ind. depth	**0.01**	**0.65**	**100**	**0**	**14**	**0**	**1**	**85**	**0.09 .**
Plot depth	2E-03	0.66	100	0	14	0	1	85	1.00

Aspect (beers)	**-0.20**	**0.64**	**98**	**0**	**12**	**0**	**1**	**87**	**0.08 .**
Slope angle	**-0.01**	**0.65**	**99**	**0**	**13**	**0**	**1**	**86**	**0.09 .**
VC plot	-0.63	0.64	98	0	11	0	2	87	0.21
WP plot	-1E-05	0.65	100	0	13	0	1	85	1.00
WP Dale	-6E-04	0.67	103	3	13	0	1	83	1.00
VC Dale	-1.29	0.65	100	0	14	0	1	85	0.38
Precipitation Dale	0.00	0.68	104	3	13	0	1	82	1.00

Full Model: Aspect (-.23) + Slope angle (-.01) + Ind. Depth (.01) * 94%**

D. Rosette

Model	Fixed effect Value	Total Variance	% Random	Dale	Aspect	Position	Plot	Within Plot	P. Random vs. Fixed
RANDOM		0.18		0	0	79	0	21	
Ind. depth	0.01	0.18	99	0	0	80	0	20	0.51
Plot depth	0.00	0.18	100	0	0	79	0	21	0.94
Aspect (beers)	-0.14	0.18	99	0	0	79	0	21	0.77
Slope angle	**-0.03**	**0.14**	**81**	**0**	**0**	**74**	**0**	**26**	**0.02 ***
VC plot	-0.05	0.18	100	0	0	79	0	21	0.93
WP plot	0.00	0.18	100	0	0	79	0	21	0.93
WP Dale	-4.4E-04	0.18	100	0	0	79	0	21	0.79
VC Dale	-0.47	0.18	99	0	0	79	0	21	0.75

Precipitation Dale	0.00	0.18	100	0	0	79	0	21	0.95

Full Model: Slope angle (-.03) *** **81%**

E. Scape

Number

Model	Fixed effect Value	Total Variance	% Random	Dale	Aspect	Position	Plot	Within Plot	P. Random vs. Fixed
RANDOM		0.34		0	21	4	0	75	
Ind. depth	0.01	0.34	100	0	21	4	0	74	0.36
Plot depth	**-0.07**	**0.30**	**90**	**0**	**8**	**16**	**0**	**76**	**0.01 ***
Aspect (beers)	-0.14	0.33	97	0	17	7	0	76	0.36
Slope angle	**-0.02**	**0.31**	**92**	**0**	**24**	**0**	**0**	**76**	**0.08 .**
VC plot	0.42	0.34	100	0	21	3	1	75	0.62
WP plot	2.1E-04	0.34	100	0	22	1	3	75	0.74
WP Dale	-1.9E-03	0.32	95	0	14	8	0	77	0.32
VC Dale	2.06	0.32	95	0	12	8	0	79	0.20
Precipitation Dale	0.00	0.31	94	0	11	10	0	80	0.20

Full Model: Slope angle (-.03) + Plot depth (-.08) ** **78%**

F. Scape

Length

Model	Fixed effect Value	Total Variance	% Random	Dale	Aspect	Position	Plot	Within Plot	P. Random vs. Fixed
RANDOM		2.02		0	10	0	21	69	
Ind. depth	-0.01	2.02	100	0	10	0	21	69	0.36
Plot depth	-0.05	2.00	99	0	11	0	20	69	0.33
Aspect (beers)	0.01	2.02	100	0	10	0	21	69	0.97
Slope angle	-0.02	2.02	100	0	12	0	19	69	0.58
VC plot	1.50	1.99	98	0	8	0	22	70	0.38
WP plot	0.00	2.02	100	0	10	0	21	69	0.89
WP Dale	0.00	1.98	98	0	6	0	23	71	0.45
VC Dale	0.56	2.02	100	0	10	0	21	69	0.45
Precipitation Dale	0.00	2.02	100	0	9	0	21	69	0.83

Full model: NS

Chapter 5

SYNTHESIS

Many studies have examined the need and capacity of species to migrate poleward as the climate shifts (Davis and Shaw 2001, Neilson et al. 2005). The potential for local adaptation, however, remains understudied despite the recognized potential for genetic diversity to buffer species from climate-induced local extinction (Jump and Peñuelas 2005). Compared to migration, the potential for local adaptation is likely to be highest under circumstances where: a) there is sufficient local genetic variation that underlies quantitative traits relevant to climatic shifts; b) the environmental shift is more than can be accommodated by phenotypic plasticity; and c) species-level changes are restricted (e.g., from dispersal limitation) (Davis et al. 1986, Davis & Shaw 2001) or occur more slowly than population-level change (Moser et al. 2011).

Most experimental climate manipulations have led to fairly rapid species-level changes, suggesting that local adaptation is not significant (Chapin et al. 1995, Harte and Shaw 1995, Grime et al. 2000, Zavaleta et al. 2003, Klein et al. 2007, Mikkelson et al. 2008). At the Buxton Climate Change Impacts Laboratory (BCCIL) in northern England (UK), annual manipulations of temperature and rainfall have been maintained since 1993 and most species have not experienced large changes in abundance in response to experimental treatments (Grime et al. 2000, 2008, Fridley et al. 2011). Adaptation to experimental treatments has been hypothesized as one potential mechanism of species persistence (Grime et al. 2008), but it is unclear whether selection or plasticity underlies species' stability.

Most species common to this calcareous grassland system are obligate outcrossers with high local phenotypic variation that has a genetic basis (Booth and Grime 2003, Fridley et al. 2007, Bilton et al. 2010, Whitlock et al. 2010). Here I investigated the role of local adaptation as mechanism of the apparent resistance of species' to long-term climate manipulations at BCCIL using one of the more common forbs in these calcareous grasslands, *Plantago lanceolata*. I used a common garden approach to test for evidence of selection for different suites of functional traits in P. lanceolata populations exposed to over two decades of summer drought at BCCIL. The main axis of functional variation reflected a tradeoff between reproductive and vegetative allocation, consistent with drought avoidance and competitive strategies, respectively. Avoidance strategies were more prominent in droughted populations whereas competitive strategies were more prominent in populations from control treatments. Treatment differences were more moderated by soil depth which suggests that selective pressures imposed by different climate treatments are modified by fine scale edaphic heterogeneity. Furthermore, I detected parallel divergence patterns in replicate pairwise control-drought treatment comparisons (5 replicate experimental blocks) which suggests a genetic basis for the functional differentiation demonstrated in the common garden experiment..

Parallel divergence patterns in replicated pairwise control-treatment comparisons were significant for all control-treatment contrasts that included precipitation manipulations (drought and increased precipitation, including both factorial combinations with heating). Evidence of significant treatment-based genetic structure suggests that extant populations have adapted to long term experimental manipulations at BCCIL through local genetic restructuring associated with treatment based differences in soil water supply (drought, increased precipitation) and demand (factorial with heating). Evidence of significant genetic and functional structuring in relation to gradients in soil water availability suggests that local environmental heterogeneity may buffer this species from climatic change because it promotes diversity upon which climate-based selection may act.

Different soil water dynamics associated with experimental treatment and as modified by local edaphic heterogeneity reflect moisture gradients that occur at nested spatial scales in this topographically complex calcareous grassland. I expanded environmental monitoring and trait analyses of *P. lanceolata* to calcareous grassland systems in the landscape surrounding BCCIL in an effort to relate the extent and spatial structure of nested soil moisture gradients to that of functional variation in six traits that collectively reflect the main axes of functional differentiation found in the common garden study. I measured intraspecific variation in four vegetative and two reproductive traits in *P. lanceolata* populations in a series of nested plots in the landscape surrounding BCCIL. Abiotic gradients associated with soil water dynamics had a distinct spatial structure that in turn promoted hierarchical partitioning of intraspecific functional variation in five of the six traits measured. Trait-environment relationships were particularly pronounced for specific leaf area (SLA) in association with soil water availability.

Conclusion

Here I reveal significant treatment based genetic differentiation in P. lanceolata populations which suggests a genetic basis for the functional differentiation evident in the common garden study. Evidence of treatment based genetic differentiation was most pronounced in precipitation manipulation treatments, suggesting genetically based functional differentiation in P. lanceolata is associated with treatment based differences in soil water supply and demand. Taken together my results suggest a genetic basis for local intraspecific functional differentiation in *P. lanceolata* which in turn has allowed this species to adapt in situ to experimental climate manipulations. Furthermore, local and landscape scale gradients in factors related to climate change (e.g., soil moisture) promote functional trait variation at associated scales which may buffer this species from future climatic change.

Although results suggest some populations may have the capacity to adapt to climate change and thus resist climate-induced local extirpation given adequate levels of genetic variation, this does not mean that these systems are inherently stable. In particular, barriers to dispersal limit the invasion of southerly species, which may over longer time scales cause species-level shifts even if extant populations are able to adapt to new environmental regimes (Moser et al. 2011). The likelihood of long-term population persistence in the face of new species immigration over the coming decades remains a key unresolved area in global change research.

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Education

2008-present	PhD candidate in Biology, Syracuse University Advisor: Dr. Jason Fridley Dissertation: *Local adaption to long-term climate change in a calcareous grassland system.*
2008	MS in Forest and Wildlife Ecology, University of Wisconsin-Madison Advisor: Dr. David Mladenoff Thesis: *Modeling the interaction of climate change and forest management in northeastern Minnesota using a spatially dynamic model.*
2005	MS in Environmental Science, University of California, Santa Barbara
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Fellowships and Awards

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Publications

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Ravenscroft C.R., Scheller R.M., Mladenoff D.J. and White M.J. (2010) Simulating forest restoration in a mixed ownership landscape under climate change. *Ecological Applications* 20: 327-346.

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2009	Functional trait differentiation reveals local adaptation to long-term climate change. Ecological Society of America Annual Meeting, Albuquerque, NM.
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2007	An assessment of regional conservation priorities under climate change. The Nature Conservancy Global Climate Initiative Annual Meeting. Portland, OR
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Professional Experience

2006-2008	Graduate Research Assistant: University of Wisconsin Madison, Forest Landscape Ecology Lab
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