Ecological Effects of Genotypic Diversity on Community and Ecosystem Function

Megan K. Kanaga
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ECOLOGICAL EFFECTS OF GENOTYPIC DIVERSITY ON COMMUNITY AND ECOSYSTEM FUNCTION

by

Megan K. Kanaga

A dissertation submitted in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Ecology

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UTAH STATE UNIVERSITY
Logan, Utah
2009
ABSTRACT

Ecological Effects of Genotypic Diversity on Community and Ecosystem Function

by

Megan K. Kanaga, Doctor of Philosophy
Utah State University, 2009

Major Professor: Dr. Michael E. Pfrender
Department: Biology

Genotypic diversity within populations can have important evolutionary consequences, but the ecological effects of intraspecific genetic variation on community and ecosystem function have only been studied in a few systems. I present the results of a three-year study designed to address the ecological impacts of genotypic diversity in quaking aspen (Populus tremuloides Michx.), using aspen genotypes planted across genotypic diversity levels (monoculture and mixture) and watering treatment levels (well-watered and water-limited). First, I demonstrated that significant variation exists among genotypes for a wide range of growth, morphological and physiological traits, and quantified high heritability and coefficient of genetic variation values for those traits. This demonstrates that heritable phenotypic variation exists within an aspen population, which could potentially have community and ecosystem implications. Secondly, I collected ground-dwelling arthropods across experimental treatment levels to determine if there are any community-level implications of genotypic diversity and watering treatment. Ground-dwelling arthropods were significantly affected by the genotypic diversity × watering treatment interaction, such that arthropod taxonomic diversity was lowest in water-limited genotypic mixtures. This result runs counter to the bulk of the plant diversity-arthropod diversity literature, which predicts that plant and arthropod diversity should be positively correlated, and highlights...
the importance of environmental conditions in mediating the plant-arthropod diversity relationship. Lastly, I show that there are no overall effects of genotypic diversity or watering treatment on tree growth patterns. Instead, there are high levels of variation among genotypes in their responses to treatments (significant genotype × diversity × watering treatment interactions), which are often opposing in direction. I also show that there are significant collection site × diversity × watering treatment interactions, demonstrating that genotypes vary in their response to experimental treatments based in part on their original collection site conditions in the field. This study demonstrates that aspen populations contain high levels of genotypic diversity, but that the ecological effects of genotypic diversity are mediated by the environment (in this case, watering treatment) and can be considerably more complicated than found in most previous studies.

(98 pages)
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Megan K. Kanaga
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CHAPTER 1

INTRODUCTION

As global biodiversity has declined, increasing focus in the scientific community has been placed on determining the effects of biodiversity on various ecological and evolutionary processes. There is a long history of biodiversity research in the field of ecology, starting with a basis in theoretical work prior to the 1970s, and continuing with the incorporation of newer views of nonequilibrium population fluctuations and food web dynamics in more recent work (McCann 2000). A large body of evidence has accumulated suggesting that biodiversity increases ecosystem functions, including stability, productivity and nutrient retention (reviewed in Hooper et al. 2005). Additionally, biodiversity at one trophic level (particularly primary producers) can impact biodiversity at other trophic levels by creating a greater variety of feeding resources or increasing habitat heterogeneity (Andow 1991), an important community-level function. Positive diversity-function relationships have been demonstrated at a variety of scales, including empirical studies at local scales (Siemann et al. 1998; Hector et al. 1999; Tilman 1999; Loreau et al. 2001), model predictions over wide spatial scales incorporating environmental heterogeneity (Loreau et al. 2003), and even long-term historical stability in Phanerozoic reefs (Kiessling 2005). Although early studies focused almost exclusively on diversity-ecosystem function relationships at the species level, phenotypic diversity (sometimes referred to as functional diversity) actually drives the positive diversity-function relationship (Tilman et al. 1997; Diaz and Cabido 2001; Heemsbergen et al. 2004). In grassland diversity experiments, for instance, nitrogen-fixing plants play an important role in providing a limiting resource, and exhibit a disproportionate influence on primary productivity (Tilman et al. 1997; Hector et al. 1999). At the community level, diverse plant assemblages that exhibit greater architectural complexity, a measure of phenotypic diversity, can increase the diversity of associated arthropods (Lawton 1983; Gardner et al. 1995; Tews et al. 2004).
Biodiversity can influence ecosystem functioning through two major mechanisms: resource partitioning or facilitation among individuals that differ in their resource use patterns in space or time (complementarity), or dominance by particular individuals that strongly affect ecosystem functions (the selection effect). Phenotypic trait variation is an essential component of the relationship between biodiversity and ecosystem functioning (Loreau 2000), and where phenotypic variation is greater, biodiversity effects could potentially be stronger. It is well known that phenotypic trait variation can exist at the species or functional group levels, but intraspecific genetic variation can also contribute substantially to ecologically-relevant phenotypic variation (Chapter 3; Hughes et al. 2009). Genetic diversity is important to the persistence of species because heterozygosity increases the fitness of individuals and within-population variation allows species to adapt to changing environmental conditions (Lynch and Lande 1993; Burger and Lynch 1997). In addition, genetic diversity can also increase phenotypic variation within species, resulting in a greater range of functional roles being filled and the potential for positive biodiversity effects.

There are many studies in the literature that report a positive diversity-ecosystem function relationship, but there is still debate about the role of diversity in ecosystem functioning, the magnitude of diversity effects, and the mechanisms by which diversity affects ecological function (Aarsen 1997; Huston 1997; Huston et al. 2000; McCann 2000; Jiang et al. 2009). Several studies of species diversity show negative relationships, mixed results, or weak effects of diversity on various community and ecosystem responses (Hooper 1998; Huston et al. 2000; Aarsen et al. 2003; Fox 2003; Crawley et al. 2005; Zhang and Zhang 2006; Jiang et al. 2008; Creed et al. 2009; Valdivia and Molis 2009). Studies of forest tree diversity are underrepresented in the diversity-function literature, but the studies that exist show variable patterns in the relationship between tree diversity and ecosystem function, including positive relationships (Erskine et al. 2006; Vila et al. 2007), negative relationships (Huston 1980; Firn et al. 2007), and
neutral or species-specific responses (Vila et al. 2003; Redondo-Brenes and Montagnini 2006). Some studies suggest that the identity of the particular individuals in mixtures is more important than the actual number of species (Tilman et al. 1997; Diaz and Cabido 2001; Goodsell and Underwood 2008). Other studies highlight the importance of environmental factors such as disturbance and stress, which can alter the diversity-function relationship (Cardinale et al. 2000; Norberg et al. 2001; Wang et al. 2007). These studies suggest that many factors can affect the relationship between biodiversity and ecosystem function, and that a universal positive relationship between diversity and ecological processes may be unlikely (Goodman 1975; Murdoch 1975; Jiang et al. 2008; Jiang et al. 2009).

Many studies have documented important effects of the genetic structuring of dominant and keystone species at levels above the population (Whitham et al. 2003). Examples from hybrid complexes of cottonwood trees (Floate and Whitham 1994; Bailey et al. 2004; Wimp et al. 2005; Wimp et al. 2007), eucalyptus trees (Dungey et al. 2000), willows (Hochwender and Fritz 2004), and primrose (Johnson and Agrawal 2005; 2007) have demonstrated strong correlations between plant genotype and various ecological properties. In cottonwood common garden studies, insect communities were distinctively and consistently different on each of two parental species and their F1 hybrids, and in one case the prediction of tree genotype based on insect community alone showed a 98% agreement with morphological and genetic characterization (Floate and Whitham 1994). In another example of community genetic structuring, Bailey et al. (2004) found that beavers selectively forage on cottonwood trees of genotypes with lower genetically-determined tannin concentrations, altering the stand genotype composition, age composition and spatial structure of cottonwoods. These findings led to the development of the ‘community genetics’ field to study the effects of genes on organizational levels above the population – i.e. communities and ecosystems (Antonovics 1992, 2003; Neuhauser et al. 2003; Whitham et al. 2003).
More recently, several studies have merged the community genetics framework with the study of biodiversity-ecosystem function, by manipulating genetic diversity levels in plots or stands (usually by manipulating the number of genotypes, analogous to species richness). These studies have demonstrated that intraspecific genetic diversity can increase ecosystem stability in a similar way to species diversity (Hughes and Stachowicz 2004; Reusch et al. 2005; Agashe 2009). Reusch et al. (2005) suggest that, in species-poor communities, genotypic diversity fills a similar role to species or functional group diversity of species-rich communities, acting as a buffer to environmental perturbation. Other similar studies have shown that increasing plant genotypic diversity also increases productivity and positively affects arthropod abundance and diversity (Wimp et al. 2004; Crutsinger et al. 2006; Johnson et al. 2006; Crawford et al. 2007).

The incorporation of genotypic diversity-ecosystem function studies into the field of biodiversity research is a logical extension of the theory underlying previous diversity studies, recognizing that genetic diversity is one of the fundamental levels of biodiversity. The effects of genetic diversity on community and ecosystem function can be equal or greater in magnitude to studies of species diversity (Hughes et al. 2008), emphasizing the important role that genotypic diversity can play in ecological processes.

I used quaking aspen (*Populus tremuloides* Michx.) as a study system to elucidate the effects of genotypic diversity in community and ecosystem processes for several reasons. Forest trees are underrepresented in the biodiversity-ecosystem function literature, despite their widespread extent and ecological and economic importance, and this study helps fill a key knowledge gap. Aspen is a dominant and ecologically important species in high elevation forests throughout North America and has an exceptionally wide geographic distribution and ecological tolerance range (Jones 1985; Lieffers et al. 2000). Aspen have many useful experimental traits, such as their fast growth rate and ability to reproduce clonally, allowing propagation of many replicates of each genotype and thus the separation of genetic from environmental effects on plant
phenotype. They are also extremely genetically variable based on molecular markers (Cheliak and Dancik 1982; Jelinski and Cheliak 1992) and morphological traits (Chapter 3; Barnes 1975; Jones and DeByle 1985), and dramatic differences between genotypes in traits such as secondary chemical compound production have been found to have important ecological effects (Lindroth 2000). Recent population genetic studies of aspen throughout their range have revealed high levels of genotypic diversity (large numbers of genotypes) within stands (Namroud et al. 2005; Suvanto and Latva-Karjanmaa 2005; Mock et al. 2008), contrary to the historical idea of only a few large aspen clones covering large swaths of the landscape. Aspen are also important in a conservation context, as they are declining rapidly in the western United States due to a combination of fire suppression, disease and livestock grazing (Frey et al. 2004; Romme et al. 2005).

For my dissertation research, I used a common garden experiment planted with quaking aspen to investigate the genetic basis of aspen phenotypic variation, and determine the interactive effects of genotypic diversity and water stress on community and ecosystem processes. In the second chapter of this dissertation, I give a detailed description of tree propagation methods, the common garden experimental setup, and watering treatments implemented in the experiment.

The third chapter is the first data chapter, in which I quantify the heritable genetic basis of aspen growth, morphological, physiological and structural traits. I ask whether aspen have high levels of within-population quantitative-genetic variation in growth, morphological, structural and physiological traits, as suggested by field studies that note high levels of phenotypic diversity among genotypes in the field (Barnes 1975). Documenting levels of quantitative-genetic variation sets the stage for determining the effects of aspen genotypic diversity on community and ecosystem processes.

In the fourth chapter, I focus on the effects of aspen genotypic variation and watering treatment on the ground-dwelling arthropod community during the second year of the common
garden study. I expected that experimental treatments (plant genotypic diversity and watering treatment) may affect ground-dwelling arthropods, either through direct effects or indirect effects of plant phenotypic traits (Figure 1.1).

![Diagram](image)

**Figure 1.1.** Schematic showing potential effects of experimental treatments on the ground-dwelling arthropod community. Direct effects are shown with a solid black arrow and indirect effects mediated by plant phenotype with a dashed arrow. Other abiotic factors shown in the gray box are not measured directly in this study.

The fifth chapter documents the effects of genotypic diversity and watering treatment on ecosystem function three years after establishing the experiment (Figure 1.2). I hypothesized that high levels of variation in phenotypic traits among genotypes would lead to positive effects of genetic diversity on aspen growth and physiology, and more efficient resource use in genetic mixtures.
Figure 1.2. Schematic showing potential effects of experimental treatments on ecosystem function. Ecosystem responses were measured at the individual tree level (growth and physiological traits, left) and at the plot level (aggregated growth and survivorship traits, and soil nitrogen, right).

To conclude in the sixth chapter, I briefly summarize the major results of the study. I place the results in the context of my original hypotheses and discuss the implications of this study and need for further research.
CHAPTER 2
COMMON GARDEN EXPERIMENT

Collection of Aspen Root Stock

Aspen roots were collected for propagation from 60 genotypes within a 40 km² area of native aspen forest in Iron County, Utah, USA. A landform map for the area was developed combining elevation, slope, and aspect, and sites were selected from dry, south-facing slopes (dry sites) and moist, north-facing slopes (wet sites). Within the mapped sites, lateral root segments from distinct aspen stands were collected and cut into roughly 30cm sections. Roots were stored in a refrigerator until use, wrapped in slightly moist paper towels and plastic bags.

Propagation of Aspen Trees

Shoots were propagated vegetatively following a modified procedure of Schier (1978) during the winter and spring of 2006. Lateral root segments from each genotype were planted horizontally in trays filled with vermiculite, such that the root was covered with approximately 1-4cm of vermiculite. The root segments sprouted shoots vegetatively off the root segments, and varied widely in their shoot production, ranging from just a few to several hundred shoots. Once shoots had reached 3-8 cm in height, shoots were cut from the root segment using a sterile razor and planted in trays, where they each developed an independent root system. Trays contained a mixture of potting soil, vermiculite and perlite to provide both adequate moisture and drainage. Fitted clear plastic covers were used on all trays to maintain high humidity and reduce plant water stress until the root system was developed. Shoots grew best and mortality was lowest when the soil matrix was packed very firmly into trays, allowing the cut shoots good contact with the soil and access to moisture. As shoots sprouted independent root systems and began to grow, they were transplanted into a 50-50 mixture of potting soil and field soil, and sprayed with a fertilizer containing micronutrients. All shoots were grown in a greenhouse until the late spring of 2006,
when they were moved outside and planted in the common garden experiment. The genotypes that produced the largest number of shoots were selected for the experiment. I originally planted 13 putative genotypes in the experiment, but microsatellite DNA analysis showed that two of the genotypes were genetically identical and had been collected in close proximity to each other. All 13 putative genotypes are used in statistical analyses for Chapter 3, but I verified that correcting the number of genotypes to 12 does not qualitatively change the results or levels of significance. A list of all genotypes planted in the experiment can be found in Table 2.1. Seven of the 12 experimental genotypes were collected from wet sites and 5 from dry sites. There were high levels of genotypic variation at every stage of the propagation process; genotypes varied in their ability to propagate shoots, the number of shoots produced, and their growth rates and patterns.

**Table 2.1.** List of the 12 aspen genotypes planted in the common garden experiment, their collection site type, and whether they are fully replicated across well-watered and water-limited blocks (cross-environment subset) or present only in water-limited blocks (drought only). The genotype number corresponds to monoculture numbers in Figure 2.2 (i.e. the monoculture plot for genotype CD26 is labeled M6).

<table>
<thead>
<tr>
<th>Genotype</th>
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<th>Collection Site</th>
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<th>Notes</th>
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<td>CD11</td>
<td>5</td>
<td>dry</td>
<td>cross-environment</td>
<td></td>
</tr>
<tr>
<td>CD26</td>
<td>6</td>
<td>dry</td>
<td>cross-environment</td>
<td></td>
</tr>
<tr>
<td>CW7</td>
<td>1</td>
<td>wet</td>
<td>cross-environment</td>
<td>all individuals died in 2006-07</td>
</tr>
<tr>
<td>CW24</td>
<td>4</td>
<td>wet</td>
<td>cross-environment</td>
<td>same as putative genotype CW14</td>
</tr>
<tr>
<td>CW27</td>
<td>3</td>
<td>wet</td>
<td>cross-environment</td>
<td></td>
</tr>
<tr>
<td>CW12</td>
<td>2</td>
<td>wet</td>
<td>cross-environment</td>
<td></td>
</tr>
<tr>
<td>CD20</td>
<td>12</td>
<td>dry</td>
<td>drought only</td>
<td></td>
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<td>CD30</td>
<td>13</td>
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<td>CD5</td>
<td>11</td>
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<td>7</td>
<td>wet</td>
<td>drought only</td>
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Common Garden Experimental Setup

Trees were planted in a flat agricultural field in northern Utah in the spring of 2006. The soil at the study site consisted of a fairly uniform silt loam with few rocks. The experiment was set up to investigate the effects of two levels of plant genotypic diversity, two levels of watering treatment, and the interaction of plant genotypic diversity with watering treatment.

Four experimental blocks were set up: two with high levels of irrigation (referred to as well-watered blocks) and two with low irrigation (referred to as water-limited blocks). Within each block, trees were planted in plots with two diversity levels: monocultures planted with replicates of a single genotype, and mixtures planted with a combination of six genotypes. Each diversity plot (genotypic monoculture or mixture) was set up as a 2.5m² hexagonal-shaped group of 19 trees, all placed 50 cm apart on a grid in which each tree had six equidistant neighbors. Monoculture plots consisted of 19 genetically identical trees, and mixture plots contained between two and four individuals of each of the six genotypes, configured so no two replicates of the same genotype were adjacent. A single row of border trees of other non-experimental genotypes was placed between all plots and around the outside of each block at 50 cm spacing to minimize edge effects (Figure 2.1).

Twelve experimental genotypes were planted in the experiment, but due to limitations on the number of aspen shoots that could be clonally propagated, the final design included two distinct data subsets. Six genotypes produced enough ramets (a minimum of ~100) to replicate across all blocks, and therefore six monocultures and the mixture of those six genotypes were each planted across all four blocks. I refer to this data subset as the cross-environment data, because genotypic monocultures and mixtures are replicated across both well-watered and water-limited watering treatments. Six other genotypes produced fewer shoots, and were planted only in water-limited blocks (Figure 2.1, Figure 2.2). Putative genotype CW14 (Table 2.1) was excluded from the cross-environment data set for simplicity, and I verified that omitting or
including CW14 did not qualitatively change the results or conclusions of the study. Therefore, the well-watered blocks each contained a total of 6 monocultures and one mixture, and the water-limited blocks contained all 11 monocultures (including the 6 in well-watered blocks) and 8 mixtures of different genotypic combinations. The cross-environment data set included 6 genotypes, with 4 replicates of each monoculture (1 in each block) and 6 replicates of the mixture of all genotypes (2 in each well-watered block and 1 in each water-limited block). However, all individuals of one of these cross-environment genotypes (CW7) died in the first winter of the

**Figure 2.1.** Setup of the common garden experiment, showing the configuration of rectangular blocks and hexagonal plots. The left part of the figure shows layout of blocks and plots across the whole experiment, with watering treatment noted with text. Monoculture plots are depicted as open hexagons and mixtures as shaded hexagons, and red plots are part of the cross-environment data set. The middle panel of the figure shows seven plots more closely, with mixtures (“mix”) and monocultures (“mono”) shown, and a dashed line depicting the row of border trees between each plot. The right panel shows each individual tree in two different plots: a mixture (top) and monoculture (bottom), where each lowercase letter represents a tree of a particular genotype.
study, resulting in open monocultures with no plant cover. Therefore, only 5 live genotypes are represented in the cross-environment data set in Chapters 4 and 5. The drought-only data set consisted of 11 genotypes, including each of the 5 cross-environment genotypes planted in water-limited blocks (excluding genotype CW7 that died), and 6 other genotypes that were planted in water-limited blocks only. The drought-only data subset contained monocultures of each genotype replicated twice (1 in each water-limited block), and 7 mixtures, each replicated twice (1 in each water-limited block). There were two additional mixtures (one in each water-limited block) that were not truly replicated since they differed by one genotype, due to limitations on the number of shoots of each genotype that could be propagated (Figure 2.1, Figure 2.2). The two distinct data sets were analyzed separately in statistical analyses. The cross-environment data set is limited in the number of genotypes and only contains one mixture composition, but can test for diversity, watering treatment and interactive effects. The drought-only data set contains more genotypes and genotypic mixtures, but is not replicated across watering treatments so environmental and interactive effects of experimental treatments cannot be examined. For Chapters 4 and 5, only the cross-environment data set was used, because the interaction of plant genotypic diversity and watering treatment were of interest in both studies.

A thin layer of wood chips was placed on the ground in all plots to simulate a litter layer, and weeds were removed manually. All live trees were fertilized with 1 teaspoon of chelated iron fertilizer during leaf flush in 2007 and 2008, as the alkaline soils at the study site are known to cause iron deficiency in aspen trees.

**Watering Treatments**

The experimental site is located at an elevation of 1400 meters, lower than native aspen forest, and thus the site experiences hotter, drier summers than adjacent upland forests. All trees were watered equally in the first year of the study (2006) to allow establishment of saplings, and irrigation treatments were implemented in 2007 and 2008. During the summer of 2007 well-
watered blocks were given approximately 58cm of water and water-limited blocks 37cm. In the summer of 2008, drought treatments were intensified, with well-watered blocks receiving 28cm water and water-limited blocks only 6cm. Note that collection site (wet-site and dry-site genotypes) refers to the moisture conditions in the field where the initial root stock for each genotype was collected, whereas watering treatment (well-watered and water-limited blocks) refers to the irrigation level implemented in the common garden experiment. Both wet-site and dry-site genotypes were planted in well-watered and water-limited blocks.

![Diagram of garden experiment setup]

**Figure 2.2.** Spatial configuration of plots in the common garden experiment. Each plot labeled with an M is a monoculture, and each plot labeled with a C is a mixture. Numbers represent unique plots (i.e., all plots labeled M5 are monocultures of genotype CD11, and all plots labeled C1 are mixtures of the cross-environment genotypes). All plots are replicated at least twice, except mixtures C9 and C10, which differ by one genotype due to limited replication of shoots. Monoculture numbers correspond to Genotype numbers in Table 2.1.
CHAPTER 3
QUANTITATIVE-GENETIC VARIATION IN MORPHOLOGICAL AND PHYSIOLOGICAL TRAITS WITHIN A QUAKING ASPEN (POPULUS TREMULOIDES) POPULATION

Abstract

Genetic diversity within populations is an important component of adaptive evolution, and recent research has demonstrated that genetic variation within plant populations can have important ecological effects. In this study we investigate quantitative-genetic variation in several traits within a quaking aspen (Populus tremuloides Michx.) population. A common garden experiment was planted with replicates of 13 aspen genotypes collected from wet and dry sites within a population in southern Utah, USA. Ten growth, leaf, physiological, and structural traits were measured. There were significant, heritable phenotypic differences among genotypes in every measured trait and differences in 4 of the 10 traits among genotypes originating from wet and dry collection sites. The data were compared with other published studies, showing that aspen heritability ($H^2$) estimates and coefficients of genetic variation ($CV_G$) were comparable or higher than other Populus species and hybrid F$_1$ Populus genotypes, indicating a large amount of quantitative-genetic variation in aspen.

Introduction

Genetic variation within populations is an important but often overlooked aspect of ecological studies. Genetic variation has two important consequences at the population level: heterozygosity tends to increase fitness of individuals, and genetic variation provides the evolutionary potential for populations to track environmental fluctuations and persist over time (Lynch and Lande 1993; Burger and Lynch 1997). There is also increasing evidence that quantitative-genetic variation in hybrid plants can strongly affect community composition of
species such as arthropods and nesting birds (Martinsen and Whitham 1994; Hochwender and Fritz 2004) and ecosystem-level processes such as soil nutrient retention and decomposition (Driebe and Whitham 2000; Schweitzer et al. 2004). These studies suggest that genetic variation can have important effects; however, to fully understand the ecological and evolutionary implications of phenotypic variation, the heritable genetic component must be characterized.

Quaking aspen (Populus tremuloides Michx.) is a widely distributed and dominant tree species throughout North America and has important effects on community structure and wildlife diversity in the western United States (DeByle 1985). Studies characterizing genetic variation based on isozyme (Jelinsky and Cheliak 1992) and microsatellite (Wyman et al. 2003; Cole 2005) markers indicate that aspen is one of the most genetically variable plant species. There is also marked variation in quantitative traits, and both field studies (Barnes 1975; Mitton and Grant 1996) and controlled experiments in a common environment (King et al. 1999; Donaldson and Lindroth 2004) reveal substantial phenotypic variation in aspen. Still, the degree to which the phenotypic variation is due to heritable genetic variation is not known.

In this study, we quantify within-population quantitative-genetic variation of quaking aspen to determine whether the high degree of phenotypic variation found in natural aspen stands has a significant heritable genetic basis. In a common garden study, broad-sense heritabilities ($H^2$) and coefficients of genetic variation ($CV_G$) were calculated to characterize genetic variation in 10 growth, leaf, physiological, and structural traits. Root stock was collected from both wet and dry sites, allowing the assessment of overall genetic variation as well as differences between genotypes that established on sites with differing levels of soil moisture. We hypothesized that aspen genotypes would exhibit substantial heritable genetic variation in phenotypic traits, and that genotypes collected from wet sites and dry sites would exhibit heritable differences only in traits that strongly affect water relations. To provide perspective on the amount of genetic variation in
western aspen relative to other related species, we compare our \( H^2 \) and \( CV_G \) values with other published studies that report quantitative-genetic variation in the genus *Populus*.

**Materials and Methods**

The common garden experiment was set up as described in Chapter 2. Data for this chapter were collected in 2006, before watering treatments were implemented, and thus all trees were watered equally during the period of this study. Additionally, for the purposes of this chapter, diversity plots were ignored because trees had been interacting with neighbors for only two months, and were not likely to experience the effects of plot diversity.

**Traits Measured**

Measurements were taken in mid-August 2006 to characterize phenotypic traits of each aspen genotype (Table 3.1). Between 14 and 20 ramets per genotype were sampled for leaf traits and internode length, and 31-135 ramets per genotype were measured for growth traits and leaf number. From phenotypic measurements, total stem length, relative growth rate, single leaf area, and leaf width/length ratio \( (L_w/L_l) \) were calculated as shown in Table 3.1, and a total of 10 traits were used in analyses. Stem structure was coded as a categorical variable, classified into branching and unbranching patterns. Leaf ultraviolet-A (UV-A) transmittance was measured using a portable UV-A-PAM chlorophyll fluorometer (Gademann Enterprises, Wuerzburg, Germany), which uses the calibrated ratio of UV-A-excited fluorescence (375 nm excitation) to blue-green-excited fluorescence (470 nm excitation) to determine the percent UV-A shielding provided by protective pigments in the leaf epidermis. The UV-A epidermal transmittance is 100 minus this ratio: lower values indicate higher mesophyll protection from UV radiation. UV-A transmittance was measured for 3-17 trees per genotype in a single morning prior to direct sunlight. Plant water use was inferred from \( \delta^{13}C \) stable isotope ratios of leaf tissue, which provides a long-term indicator of stomatal conductance and plant water use (Hubick et al. 1988).
More negative $\delta^{13}$C values indicate high internal leaf concentration of CO$_2$ and greater discrimination against $^{13}$C by rubisco, an enzyme essential in photosynthesis (Farquhar and Richards 1984). More negative $\delta^{13}$C values are associated with high stomatal conductance (i.e., biochemical limitation on photosynthesis), whereas less negative values indicate lower stomatal conductance (i.e., carbon limitation). The $\delta^{13}$C values were generated from desiccated leaf tissue of five or six individuals per genotype using an isotope ratio mass spectrometer. Carbon stable isotope values are expressed using the delta notation ($\%e$) against the Pee Dee Belemnite standard.

Table 3.1. Traits measured in the common garden experiment

<table>
<thead>
<tr>
<th>Trait</th>
<th>Unit</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Growth</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial height ($H_i$)</td>
<td>cm</td>
<td>height of leading shoot at start of experiment</td>
</tr>
<tr>
<td>Total height ($H_t$)</td>
<td>cm</td>
<td>height of leading shoot at end of year</td>
</tr>
<tr>
<td>Branch length (BL)</td>
<td>cm</td>
<td>length of all branches</td>
</tr>
<tr>
<td>Total stem length (SL)</td>
<td>cm</td>
<td>$H_t + BL$</td>
</tr>
<tr>
<td>Relative growth rate (RGR)</td>
<td>cm/mo</td>
<td>($SL-H_t$) / 3</td>
</tr>
<tr>
<td>Internode length ($I$)</td>
<td>cm</td>
<td>Mean internode length along stem</td>
</tr>
<tr>
<td><strong>Leaf Traits</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf number ($L_n$)</td>
<td>cm</td>
<td>Number of mature leaves</td>
</tr>
<tr>
<td>Leaf length ($L_l$)</td>
<td>cm</td>
<td>Mean leaf blade length of largest leaf cohort</td>
</tr>
<tr>
<td>Leaf width ($L_w$)</td>
<td>cm</td>
<td>Mean leaf blade width of largest leaf cohort</td>
</tr>
<tr>
<td>Leaf width/length ratio ($L_w/L_l$)</td>
<td>cm</td>
<td>$L_w/L_l$</td>
</tr>
<tr>
<td>Single leaf area ($L_{A_s}$)</td>
<td>cm$^2$</td>
<td>$\pi (L_l/2) \times (L_w/2)$</td>
</tr>
<tr>
<td><strong>Physiology</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UV-A transmittance ($T$)</td>
<td>%</td>
<td>UV-A protection of leaf epidermis</td>
</tr>
<tr>
<td>Water use ($\delta^{13}$C)</td>
<td>%</td>
<td>$\delta^{13}$C stable isotope ratio</td>
</tr>
<tr>
<td><strong>Stem structure</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data Analysis

Note that data analyses for this chapter were conducted prior to microsatellite analysis that showed genotypes CW24 and CW14 to be identical (see Chapter 2; Appendix A1). Therefore, analyses are based on 13 genotypes instead of 12, the true number of genotypes.
However, subsequent analyses showed that combining genotypes CW24 and CW14 did not qualitatively alter the results, and changed heritability and coefficient of genetic variation values very little (data not shown).

All phenotypic traits (excluding stem structure) were analyzed using the SAS general linear model procedure (SAS Institute 2003) using a two-factor analysis of covariance (ANCOVA), with separate analyses for genotype and site effects. Block was included as a factor in both models, and initial height was treated as a covariate because of significant variation among genotypes at the time of planting. Only traits that did not show significant departures from normality were used in analyses (limiting the number of traits to 10), and P-values were reported based on type III sum of squares estimates. Because the experimental design included replication within clones, the within-clone and among-clone variance could be directly interpreted as the environmental and genetic variation, respectively. From the among-clone variance (genetic variance component – $\sigma^2_G$), coefficients of genetic variation ($CV_G$) for each trait were calculated as: $CV_G = (\sigma_G)/\text{mean}$. Broad-sense heritability ($H^2$) estimates were calculated as $H^2 = \sigma^2_G/\sigma^2_P$, where $\sigma^2_P$ is the total phenotypic variance for a trait (both genetic and environmental). $H^2$ was calculated with the program H2boot, using bootstrapping to generate standard errors (Phillips 2001). $H^2$ and $CV_G$ estimates together provide a strong measure of population variation; $H^2$ gives a ratio of genetic to total variance, and $CV_G$ provides a measure of the magnitude of variation standardized by the trait mean. Stem structure was analyzed as a two-level categorical variable using a chi-square test for independence and, thus, is not included in Tables 3.2 and 3.3. The estimates of genetic variation are based on replicated clonal individuals derived from root stock taken from natural populations, and therefore maternal effects cannot be partitioned from genetic variation. These effects potentially inflate our estimates of genetic variation among clones, although many other quantitative-genetic studies in trees also have the same limitation.
Results

There were significant phenotypic differences among genotypes in every trait based on ANCOVA results (Table 3.2). Structural type also varied significantly among genotypes ($\chi^2 = 335.9$, df =12, $P<0.001$). Variation in all traits (except stem structure, a non-numerical variable) had a significant genetic component and a broad range of observed values. Broad-sense $H^2$ estimates were significantly different from zero for all traits, with a range from 0.17 to 0.56 (Table 3.3). The $H^2$ was greatest for internode length (0.50), height (0.45), and leaf morphology (average of $L_w/L_d$ and $L_A$: 0.52). The $CV_G$ values, which ranged from 1.9% to 41.1% (Table 3.3), were high for all growth traits (mean 22.3%) and most leaf traits (mean 16.9%), but low for water use (mean 1.9%).

Table 3.2. $F$-values, degrees of freedom, and $P$-values for aspen phenotypic traits (excluding structure) from analysis of covariance (ANCOVA) by genotype and ANCOVA by collection site (wet and dry site type)

<table>
<thead>
<tr>
<th>Trait</th>
<th>Genotype F-value</th>
<th>df</th>
<th>P-value</th>
<th>Collection site F-value</th>
<th>df</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$l$</td>
<td>30.68</td>
<td>12</td>
<td>&lt;0.0001</td>
<td>1.44</td>
<td>1</td>
<td>ns</td>
</tr>
<tr>
<td>RGR</td>
<td>20.51</td>
<td>12</td>
<td>&lt;0.0001</td>
<td>0.71</td>
<td>1</td>
<td>ns</td>
</tr>
<tr>
<td>$H_l$</td>
<td>37.79</td>
<td>12</td>
<td>&lt;0.0001</td>
<td>5.53</td>
<td>1</td>
<td>0.0188</td>
</tr>
<tr>
<td>SL</td>
<td>20.50</td>
<td>12</td>
<td>&lt;0.0001</td>
<td>0.71</td>
<td>1</td>
<td>ns</td>
</tr>
<tr>
<td>Leaf Traits</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$L_n$</td>
<td>26.35</td>
<td>12</td>
<td>&lt;0.0001</td>
<td>46.37</td>
<td>1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>$L_w/L_d$</td>
<td>18.95</td>
<td>12</td>
<td>&lt;0.0001</td>
<td>0.13</td>
<td>1</td>
<td>ns</td>
</tr>
<tr>
<td>$L_A$</td>
<td>28.06</td>
<td>12</td>
<td>&lt;0.0001</td>
<td>2.85</td>
<td>1</td>
<td>ns</td>
</tr>
<tr>
<td>Physiology</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$T$</td>
<td>2.21</td>
<td>12</td>
<td>0.0158</td>
<td>0.28</td>
<td>1</td>
<td>ns</td>
</tr>
<tr>
<td>$\delta^{13}$C</td>
<td>4.79</td>
<td>12</td>
<td>&lt;0.0001</td>
<td>7.03</td>
<td>1</td>
<td>0.0101</td>
</tr>
</tbody>
</table>

Note: see Table 3.1 for trait abbreviations
Total height, leaf number, leaf $\delta^{13}$C, and stem structure differed significantly between genotypes from wet and dry collection sites (Table 3.2). Total height was greater for wet site genotypes (mean 67.09 cm for wet sites; 64.18 cm for dry sites), but wet and dry-site genotypes did not differ in measures incorporating growth of branches (relative growth rate and total stem length). In contrast, dry-site genotypes had significantly greater structural complexity ($\chi^2 = 39.6$, df =1, $P<0.001$), tending to grow a greater number of branches rather than increasing their vertical height. Genotypes from dry sites also had a significantly greater number of leaves (mean 31.75 for dry sites; 27.41 for wet sites) that tended to be smaller in size (marginally nonsignificant trend, single leaf area $P=0.092$). Genotypes from wet collection sites had greater discrimination for $^{13}$C (more negative $\delta^{13}$C values), reflecting greater stomatal aperture and plant water use (mean -27.49‰ for wet sites; -26.87‰ for dry sites). Block effects were not significant.
at $\alpha=0.05$ for any of the traits except $L_w/L_d$ and UV-A transmittance in the ANCOVA by genotype, and total height in the ANCOVA by site.

**Discussion**

We show that western aspen populations can have high levels of phenotypic variation with a strongly heritable genetic component. Every measured trait, including growth, leaf, physiology, and structural characteristics, showed significant phenotypic differences among aspen genotypes. The measured traits had significant heritability estimates and a wide range of phenotypic variation as measured by coefficients of genetic variation, showing that aspen stands carry a substantial amount of heritable quantitative-genetic variation. Genotypes collected from wet and dry site types exhibited heritable differences in 4 of the 10 phenotypic traits (total height, leaf number, water use, and stem structure). Selection seems to favor genotypes with greater height growth and water use at wet sites, while favoring genotypes with more conservative water use and highly branching growth forms at dry sites, consistent with local adaptation to variation in soil moisture.

It is important to note that the 13 genotypes in this study represent an extremely small subset of the actual population, and almost certainly underestimate the levels of genotypic and phenotypic variation in western aspen stands. Furthermore, only genotypes that exhibited prolific suckering (clonal reproduction) ability in the greenhouse were used in the experiment, likely introducing selection that may bias the magnitude of variation downward. Phenotypic plasticity also can contribute to levels of phenotypic variation, and considerable phenotypic plasticity has been found in previous studies of *Populus* hybrids (Marron et al. 2006). Plasticity can add additional phenotypic variation through the effects of genotype $\times$ environment interactions, and thus the levels of phenotypic variation in natural aspen stands may be higher than documented here.
To provide perspective on the amount of genetic variation among aspen genotypes in this study, we compared the variation found in this study to published data from other Populus species. Three published studies that report quantitative-genetic variation for 1- or 2-year-old trees were used: a study of a natural population of Populus deltoides Bartr. (Wilcox and Farmer 1967), and two studies of a breeding population of Populus trichocarpa Torr. & Gray × Populus nigra L. and P. trichocarpa × P. deltoides F₁ hybrids (Marron et al. 2006; Marron and Ceulemans 2006). H² and CV_G were compared for three traits common among studies: total height, internode length and single leaf area. The H² and CV_G values for tree height were roughly twice as high in aspen as in P. deltoides, and aspen had higher CV_G and comparable H² values across three traits when compared with F₁ Populus hybrids (Table 3.4). Although we recognize that direct comparisons between our study and other published data are imperfect because of differences in population structure and breeding designs, we found that the genetic variation among the 13 aspen genotypes was generally comparable to, or higher than, the variation observed in both a congener and F₁ hybrid crosses.

Table 3.4. A comparison of broad-sense heritability estimates (H²) (with SEs given in parentheses, except where not available) and coefficients of genetic variation (CV_G) for three traits among Populus tremuloides in the current study, published data from a natural population of P. deltoides, and published data for F₁ Populus hybrids.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Aspen (Populus tremuloides)</th>
<th>Populus deltoides</th>
<th>F₁ Populus hybrids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H² (SE) CV_G (%)</td>
<td>H² CV_G (%)</td>
<td>H² (SE) CV_G (%)</td>
</tr>
<tr>
<td>l</td>
<td>0.50 (0.10) 12.5</td>
<td>0.43 (0.09) 0.7</td>
<td></td>
</tr>
<tr>
<td>LA_s</td>
<td>0.56 (0.11) 24.2</td>
<td>0.47 (0.09) 17.7</td>
<td></td>
</tr>
<tr>
<td>H_t</td>
<td>0.45 (0.09) 19.3</td>
<td>0.28 6.7</td>
<td>0.35 (0.03) 9.1</td>
</tr>
</tbody>
</table>


Note: see Table 3.1 for trait abbreviations
Phenotypic variation within populations can have important functional consequences, and our study shows that there is a large amount of heritable genetic variation within an aspen population. Traits such as growth and plant water use can affect competitive interactions among plants (Cohen 1970), and structural characteristics are important for species such as nesting birds (Martinsen and Whitham 1994). We report data only for young aspen trees in the first year of growth, but emphasize that early variation in traits such as structural and height characteristics will strongly influence subsequent years of growth. Recent work has brought plant hybrid zones to the attention of ecologists, showing that variation among plant genotypes can have important community and ecosystem effects (Whitham et al. 2003). Our study demonstrates high levels of within-population genetic variation among aspen genotypes, and more work is needed to determine the ecological and evolutionary implications of this genetic variation in natural aspen landscapes.
CHAPTER 4

PLANT GENOTYPIC DIVERSITY AND ENVIRONMENTAL STRESS INTERACT TO NEGATIVELY AFFECT ARTHROPOD COMMUNITY DIVERSITY

Abstract

Many studies have found positive relationships between plant diversity and arthropod communities, but the interactive effects of plant genetic diversity and environmental stress on arthropods are not well documented. In this study, we investigated the consequences of plant genotypic diversity, watering treatment, and its interaction for the ground-dwelling arthropod community in an experimental common garden of quaking aspen (*Populus tremuloides* Michx.). We found that varying plant genotypic diversity and watering treatment altered multivariate arthropod community composition and structure. Arthropod biodiversity and richness showed a distinct response to the plant diversity × watering treatment interaction, declining sharply in water-limited genotypic mixtures. Abundance of arthropod functional groups did not show any response to diversity or the plant diversity × watering treatment interaction, but varied in their response to watering treatment, with predator and detritivore abundance increasing and parasitoid abundance decreasing in well-watered blocks. Our results conflict with most previous studies, and suggest that environmental stress can substantially change the nature of the plant-arthropod diversity relationship. Additionally, we suggest that the plant-arthropod diversity relationship is dependent on the type of plant and arthropod species sampled, and that the association between tree diversity and ground-dwelling arthropods may be much different than more commonly studied grassland species and herbivorous arthropods.

Introduction

With growing concerns about species extinctions, many researchers have sought to understand the community and ecosystem effects of biodiversity, particularly in plant
communities (reviewed in Hooper et al. 2005). Arthropods in particular are strongly affected by the characteristics of plant communities (Murdoch et al. 1972; Southwood et al. 1979). Many plant traits are known to be important in structuring arthropod communities, from defensive chemical compounds (Hwang and Lindroth 1997; Wimp et al. 2007) to whole-plant architecture (Lawton 1983; Gardner et al. 1995; Tews et al. 2004). Additionally, plant community properties also influence arthropods, including plant species and functional diversity (Haddad et al. 2001; Wenninger and Inouye 2008), and community composition (Perner et al. 2005; Vehvilainen et al. 2008).

Although most plant-arthropod research has focused on interspecific plant variation, many recent studies have also found strong effects of intraspecific genetic variation on arthropod communities (Hochwender and Fritz 2004; Johnson and Agrawal 2005; Wimp et al. 2005; Bangert et al. 2006). In one study, the genotype of hybrid cottonwood trees could be predicted with 98% accuracy based on the arthropod assemblage associated with the tree (Floate and Whitham 1994), demonstrating a remarkable concordance between tree genotype and the composition of the arthropod community. The strong structuring effects of plant genotype extend to larger scales, where genetically diverse hybrid cottonwood stands harbor a greater species diversity of arthropods (Wimp et al. 2004). Additionally, experimental studies manipulating plant genotypic diversity in a common environment have shown that genetically diverse mixtures of plants harbor higher arthropod diversity and abundance than genetic monocultures (Reusch et al. 2005; Crutsinger et al. 2006; Johnson et al. 2006). In some cases, plant genotypic diversity can have even stronger structuring effects on the arthropod community than those of plant species diversity (Crutsinger et al. 2006).

Environmental conditions can dramatically alter interactions among organisms, making the environmental context in which species assemblages operate very important. For instance,
there is evidence that plant-plant interactions shift from competition to facilitation as environmental stress increases (Callaway et al. 2002). Across trophic levels, drought stress can alter plant susceptibility to herbivory (Koricheva et al. 1998), and can cause widespread changes across whole food webs, such as altering biomass distribution among different trophic groups (Priesser and Strong 2004). Additionally, plant phenotypic diversity, whether at the functional, species or genetic level, can ameliorate the effects of stress through the insurance effect, where assemblages with higher trait diversity maintain a decreased probability of losing all individuals that fill a particular functional role (Yachi and Loreau 1999). If this is the case, associated arthropod communities may also be buffered against the effects of stress in more diverse plant assemblages. Drought is one of the most prevalent forms of environmental stress, and often negatively impacts arthropod communities as a whole, although individual arthropod species vary widely in their drought responses (Schowalter et al. 1999; Trotter et al. 2008).

Although plant diversity and stress are both important in structuring arthropod communities, the multitrophic consequences of the interaction between plant diversity and stress has received little attention. Wenninger and Inouye (2008) compared an invasive grass monoculture with native grass mixtures under differing irrigation regimes, and determined that mixtures under irrigation generally harbored the greatest abundance and diversity of arthropods. Their study manipulated both plant diversity and stress, but did not replicate species from mixtures in monocultures, making it difficult to predict the effects of native grass monocultures or intermediate diversity levels. Reusch et al. (2005) showed that arthropod abundances were higher in more genetically diverse eelgrass plots during a heat wave. In their study, plant genotypic diversity appeared to ameliorate the effects of high temperatures that may have otherwise negatively affected the arthropod community, but the study lacked a cooler control to simultaneously test for the interactive effects of genotypic diversity and stress.

To examine the effects of plant genotypic diversity, water limitation, and their interaction
on the arthropod community, we established an experimental common garden with genotypes of quaking aspen (*Populus tremuloides*), a deciduous tree species common throughout western North America. Aspen genotypes exhibit high levels of variation in morphological and physiological traits (Chapter 3) and defense against herbivores (Stevens et al. 2007), traits that can have important ecological effects on the arthropod community (Bangert et al. 2006). In this study, arthropods were collected from pitfall traps, which primarily sample ground-dwelling arthropods. We expected that plant genotypic diversity would indirectly influence ground-dwelling arthropods, either via plant architecture, which can modify the environment on the ground, or through plant nutritional quality and chemical composition, which influences grazer and decomposer food chains.

**Methods**

The common garden experiment was set up and watering treatments implemented as described in Chapter 2. Only the cross-environment data subset was used for this chapter.

**Collection and identification of arthropod samples**

To sample the arthropod community, three 7cm diameter \( \times \) 8cm deep pitfall traps were installed in each plot. Traps were filled with ethylene glycol (1:1 diluted antifreeze) for a five day sampling period once a month in July, August, and September of 2007. Arthropods caught in pitfall traps were sorted to order and counted. Orders represented in pitfall traps included Collembola, Archaeognatha, Dermaptera, Orthoptera, Hemiptera, Coleoptera, Diptera, Lepidoptera, Hymenoptera, Araneae, Lithobiomorpha, and Isopoda. Arthropods of the order Hemiptera were divided into two suborders: Heteroptera and Auchenorrhyncha/Sternorrhyncha, corresponding to the traditional classification of these suborders as orders Homoptera and Homoptera. Collembola and Acarina (mites, in the order Araneae) were not included in analyses due to extremely large numbers and difficult detection in samples, resulting in inaccurate counts.
Collembola and Acarina were found in every sample, and thus their exclusion is unlikely to alter our comparisons of arthropod taxonomic richness.

We also identified all arthropods to a sufficient taxonomic level to place them into feeding functional groups. We placed orders Orthoptera and Hemiptera (both suborders; see above), along with Coleoptera families Chrysomelid, Curculonid, Cerambycid, and Elaterid in the herbivore functional group. Orders Araneae and Lithobiomorpha, along with Coleoptera families Carabidae, Coccinellidae, and Dyticidae, were considered predators. Arthropods of the order Hymenoptera, family Vespidae (wasps) were placed in the parasitoid functional group. Omnivores included Hymenoptera of the family Formicidae (ants) and Dermaptera. Lastly, arthropod orders Archaeognatha and Isopoda, along with Coleoptera families Tenebrionidae and Scarabidae were considered detritivores. The majority of the dipterans caught in pitfall traps belonged to the family Chironomidae, and were not included in functional group analyses because adults rarely feed (Armitage 1995) and the order is extremely functionally diverse. Lepidopterans were also omitted from functional group analyses, as all individuals caught were adults, and nectar sources for feeding were not present in our experimental plots.

Categorization of foliage density, plant biomass and survivorship

Foliage density and biomass were quantified based on structural traits of the trees in September 2007, and were summed across all trees in each plot. Foliage density was quantified as: \( BL \times LA_s \), where \( BL \) is the estimated length of all branches (average branch length multiplied by branch number, for primary, secondary and tertiary branches), and \( LA_s \) is average single leaf area (calculated as: \( \pi \left( \frac{L_l}{2} \right) \times \left( \frac{L_w}{2} \right) \), where \( L_l \) is average leaf length and \( L_w \) is average leaf width). Our foliage density metric provides a relative measure of cover or shading experienced by ground-dwelling arthropods. Woody biomass of each tree was estimated by the volume of a cone: \( 1/3 \pi r_{stem}^2 h_{stem} + 1/3 \pi r_{branch}^2 h_{branch} \), where \( h_{stem} \) and \( h_{branch} \) are stem height and estimated
total branch length, and $r_{stem}$ and $r_{branch}$ are basal radius measurements of the stem and branches.

Survivorship was calculated as the percentage of trees alive in each plot at the end of the 2007 growing season.

**Plant physiological measures**

Mature leaves from throughout the plant canopy were collected for stable isotope analysis, leaf nutrient content, and phytochemistry in August 2007. Plant water use was inferred from $\delta^{13}C$ stable isotope ratios of leaf tissue, which provides a long-term indicator of stomatal conductance and plant water use (Farquhar and Richards 1984). $\delta^{13}C$ values were generated from desiccated leaf tissue of 93 trees using an isotope ratio mass spectrometer. Leaf carbon and nitrogen content were determined with a Thermo Finnigan Flash 1112 elemental (CN) analyzer. High-performance thin layer chromatography was used to quantify levels of the phenolic glycosides salicortin and tremulacin, compounds produced by plants to deter insect herbivory, using purified aspen phenolic glycoside standards (Lindroth et al. 1993). Condensed tannins were extracted from leaf tissue with 70% acetone at 4ºC, and quantified using acid butanol (Porter et al. 1986) and purified aspen tannin standards. Assays for leaf carbon and nitrogen were generated from 35 trees, and concentration of tremulacin, salicortin and condensed tannins from 20 trees.

**Statistical analyses**

We conducted permutational multivariate analyses of variance (MANOVA) using the Adonis function in the Vegan package of Program R (Anderson 2001; McArdle and Anderson 2001; Oksanen et al. 2008) to test for the effects of genotypic diversity, water limitation, the diversity × watering treatment interaction, sampling date, and experimental block on the multivariate arthropod community. The Adonis function takes a dissimilarity matrix describing the multivariate community and statistically tests for experimental effects by identifying relevant
centroids and calculating the squared deviations from those points. Two forms of community data were used for MANOVA analyses: a presence/absence community matrix describing community composition, and a community matrix incorporating abundance of arthropod groups, describing community structure (Table 4.1). Distance matrices for use in MANOVA were constructed using the Bray-Curtis index, and P-values were generated using F-tests based on sequential sums of squares from 1000 permutations of the raw data. Note that MANOVA analyses produced P-values and R² for each factor, but indicated only whether or not there were differences among experimental treatments, not the directionality of any changes.

To assess the directionality of change due to experimental treatments, arthropod order data were used to generate three diversity metrics: the Shannon-Weiner diversity index based on richness and evenness of arthropod orders (hereafter termed “biodiversity” to differentiate arthropod diversity from plant genotypic diversity), taxonomic richness (number of arthropod orders), and total arthropod abundance (Table 4.1). Because multivariate analyses showed that sample date explained the greatest proportion of the data, each sample date (July, August, and September) was analyzed separately for all diversity metrics. Three-factor ANOVA was conducted using proc mixed in SAS v. 9.1 (SAS Institute 2003) to test for fixed effects of plant diversity, watering treatment and experimental block (nested within watering treatment), and the diversity × watering treatment interaction. Functional group analyses were conducted for arthropod abundance within each of the five functional groups, using a four-factor ANOVA model with plant diversity, watering treatment, block, and the diversity × watering treatment interaction as fixed effects, and month as a random effect in proc mixed (SAS Institute 2003). We did not assess diversity or richness within functional groups due to low numbers of arthropod orders in most functional groups.

To determine the effects of plant diversity, watering treatment and the diversity × watering treatment interaction on plant phenotype (foliage density, biomass, and survivorship)
and physiological measures (water use, leaf nutrients, and leaf phytochemical content), we used the same three-factor ANOVA as described above, with treatment, diversity and experimental block as fixed effects. We further examined the relationship between plant structure and arthropods by regressing foliage density, biomass and survivorship against arthropod biodiversity, richness and abundance in separate simple linear regressions. When necessary, response variables were square root transformed to meet the assumption of normality of residual distribution.

Table 4.1. Definitions of terms used to characterize the arthropod community

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Community Composition</td>
<td>Multivariate measure of the arthropod community using presence/absence of orders</td>
</tr>
<tr>
<td>Community Structure</td>
<td>Multivariate measure of the arthropod community incorporating abundance of orders</td>
</tr>
<tr>
<td>Biodiversity</td>
<td>Shannon-Weiner diversity index calculated for arthropod orders</td>
</tr>
<tr>
<td>Taxonomic Richness</td>
<td>Number of arthropod orders</td>
</tr>
<tr>
<td>Abundance</td>
<td>Total arthropod abundance</td>
</tr>
</tbody>
</table>

Results

Effects of plant diversity and watering treatment on plant phenotype

Trees in the experiment averaged 1.2m tall with 22cm average primary branch lengths at the end of the 2007 growing season. Genotypic mixture plots had slightly lower foliage density than monocultures across watering treatments ($F=3.59$, df=1, $P=0.062$), but there were no other significant main effects of genotypic diversity on plant phenotype or physiology. Watering treatments did not significantly affect foliage density, biomass or survivorship, but several physiological traits showed significant effects of watering treatment. Well-watered trees had a significantly more negative $\delta^{13}C$ stable isotope ratio (mean -27.54‰) than water-limited trees.
(mean -27.06‰; \( F=12.07, \text{df}=1, P<0.001 \)). Leaf tissue concentrations of tremulacin and salicortin, phenolic glycoside compounds that deter herbivory, were on average 28% and 18% higher, respectively, among trees in water-limited blocks compared to well-watered blocks, although the change in salicortin was not statistically significant (tremulacin: \( F=7.76, \text{df}=1, P=0.015 \); salicortin: \( F=2.56, \text{df}=1, P=0.132 \)). Leaf carbon content was 1% higher among plants in water-limited blocks (\( F=4.18, \text{df}=1, P=0.050 \)), but leaf nitrogen and condensed tannins showed no significant treatment effects (\( P>0.2 \)). The plant diversity \( \times \) watering treatment interaction did not affect any aspect of plant phenotype or physiology except leaf carbon concentrations, with water-limited mixtures maintaining the highest levels of leaf carbon (\( F=4.60, \text{df}=1, P=0.040 \)).

**Table 4.2.** \( R^2 \) and \( P \)-values from MANOVA analysis of arthropod community composition (based on presence/absence) and community structure (incorporating abundance)

<table>
<thead>
<tr>
<th></th>
<th>Community Composition</th>
<th>Community Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( R^2 )</td>
<td>( P )</td>
</tr>
<tr>
<td>Plant Genotypic Diversity</td>
<td>2.29</td>
<td>0.039</td>
</tr>
<tr>
<td>Watering Treatment</td>
<td>4.35</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diversity( \times )Treatment</td>
<td>2.04</td>
<td>0.055</td>
</tr>
<tr>
<td>Sample Date</td>
<td>14.46</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Block</td>
<td>2.68</td>
<td>0.154</td>
</tr>
</tbody>
</table>

Arthropod community composition based on presence/absence of arthropod orders showed marginally significant effects of plant genotypic diversity and significant effects of watering treatment (Table 4.2). Community structure based on abundance was significantly altered by both plant diversity and watering treatment. The multivariate genotypic diversity \( \times \) watering treatment interaction also significantly affected arthropod community composition, but not community structure. Community composition and structure varied widely across months, as evidenced by the highly significant terms and highest \( R^2 \) values for sample date in both models.
Interactive effects of plant genotypic diversity and water limitation on the arthropod community

Across the three sample dates, average arthropod biodiversity declined by roughly 25% in water-limited genotypic mixtures (Figure 4.1A). The interaction between plant genotypic diversity and watering treatment was significant in July and September, whereas in August the effects of water limitation decreased arthropod biodiversity in both monocultures and mixtures. Arthropod taxonomic richness showed a similar trend in July and September, with richness values roughly 30% lower in water-limited genotypic mixtures (Figure 4.1B). August samples showed little difference among plant diversity or watering levels. Arthropod abundance did not change with plant genotypic diversity or watering treatment level throughout the summer, but declined across the three months to half the number of arthropods caught at the beginning of the study (Figure 4.1C).

Table 4.3. Degrees of freedom, F-statistics and P-values for effects of experimental treatments and interactions on the abundance of arthropod functional groups

<table>
<thead>
<tr>
<th></th>
<th>Plant diversity</th>
<th>Watering treatment</th>
<th>Diversity × treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>Herbivores</td>
<td>1</td>
<td>0.24</td>
<td>0.625</td>
</tr>
<tr>
<td>Predators</td>
<td>1</td>
<td>0.40</td>
<td>0.531</td>
</tr>
<tr>
<td>Parasitoids</td>
<td>1</td>
<td>0.03</td>
<td>0.858</td>
</tr>
<tr>
<td>Omnivores</td>
<td>1</td>
<td>0.36</td>
<td>0.551</td>
</tr>
<tr>
<td>Detritivores</td>
<td>1</td>
<td>2.30</td>
<td>0.134</td>
</tr>
</tbody>
</table>

None of the five arthropod functional groups responded to changes in plant genotypic diversity or the diversity × watering treatment interaction (Table 4.3). However, three functional groups showed responses to watering treatment but differed in the directionality of their response. Detritivores and predators reached 28% and 4.6% higher abundances, respectively, in well-
Figure 4.1. Arthropod biodiversity (A), richness (B) and abundance (C) compared between plant diversity levels (genotypic monocultures and mixtures) and watering treatments (well-watered and water-limited). Error bars represent 1 SE. Tables below each graph report F values and P-values for each experimental treatment, and asterisks in the graphs denote significant plant diversity x watering treatment interactions.
watered blocks, although predator response to watering treatment was only marginally statistically significant ($P=0.026$ and $P=0.063$). Conversely, parasitoids reached higher abundances in water-limited blocks, increasing their average abundance by 27% ($P=0.051$).

**Effects of plant structure on the arthropod community**

There was no significant relationship between foliage density, biomass or survivorship and arthropod biodiversity, richness or abundance ($P>0.169$).

**Discussion**

A long-standing body of theory predicts that more diverse plant assemblages should provide a greater diversity of resources (both food and shelter), which should increase the diversity of associated herbivores as well as their natural enemies (Andow 1991). In contrast, our study found that aspen genotypic diversity and its interaction with water stress negatively affected the arthropod community. Arthropod biodiversity and richness declined substantially in genotypic mixtures under water limitation, particularly in the months of July and September. This interaction is driven by a decline in the number of arthropod groups, not a change in their abundances (Table 4.2, Figure 4.1). Our results contrast with many other studies showing positive relationships between plant and arthropod diversity in experiments manipulating both plant species diversity (Siemann et al. 1998; Knops et al. 1999; Wenninger and Inouye 2008) and plant genotypic diversity (Crutsinger et al. 2006; Johnson et al. 2006).

Arthropod functional groups show little response to diversity or the diversity × watering treatment interaction, but three of the five functional groups responded to watering treatment main effects. Predators and detritivores responded positively to irrigation, reaching higher abundances in well-watered blocks, whereas parasitoids attained higher abundances in water-limited blocks. This variation in response to water stress is consistent with some previous studies that have found that many arthropod species respond differently to water stress, but the majority
tend to perform better under conditions of low water stress (Schowalter et al. 1999; Trotter et al. 2008). Interestingly, the only effects of experimental treatments on arthropod abundance appear at the functional group level, with no significant effects of any treatments on overall abundance (Figure 4.1C). The striking diversity \( \times \) treatment interaction effects only become apparent when analyzing the community based on diversity indices of arthropod orders, not at the functional group level.

Our results conflict with the insurance hypothesis, which predicts a buffering effect of plant diversity under conditions of stress. In our study, water limitation negatively impacted the arthropod community, mostly through its interaction with plant diversity in water-limited genotypic mixtures. The differences in water use, defensive compound production, and leaf nutritional quality between watering treatments indicate that water-limited trees were subjected to enough water stress to affect their physiology, although they maintained similar growth patterns to well-watered trees. Less negative carbon stable isotope ratios indicate that photosynthesis in trees planted in water-limited blocks was likely limited by availability of CO\(_2\) due to smaller stomatal apertures during times of water stress (Farquhar and Richards 1984). Higher levels of leaf tremulacin, a phenolic glycoside compound that deters herbivory, were detected in water-limited blocks, indicating that water-stressed plants contained better defended tissue. However, rather than diversity providing a buffer against environmental stress, arthropods in plots with higher genotypic diversity were more adversely affected by water stress.

Few previous studies have reported negative relationships between plant diversity and arthropod biodiversity or abundance. In a grassland experiment manipulating plant species diversity, Koricheva et al. (2000) found that the abundance of predators caught in pitfall traps decreased with increasing plant species diversity, a trend they attributed to higher temperatures in monoculture plots and thus greater arthropod activity. However, in our study, plant diversity affected only arthropod biodiversity and richness, with no significant changes in arthropod
abundance. In another experimental manipulation of grassland species diversity, Siemann (1998) found that arthropod species richness decreased with increasing plant species richness, although in their study species richness was not directly manipulated but was created by varying historical fertilization treatments. Neither of these studies investigated the interaction of plant diversity and water stress, but they show that negative relationships between plant diversity and arthropod communities have been documented.

We also found no significant relationships between the arthropod community and foliage density, biomass or survivorship, despite many studies (Lawton 1983; Gardner et al. 1995; Tews et al. 2004) that have shown plant architecture to be important in structuring arthropod communities. However, several trends in the data suggest that plant traits may affect the arthropod community. Genotypic mixture plots had marginally significantly lower foliage density than monocultures across watering treatments, and water-limited mixtures had the lowest foliage density of all combinations of diversity and watering treatment. This finding runs counter to previous studies that have found increasing plant productivity in diverse mixtures, particularly under water stress (Tilman and Downing 1994; Tilman et al. 1996). The discrepancy in our results may stem from high levels of variation in the response of aspen genotypes to conspecifics, where different aspen genotypes show a diversity of positive, neutral and negative responses to genotypic diversity and watering treatment (Chapter 5). A high level of response variation among genotypes could produce an overall weak negative effect of genotypic diversity on plant cover, as found in our study. Leaf carbon also showed a significant plant diversity × watering treatment interaction in which water-limited mixtures had the highest levels of leaf carbon, possibly indicating lowered nutritional quality. Leaf tremulacin and salicortin concentration also showed the same nonsignificant trend, with the highest levels of defensive compounds found in water-limited genotypic mixtures. Although many of these patterns are not statistically significant, they suggest that the decline in arthropod biodiversity and richness may have been
partially explained by lower foliage density and heightened levels of defensive compounds in trees found in water-limited genotypic mixtures. We do not have data on the nutrient or phytochemical content of leaf litter, and therefore it is unknown whether the same leaf quality patterns apply to leaf litter on the ground.

We use our measure of foliage density as a surrogate for shade or cover experienced by ground-dwelling arthropods, but many other abiotic factors may differ between experimental treatments, including temperature, moisture and humidity. We measured ground-level humidity at several locations across all blocks using a Kestrel 3000, but variance among measurements was too high to make any robust conclusions about the effects of experimental treatments or plant structure on humidity levels. Our lack of reliable abiotic environmental data weakens our power to test mechanistic hypotheses about the how the effects of tree characteristics on arthropods may be mediated by changes in the abiotic environment. However, we were able to test more indirectly for potential abiotic effects mediated through plant phenotype using regressions of plot-level plant biomass, foliage density and survivorship. The positive regression between foliage density and arthropod biodiversity, although only marginally significant, suggests that plant traits are affecting the arthropod community, either by modifying the abiotic environment or providing greater habitat structure for foraging and/or predator avoidance.

We collected arthropods by pitfall trapping, which primarily samples ground-dwelling arthropods, and the discrepancy in our results compared to other diversity studies may partially stem from sampling methods and differences in focal arthropod groups. Pitfall traps sample arthropods based on both their abundance and their mobility (Southwood 1978), and therefore highly mobile taxa are overrepresented in pitfall catches compared to less mobile taxa. If pitfall catches actually reflect arthropod activity more than abundance, the expected relationships between plant diversity and arthropod communities may not hold. Previous studies using multiple methods of arthropod collection found that trends observed based on pitfall trap catches
can differ substantially from the results of sweep net and suction sampling methods (Koricheva et al. 2000, and references therein). This suggests that either ground-dwelling arthropods show fundamentally different relationships with plant diversity, or that pitfall trapping methods may cause significant biases that could alter the plant-arthropod diversity relationship. However, we primarily detected effects of experimental treatments on arthropod biodiversity and richness, not on arthropod abundance (except the effects of watering treatment on abundance of some functional groups), suggesting that our results are not explained solely by pitfall trapping biases. Additionally, tree species have very different growth, morphology and life history patterns than more commonly studied herbaceous species (Scherer-Lorenzen et al. 2007), and the effects of diversity may be quite different for different groups of plants. These previous studies suggest that the expectation for the relationship between plant diversity and arthropods may depend on both the type of plants and the type of arthropods under consideration (Vehvilainen et al. 2008).

The community effects of plant diversity that we detected in this study are of a smaller magnitude than many previous studies. We expected that the effects of plant diversity on ground-dwelling arthropods would be more diffuse than previous studies of phytophagous arthropods because interactions between plants and ground-dwelling arthropods are indirectly mediated through trophic dynamics and/or physical characteristics of the plant. Consistent with our expectations, the $R^2$ values for community composition and structure are low for plant diversity and watering treatment when pooled across the three sample dates, and the amount of variation explained by sample date is much larger (Table 4.2). We were not able to generate reliable $R^2$ values for each separate month due to small sample sizes, but the amount of variation explained by plant diversity and watering treatment is likely to be substantially higher for each individual month.

Due to small plot sizes and continuous planting of trees between plots, some of the more mobile arthropods were almost certainly able to move between plots and may have experienced
multiple diversity or watering treatment levels. Although we recognize this as a possible source of error, we maintain that it will only result in a dampening of experimental effects, causing our estimates of experimental treatment effects to be more conservative rather than overestimating effect sizes or generating spurious results. Also, our sampling encompassed the middle and late portions of the growing season, from July through September, and therefore may have missed important dynamics between plant diversity and arthropods in the early season. Vehviläinen et al. (2007) found that effects of tree species diversity on arthropod herbivores were more pronounced in older trees, early season sampling, larger plot sizes and low planting density, suggesting that our study design and sampling dates may have biased against finding a relationship between plants and arthropods. Despite these experimental biases, we still detected significant effects of plant genetic diversity and water limitation, suggesting that diversity and water limitation have important effects on the arthropod community.

In this study, we add to the existing literature showing that intraspecific plant genotypic diversity can have important community-level effects, and we extend previous knowledge by showing that the plant-arthropod diversity relationship can vary dramatically across environmental stress levels. Few studies have investigated the environmental dependence of the plant-arthropod diversity relationship, and our results show that there is a greater need for studies across varying environments, particularly across stress gradients. We also suggest that the types of plant and arthropod species used in biodiversity experiments can greatly impact the nature of the plant-arthropod diversity relationship, and that the generalities suggested by grassland studies may not extend across all types of ecological systems.
CHAPTER 5
HIGH VARIABILITY AMONG ASPEN GENOTYPES ALTERS THE
DIVERSITY-ECOSYSTEM FUNCTION RELATIONSHIP

Abstract

The impacts of species and functional diversity on ecosystem function have been extensively studied over the past several decades, but there is still debate as to the strength, directionality, and mechanistic basis of diversity effects. Recent studies have documented widespread community and ecosystem impacts of genetic variation within species, and indicate that genotypic diversity can play a major role in ecosystem functioning. However, genetic diversity-ecosystem function relationships have been tested in relatively few ecological systems, and few studies have tested how the diversity-ecosystem function relationship may change across levels of a limiting resource. We used a common garden of quaking aspen (*Populus tremuloides*) to examine the effects of genotypic diversity and watering treatment on ecosystem function in this ecologically important tree species. There were no main effects of plant diversity or watering treatment on tree growth across all genotypes. However, there were significant interactive effects between genotype, diversity and watering treatment for all growth traits, indicating that aspen genotypes varied widely in their growth responses to experimental treatments and their interactions. Genotype-specific responses were often opposing, cancelling out any overall effects of diversity or watering treatment. Genotypes collected from different site types in the field (wet-sites and dry-sites) also exhibited significant interactive effects of collection site, diversity and watering treatment, suggesting that previous microsite conditions and/or local adaptation can affect the diversity-ecosystem function relationship. Plant physiological responses and soil nitrate concentrations suggest that both well-watered blocks and genotypic mixtures provided slightly more favorable environments for tree growth. Our results suggest that, in a highly phenotypically variable species, the genetic diversity-ecosystem function relationship is
environmentally-dependent, and may be driven more by genotypic composition and the particular phenotypic traits of each genotype than by diversity effects per se.

Introduction

As species diversity has declined over the past few decades, one of the central questions in ecology has been the relationship between biodiversity and ecosystem functioning (MacArthur 1955; Loreau 2000; Hooper et al. 2005). An extensive body of literature has shown that biodiversity can enhance ecosystem functions such as productivity (Tilman 1996; Loreau and Hector 2001; Cardinale et al. 2006), nutrient cycling (Fargione and Tilman 2006) and stability (Tilman and Downing 1994; Yachi and Loreau 1999; McCann 2000; Kiessling 2005). However, there is still debate about the role of diversity in ecosystem functioning (Huston 1997; McCann 2000; Naeem 2002), and many studies show contrary or mixed results (Hooper 1998; Huston et al. 2000; Aarsen et al. 2003; Fox 2003; Zhang and Zhang 2006; Jiang et al. 2008; Valdivia and Molis 2009). In studies of forest tree species diversity, a variety of patterns have been reported between diversity and ecosystem function, including positive relationships (Erskine et al. 2006; Vila et al. 2007), negative relationships (Huston 1980; Firn et al. 2007), and neutral or species-specific responses (Vila et al. 2003; Redondo-Brenes and Montagnini 2006). Some studies suggest that the identity of the particular individuals or functional groups in mixtures is more important than the actual number of species (Tilman et al. 1997; Diaz and Cabido 2001; Goodsell and Underwood 2008), emphasizing important functional traits over diversity per se.

Phenotypic trait variation is an essential component of the relationship between biodiversity and ecosystem functioning (Loreau 2000). Although ecologists have traditionally considered trait variation to exist primarily at the species or functional group level, genetic variation within species can contribute substantially to ecologically-relevant phenotypic variation (Chapter 3; Hughes et al. 2009). Evidence is also accumulating that genetic variation within plant species and hybrid complexes can have important community and ecosystem effects, from
structuring arthropod communities to altering soil decomposition rates (Madritch and Hunter 2002; Schweitzer et al. 2004; Wimp et al. 2004, 2005; Bangert et al. 2006). Some recent studies have assessed the ecosystem function impacts of intraspecific genotypic diversity (Hughes and Stachowicz 2004; Reusch et al. 2005; Crutsinger et al. 2006; Johnson et al. 2006; Gamfeldt and Kallstrom 2007; Lankau and Strauss 2007; Hajjar et al. 2008), and the results of these studies demonstrate that genotypic diversity can affect a variety of ecosystem functions. The ecological effects of genetic diversity can be even greater in magnitude than manipulations of species diversity (Hughes et al. 2008), demonstrating that diversity at the genetic level can have profound effects on ecological processes. Still, there are relatively few genetic-diversity ecosystem function studies in the literature, and the generality of these patterns across systems is still unclear.

In this study, we present the results from a three-year experiment that examined the interactive effects of genotypic diversity and watering treatment on growth, physiology and soil nutrient retention in young quaking aspen (Populus tremuloides) trees. Quaking aspen is a dominant and ecologically important species throughout the western United States. Despite the widespread belief that western aspen populations reproduce almost exclusively by vegetative reproduction (Barnes 1966; Schier 1973), many aspen stands are comprised of a large number of genotypes, arising from occasional sexual reproduction events in the primarily clonal populations (Mock et al. 2008). Aspen is an ideal study system for elucidating the ecological effects of genotypic variation, because genetically identical replicates can be produced via clonal propagation. Aspen populations also exhibit high levels of heritable phenotypic trait variation among genotypes in a variety of growth, structural, morphological and physiological traits (Chapter 3), which could potentially impact ecological processes if enough functional variation is maintained.
To examine the relationship between intraspecific genetic diversity and ecosystem function, we manipulated two levels of genotypic diversity (monocultures and mixtures) and implemented watering treatments (well-watered and water-limited). Most diversity studies to date have manipulated plant diversity within a single environment, without replicating diversity treatments across varying resource availability levels. We used watering treatments to determine if varying levels of a limiting resource affect the diversity-ecosystem function relationship. We hypothesized that genetic variation in functional traits among aspen genotypes would result in resource partitioning, leading to greater biomass production and soil nutrient use by trees in genotypic mixtures compared to genotypic monocultures. We also predicted that any positive effects of genotypic diversity may be enhanced in water-limited treatments, in accordance with the insurance hypothesis (Yachi and Loreau 1999) and studies that have found increasing positive interactions among organisms under conditions of environmental stress (Mulder et al. 2001; Callaway et al. 2002; Cardinale et al. 2002).

**Methods**

The common garden experiment was set up and watering treatments implemented as described in Chapter 2. Only the cross-environment data subset was used for this chapter.

**Plant growth**

Three morphological traits were measured at the end of the 2008 growing season, collectively termed ‘growth’: height, stem length, and foliage density. Height ($H_t$) was measured from the base to tip of the main stem. Stem length ($SL$) estimates total height and branch length of each tree, and was calculated as: $H_t + (BL^1 \times N_b^1) + (BL^2 \times N_b^2) + (BL^3 \times N_b^3)$, where $BL$ is the average length of three measured branches and $N_b$ is the number of branches, for primary, secondary, and tertiary branches (noted by the superscript number). Foliage density providing a measure of branching and leaf density, and was quantified as: $(SL - H_t) \times LA_n$, where $(SL - H_t)$ is
the estimated length of all branches, and LA, is average single leaf area. Other growth traits such as relative growth rate and biomass were also quantified, but are not presented in this paper, as the results were very similar to the three traits shown. Survivorship of all planted trees was monitored at the end of the 2008 growing season, calculated as the percentage of live trees.

**Plant physiological measures**

Mature leaves from throughout the plant canopy were collected for carbon stable isotope analysis and leaf nutrient content in August of 2008. Plant water use was inferred from δ^{13}C stable isotope ratios of leaf tissue, which provides a long-term indicator of stomatal conductance and plant water use (Farquhar and Richards 1984). More negative δ^{13}C values indicate high internal leaf concentration of CO₂ and greater discrimination against ^{13}C by rubisco, an enzyme essential in photosynthesis, and are associated with higher stomatal conductance and plant water use. δ^{13}C values were generated from desiccated leaf tissue of 93 trees distributed evenly across all genotypes and experimental treatments using an isotope ratio mass spectrometer. Leaf carbon and nitrogen content were determined with a Thermo Finnigan Flash 1112 elemental (CN) analyzer, using 66 trees across all plots.

**Soil inorganic nitrogen**

To sample soil inorganic nitrogen, we extracted two 10cm deep × 4cm diameter soil cores in the interior of each plot in July and August of 2008. Soil from each core was homogenized, and approximately 5mL of soil was added to 45mL of 2M KCl solution in the field. Samples were kept on ice in the field, shaken for 1 hour upon return to the lab, and stored overnight in a freezer. Soil nitrogen extracts were then filtered through 20-25um filter paper, and extracts were stored in a freezer. Concentrations of soil ammonium (NH₄⁺) and nitrate (NO₃⁻) were analyzed with a Lachat AE flow-injection autoanalyzer (Lachat Instruments, Milwaukee,
Quantities of soil inorganic nitrogen were reported in ugN / g soil, and were calculated as: ugN / mL × (45mL KCl extract / soil dry weight (g)).

**Statistical analyses**

To determine the effects of genotype, diversity level and watering treatment on growth and physiological traits, we conducted ANOVA in the glm procedure of the SAS statistical package (SAS Institute, 2003). Models for each response variable included genotype, plot genotypic diversity (monocultures and mixtures), watering treatment (well-watered and water-limited), block (nested within watering treatment) and all two- and three-way interactions among the three main effects. Models were simplified by removing nonsignificant interactions, starting with highest order interactions, and removing block effects where not significant. Due to significant 3-way interactions for all growth traits, each genotype was analyzed separately for effects of diversity, watering treatment, diversity × watering treatment, and block (nested within watering treatment). The same ANOVA models as described above were also used to analyze data by collection site (wet-site and dry-site), substituting collection site for genotype. Response variables were square-root or log transformed when necessary to fulfill the assumptions of parametric statistics.

To determine the effects of experimental treatments on tree survival we used chi-square tests. Chi-square tests were first performed combining all genotypes and testing for diversity and watering treatment effects. Following the overall survival and condition analyses, separate chi-square tests for diversity and watering treatment were performed separately for each genotype.

Soil inorganic nitrogen data was analyzed using a mixed model ANOVA in proc mixed (SAS Institute 2003). The model included plant diversity, watering treatment, the interaction of diversity × watering treatment and block (nested within watering treatment) as fixed effects, and replicate (two cores nested within plot) and sample date (July and August) as random effects.
Nitrate and ammonium response variables were both log transformed to fulfill residual distribution assumptions.

Results

Plant growth

There was significant variation among genotypes for the three growth traits. There were no significant main effects of genotypic diversity (monocultures vs. mixtures) or watering treatment (well-watered vs. water-limited) on any growth traits (Table 5.1). However, there were significant genotype × diversity × watering treatment interaction effects for all growth traits, demonstrating that genotypes varied in the nature of their response to plot diversity, watering treatment and the diversity × watering treatment interaction (Table 5.1, Figure 5.1). There was also a significant two-way genotype × diversity interaction for foliage density, in which some genotypes achieved greater branching in mixtures and others in monocultures. Both tree height and stem length showed significant genotype × watering treatment interactions, demonstrating that genotypes also varied in their performance under well-watered or water-limited conditions (Table 5.1). The pattern of nonsignificant main effects with significant interaction terms demonstrates that genotypes are highly variable in their growth responses to experimental treatments (Figure 5.1).

Separate analyses of diversity and watering treatment effects on each individual genotype more clearly illustrate the nature of the genotype × diversity × treatment interaction. The five genotypes show a wide range of responses to experimental treatments: one genotype grew larger in mixtures regardless of watering treatment, three genotypes showed differing growth responses to the of diversity × watering treatment interaction, and one genotype grew larger in water-limited blocks regardless of diversity level (Figure 5.1). Genotype CD11 had 74% greater foliage density and 35% longer stem length in mixtures than monocultures (foliage density: $F=14.72$, df=1, $P<0.001$; stem length: $F=3.52$, $P=0.070$), but did not show any significant differences in tree
height. There were no effects of watering treatment or the diversity × watering treatment interaction on the growth of genotype CD11. Genotype CD26 grew larger in water-limited blocks compared to well-watered blocks (height: $F=7.17$, $df=1$, $P=0.019$; foliage density: $F=4.37$, $df=1$, $P=0.060$), and had nearly 10-fold lower foliage density and 50% lower height in well-watered genotypic mixtures compared to other treatments (interaction terms – height: $F=7.87$, $df=1$, $P=0.015$; foliage density: $F=7.71$, $df=1$, $P=0.016$). Stem length analyses were omitted for genotype CD26 due to a highly nonnormal distribution of residuals that could not be ameliorated by transformation of the response variable. Genotype CW12 showed a significant interaction for height and stem length, exhibiting approximately 70% greater growth in well-watered genotypic mixtures compared to all other treatments (interaction term – height: $F=7.25$, $df=1$, $P=0.012$; stem length: $F=5.06$, $df=1$, $P=0.033$). Foliage density, however, was unaffected by experimental treatments. Genotype CW24 grew two-fold greater stem length and foliage density in well-watered genotypic mixtures (interaction terms – stem length: $F=3.82$, $df=1$, $P=0.061$; foliage density: $F=3.93$, $df=1$, $P=0.058$). The height of genotype CW24 did not differ among experimental treatments. Genotype CW27 did not exhibit interaction effects, but grew 8% taller height and 12% greater stem length in water-limited blocks compared to well-watered blocks.

<table>
<thead>
<tr>
<th>Term</th>
<th>$df$</th>
<th>Tree Height $F$</th>
<th>Tree Height $P$</th>
<th>Foliage Density $F$</th>
<th>Foliage Density $P$</th>
<th>Stem Length $F$</th>
<th>Stem Length $P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>4</td>
<td>2.37</td>
<td>0.057</td>
<td>8.43</td>
<td>0.000</td>
<td>6.39</td>
<td>0.000</td>
</tr>
<tr>
<td>Diversity</td>
<td>1</td>
<td>0.19</td>
<td>0.667</td>
<td>0.28</td>
<td>0.600</td>
<td>0.22</td>
<td>0.641</td>
</tr>
<tr>
<td>Watering treatment</td>
<td>1</td>
<td>1.26</td>
<td>0.264</td>
<td>0.03</td>
<td>0.859</td>
<td>0.04</td>
<td>0.851</td>
</tr>
<tr>
<td>Gen x Div</td>
<td>4</td>
<td>0.11</td>
<td>0.978</td>
<td>3.30</td>
<td>0.013</td>
<td>1.24</td>
<td>0.300</td>
</tr>
<tr>
<td>Gen x Water trt</td>
<td>4</td>
<td>4.49</td>
<td>0.002</td>
<td>1.36</td>
<td>0.253</td>
<td>2.49</td>
<td>0.047</td>
</tr>
<tr>
<td>Div x Water trt</td>
<td>1</td>
<td>0.00</td>
<td>0.997</td>
<td>0.02</td>
<td>0.894</td>
<td>0.10</td>
<td>0.749</td>
</tr>
<tr>
<td>Gen x Div x Water trt</td>
<td>4</td>
<td>3.87</td>
<td>0.006</td>
<td>3.05</td>
<td>0.020</td>
<td>3.59</td>
<td>0.009</td>
</tr>
</tbody>
</table>
Foliage density of genotype CW27 did not differ across experimental treatments (Figure 5.1).

**Figure 5.1.** Genotype × diversity × watering treatment interaction effects on stem length. Mean stem length values ± 1 SE are shown for five aspen genotypes in response to watering treatment (well-watered and water-limited) and diversity level (mixtures and monocultures).

Collection site (wet or dry location of root stock collection) also strongly affected the diversity × watering treatment interaction. There were highly significant main effects of collection site for all growth traits, with genotypes from dry collection sites outgrowing genotypes from wet collection sites by an average of 66% for foliage density, 71% for stem length and 17% for height, across both experimental watering treatments (Table 5.2). There was also a significant collection site × diversity × watering treatment interaction for all growth traits.
(Table 5.2, Figure 5.2). On average, dry site genotypes planted in water-limited genotypic mixture plots grew more than three times larger than wet site genotypes planted in the same combination of experimental treatments. The significant 3-way interaction demonstrates that the shape of the diversity × watering treatment response of each genotype is influenced by its original collection site in the field. However, genotypes did not consistently perform better in the environment most similar to their native conditions, i.e. genotypes from dry collection sites did not perform better in water-limited blocks, and genotypes from wet collection sites did not perform better in well-watered blocks.

Table 5.2. ANOVA results for main effects of collection site, diversity, watering treatment and their interactions on three growth traits, across all experimental genotypes

<table>
<thead>
<tr>
<th>Term</th>
<th>df</th>
<th>Tree Height</th>
<th>F</th>
<th>P</th>
<th>F</th>
<th>P</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collection Site</td>
<td>1</td>
<td>8.04</td>
<td>0.005</td>
<td>26.56</td>
<td>0.000</td>
<td>23.36</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>Diversity</td>
<td>1</td>
<td>0.07</td>
<td>0.789</td>
<td>1.58</td>
<td>0.212</td>
<td>0.43</td>
<td>0.511</td>
<td></td>
</tr>
<tr>
<td>Watering treatment</td>
<td>1</td>
<td>0.94</td>
<td>0.335</td>
<td>0.31</td>
<td>0.578</td>
<td>0.20</td>
<td>0.658</td>
<td></td>
</tr>
<tr>
<td>Site x Diversity</td>
<td>1</td>
<td>0.08</td>
<td>0.778</td>
<td>3.69</td>
<td>0.057</td>
<td>0.59</td>
<td>0.443</td>
<td></td>
</tr>
<tr>
<td>Site x Water trt</td>
<td>1</td>
<td>3.56</td>
<td>0.061</td>
<td>1.14</td>
<td>0.287</td>
<td>1.34</td>
<td>0.249</td>
<td></td>
</tr>
<tr>
<td>Diversity x Water trt</td>
<td>1</td>
<td>0.00</td>
<td>0.987</td>
<td>0.00</td>
<td>0.996</td>
<td>0.07</td>
<td>0.787</td>
<td></td>
</tr>
<tr>
<td>Site x Div x Water trt</td>
<td>1</td>
<td>6.52</td>
<td>0.012</td>
<td>4.74</td>
<td>0.031</td>
<td>5.08</td>
<td>0.026</td>
<td></td>
</tr>
</tbody>
</table>

Survival averaged 64% across all experimental treatments. Chi-square tests show that there was no significant deviation from a null hypothesis of no experimental treatment effects on survival, both when combined across all trees and for each of the five genotypes analyzed separately.
There were weak effects of genotype, plant diversity, and watering treatment on carbon stable isotope ratios ($\delta^{13}$C), used to indicate plant water use, with more negative $\delta^{13}$C ratios in well-watered blocks and in genotypic mixtures ($0.05<P<0.10; \text{Table 5.3}$). The five aspen genotypes planted in the study varied strongly in leaf carbon and nitrogen content. Leaf carbon content reached higher levels in water-limited blocks compared to well-watered blocks but was unaffected by plant diversity, and leaf nitrogen was unaffected by both experimental treatments (Table 5.3). None of the two- or three-way interactions between genotype, diversity and watering treatment were significant for plant water use or nutrient content.

**Figure 5.2.** Collection site × diversity × watering treatment interaction effects on plant stem length for genotypes from dry and wet collection sites. Mean stem length values are shown ± 1 SE.
Table 5.3. Mean values ± 1 SE, F values and P-values for main effects of plant genotypic diversity, watering treatment and genotype on leaf water use and chemistry traits. Interaction effects were not significant and are not shown

<table>
<thead>
<tr>
<th></th>
<th>( \delta^{13}C )</th>
<th>Leaf Percent N</th>
<th>Leaf Percent C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td><strong>Plant Diversity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monocultures</td>
<td>-27.74 (0.08)</td>
<td>3.02</td>
<td>0.087</td>
</tr>
<tr>
<td>Mixtures</td>
<td>-27.98 (0.11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Water Treatment</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well-watered</td>
<td>-27.99 (0.09)</td>
<td>3.80</td>
<td>0.056</td>
</tr>
<tr>
<td>Water-limited</td>
<td>-27.72 (0.11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Genotype</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD11</td>
<td>-28.05 (0.14)</td>
<td>2.23</td>
<td>0.077</td>
</tr>
<tr>
<td>CD26</td>
<td>-27.70 (0.16)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CW12</td>
<td>-27.59 (0.15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CW24</td>
<td>-28.09 (0.14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CW27</td>
<td>-27.85 (0.15)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Soil inorganic nitrogen

The effects of genotypic diversity on soil nitrogen varied between nitrate (NO$_3^-$-N) and ammonium (NH$_4^+$-N) forms of inorganic nitrogen. Soil NO$_3^-$ averaged 53% higher in monocultures than mixtures ($F=4.80$, df=1, $P=0.033$). Soil NH$_4^+$, however, showed no significant difference between mixtures and monocultures ($F=2.52$, df=1, $P=0.118$).

Discussion

Aspen genotypes exhibited strikingly different responses to the combination of diversity and environment, and those responses were influenced by the environment from which root material was collected. Most previous studies have found a positive diversity-productivity relationship, particularly under conditions of stress (McNaughton 1977; Tilman and Downing 1994; Tilman et al. 1996; Naeem and Li 1997). In our study, however, each genotype showed unique growth responses to genotypic diversity, watering treatment and their interaction, resulting in no overall effects of experimental treatments. Aspen are a highly genetically variable species (Chapter 3; Jelinski and Cheliak 1992), and we expected that complementarity in resource use among phenotypically variable genotypes would increase the productivity of genotypic mixtures (Hughes et al. 2009). However, our data indicate that varying responses of genotypes to the presence of conspecifics and water availability outweighed any overall effects of diversity. This is consistent with other studies of tree diversity, that have shown differing responses of particular species (analogous here to particular genotypes) to monoculture or mixture environments (Vila et al. 2003; Redondo-Brenes and Montagnini 2006).

We also observed differences in response to experimental treatments between genotypes from wet and dry collection sites, suggesting that some of the genotypic variation in response to diversity and watering treatment is determined by native site conditions. In our study, trees were sprouted directly from roots that were collected in the field, and therefore we cannot determine whether the collection site effect is due to local adaptation or maternal effects from root stock. In
Chapter 3, we showed that aspen genotypes from wet and dry collection sites planted in a common environment differed significantly in some traits, with genotypes from wet sites tending to grow taller and use greater amounts of water, and genotypes from dry sites growing a greater number of branches and leaves. Here we show that collection site also affects how aspen genotypes are influenced by neighboring conspecifics and water availability, suggesting that historical site conditions affect aspen growth either through maternal effects or local adaptation.

We show that both heritable genetic variation and phenotypic plasticity contribute to the observed variation in growth response to the biotic and abiotic environment. There were significant effects of genotype on all growth traits, and Chapter 3 established that aspen genotypes exhibit high levels of heritable genetic variation in growth patterns. The change in growth patterns between environments (well-watered and water-limited) and diversity levels (monoculture and mixture) also shows that some genotypes have a plastic response to their biotic and abiotic growing conditions. Phenotypic plasticity is common among plants (Schmid 1992), and all genotypes demonstrated plasticity in their growth responses to experimental treatments. In some cases these plastic responses were opposing, such as difference between the response of genotype CD26 to the interaction of diversity and watering treatment compared to the responses of genotypes CW24 and CW12 (Figure 5.1).

Leaf nutrient content, plant water use and soil nitrogen levels provided an indication of the effects of experimental treatments on tree physiology. Growth responses were manifest in complex interactions and were often specific to individual genotypes, whereas physiological responses were driven by main effects of experimental treatments. Carbon stable isotope analysis showed that trees in well-watered blocks and in genotypic mixtures had more negative δ^{13}C values, suggesting that those trees use more water. Higher water use in well-watered blocks is consistent with a greater availability of water provided by the watering treatments. Greater water use in genotypic mixtures has been found in previous studies, and suggests that genotypic
mixtures use more of the water in the rooting zone available for uptake by plants (Caldeira et al. 2001). However, δ^{13}C responses to experimental treatments in our study were only marginally significant, indicating relatively weak effects of diversity on plant water use. Soil nitrogen responses to diversity and watering treatment differed between forms of inorganic nitrogen. Levels of soil NO$_3^-$ were significantly lower in genotypic mixtures, indicating that trees in genotypic mixtures depleted soil nitrate to a lower level in mixtures than in monocultures (Tilman 1996), but there were no differences in soil NH$_4^+$ between diversity levels. Surprisingly, there was no effect of watering treatment on NO$_3^-$ or NH$_4^+$ ($P=0.926$), despite the propensity of negatively charged NO$_3^-$ ions to leach in watered soils. This may be due to low soil NO$_3^-$ levels at the onset of the experiment, making it difficult to detect differences between watering treatments, or might indicate that watering treatments affected only a very shallow layer of soil. The combination of soil nitrogen and plant water use responses to diversity suggest that there is a slight overall positive effect of genotypic diversity on aspen growth and physiology, although the wide range of variation among genotypes largely masks this effect.

Our results indicate that there may be no consistent diversity-ecosystem function relationship across all ecological systems, as suggested by some previous studies (Goodman 1975; Jiang et al. 2008; Klanderud and Totland 2008). The wide range of variation in aspen response to diversity and environment in our study canceled out any overall diversity effects. However, previous studies have found that the magnitude of diversity effects tends to increase over time (Tilman et al. 2001; Hooper and Dukes 2004), suggesting that the weak positive effects of genotypic diversity may strengthen in subsequent years of the experiment. The high levels of variability in genotype performance also suggest that genetic diversity per se may be less important than the phenotypic traits of particular genotypes. This is analogous to the conclusions of other diversity studies at the species and functional group levels that have shown composition to be more important than diversity (Tilman et al. 1997; Hooper 1998; Hector et al. 1999).
results also highlight the importance of the environment on the diversity-ecosystem function relationship, by showing that diversity often interacted with watering treatment to determine patterns of tree growth. We show that the relationship between aspen genetic diversity and ecosystem functioning can be highly variable and environmentally-dependent, suggesting that no universal diversity-ecosystem function relationship exists.
CHAPTER 6

CONCLUSIONS

Genetic variation has been studied extensively in an evolutionary context, and a long-standing body of evolutionary theory has shown that genetic variation is important to the persistence of species. There is also a long history of studies investigating the ecological effects of species diversity (MacArthur 1955; May 1973; Tilman 1999; Hooper et al. 2005), but the merging of biodiversity-ecosystem function research with the study of genetic diversity is fairly recent. A surge of interest in the ecological effects of genetic diversity has resulted in several studies documenting widespread impacts of genetic diversity, including altering community structure, affecting ecosystem processes and enhancing stability (reviewed in Hughes et al. 2008). This surge of interest stemmed from the recognition that intraspecific genetic diversity is one fundamental level of biodiversity, along with species and functional group diversity, and could potentially affect communities and ecosystems in a similar way to other levels of biodiversity through parallel processes at different levels of biological organization. There are still relatively few studies of the ecological effects of genotypic diversity in the literature, but they indicate that genotypic diversity can strongly affect community and ecosystem function. However, as the diversity-ecosystem function literature expands with more studies at the genetic, species and functional group levels, some studies are uncovering more complicated patterns and suggesting that there may be no universal diversity-ecosystem function relationship (Goodman 1975; Hooper 1998; Huston et al. 2000; Zhang and Zhang 2006; Jiang et al. 2008). This study supports the idea that the relationship between diversity and ecological processes may not be constant across all environmental conditions and ecological systems.

A common theme across the three data chapters is the highly variable nature of aspen genotypes. Previous studies had noted high phenotypic variability within natural aspen stands (Barnes 1975; Jones and DeByle 1985), but no prior studies had quantified genetic variation in a
common garden where environmental variation could be minimized. I quantified heritability values and coefficients of genetic variation, which together provided a metric of the amount of genetic variation relative to environmental variation, and the magnitude of genetic variation relative to the trait mean. I established that aspen populations contain high levels of heritable genetic variation, and that significant variation among genotypes exists for a variety of growth, morphological and physiological traits (Chapter 3), traits that could potentially affect ecological processes.

Although the high heritability values and highly significant trait variation uncovered in Chapter 3 were expected based on previous field studies, the ecological consequences of aspen genetic diversity at the plot level resulted in some surprising patterns. I expected that high levels of heritable phenotypic variation among aspen genotypes would result in increased ground-dwelling arthropod diversity, in accordance with long-established ideas about general positive relationships between plant and arthropod diversity (Siemann et al. 1998; Knops et al. 1999; Haddad et al. 2001). Instead, the interaction of water limitation and genotypic diversity decreased ground-dwelling arthropod diversity in this study (Chapter 4). I also expected that the high levels of genotypic variation would result in increased resource partitioning among genotypes, which could lead to strong positive effects of genotypic diversity on growth and ecosystem function. However, the high levels of variation among genotypes appeared to outweigh any main effects of experimental treatments, rather than enhance them (Chapter 5). This result runs counter to the findings of Hughes et al. (2009), who attributed strong genotypic diversity-stability effects (Hughes and Stachowicz 2004) to high levels of morphological variation among eelgrass genotypes. Part of the discrepancy may stem from the low number of genotypes planted in the cross-environment data set (5 genotypes), representing a more limited subset of phenotypic trait variation than considered in Chapter 3. However, there were no main effects of genotypic diversity on community and ecosystem function responses across the
drought-only data set either, which contained greater number of genotypes and thus more
phenotypic variation (11 genotypes in monoculture and 8 mixtures; data not shown). Therefore,
it is likely that the lack of main diversity effects did not stem from the low number of genotypes
considered in Chapters 4 and 5.

Although there were no significant effects of genotypic diversity on tree growth (Chapter
5), there appeared to be some positive effects of genotypic diversity on plant water use and soil
nitrogen content. These effects were weak, but may strengthen over time as more interactions
occur among trees and resources become more limited. The common garden experiment had
been established for only three years at the end of data collection, representing a short time period
for a forest tree species. Several previous biodiversity studies have found that the magnitude of
diversity effects increases over time (Tilman et al. 2001; Hooper and Dukes 2004), turning initial
weak positive effect of diversity into overyielding. It is possible that the variable effects of
genotypic diversity and watering treatment are transient effects, and that over time positive
effects of diversity may develop. However, there are enough studies emerging in the literature
showing negative and variable diversity effects (see Chapter 1) that the assumption of general
positive diversity effects may no longer be valid.

The design of the common garden experiment manipulated both plant genotypic diversity
and water treatment levels, and the interactive effects of diversity and environment uncovered
some interesting patterns that were not detectable in a single environment. By subjecting half of
the study to drought (water-limited treatments) and the other half to well-watered conditions, I
was able to directly assess the role of diversity, environment and the diversity × environment
interaction on community and ecosystem function. The most interesting patterns in Chapters 4
and 5 emerged as a result of diversity × watering treatment interaction effects, and revealed some
interesting and unexpected patterns. In Chapter 4, only the combination of water limitation and
plant genotypic diversity resulted in a strong negative impact on arthropod community, but the
main effects of diversity and watering treatment were rarely significant. The environmental-dependence of the relationship between plant and arthropod diversity would not have been evident without manipulation of both diversity and watering treatments. Similarly, in Chapter 5, the highly variable nature of aspen genotypes in response to biotic (plot-level diversity) and abiotic (watering treatment) environmental conditions was dependent on simultaneous manipulations of diversity and watering treatment. Three of the five genotypes showed a significant diversity × treatment growth response, demonstrating that aspen genotypes can exhibit plasticity in response to both their biotic and abiotic environment. Our results highlight the need for more diversity-ecosystem function studies across varying environmental conditions, particularly across levels or gradients of a limiting resource.

This study may have implications for the management of aspen stands throughout the Intermountain West. My results suggest that particular adaptive traits may be more important than diversity per se in restoring aspen forests. It also emphasizes that the microsite conditions at the location of seed collection is important in determining subsequent growth and physiology, as well as response to biotic and abiotic conditions. If restoration of aspen forests is achieved through planting of aspen seeds, collecting seeds from trees in a variety of microsite conditions will likely result in a greater range of phenotypes and tolerances to both biotic and abiotic conditions. Seeding aspen stands from a large number of genotypes collected across a range of microsite conditions will maximize phenotypic variation, and likely result in an aspen stand composed of many genotypes varying widely in their traits. Although this study suggests that genotypic diversity alone does not enhance ecosystem function, a greater number of genotypes are likely to contain high levels of phenotypic variation and allow the genotypes most suited to field conditions to thrive. Trait variation in ecologically relevant traits is important for the long-term persistence of species, and restoring aspen forests with high levels of heritable trait variation maximizes evolutionary potential under changing environmental conditions.
The genetic diversity-ecosystem function literature is relatively young and still limited in its ability to draw robust conclusions about the generality of the positive genetic diversity-ecosystem function relationship. This study is one of the first studies of the ecological effects of genotypic diversity in a forest tree species, beginning to fill an important knowledge gap in understanding forest ecosystems. It also used a relatively uncommon experimental design that simultaneously manipulated diversity levels and environmental conditions, and showed that the interaction of multiple factors can dramatically change the diversity-ecosystem function relationship. This study also challenges some of the mainstream biodiversity literature that has concluded that diversity at all levels of biological organization, across all ecosystem types, and under all environmental conditions has a positive effect on community and ecosystem function.
REFERENCES


habitat parameters on arthropod abundance in montane European grasslands. Ecography 28: 429-442.


APPENDICES
APPENDIX A

Microsatellite Molecular Analysis of Genotypes Planted in the Common Garden Experiment
Microsatellite DNA analyses were performed to verify that the genotypes planted in the common garden experiment were all unique.

Seven highly variable microsatellite loci (W-20, P-2571, P-576, W-14, G-970, W15 and P-433) were genotyped following the methods of Mock et al. (2008). Microsatellite DNA analysis showed that all genotypes in the common garden were unique, with the exception of genotypes CW24 and CW14 (combined into genotype CW24/14 in the table).

Table A.1. Multilocus genotypes of the twelve aspen genotypes planted in the common garden experiment. Putative genotypes CW24 and CW14 were combined into genotype CW24/14 based on molecular analyses.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>W-20 Alleles</th>
<th>P-2571 Alleles</th>
<th>P-576 Alleles</th>
<th>W-14 Alleles</th>
<th>G-970 Alleles</th>
<th>W-15 Alleles</th>
<th>P-433 Alleles</th>
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<tr>
<td>CD11</td>
<td>217 223</td>
<td>89 99</td>
<td>156 163</td>
<td>211 230</td>
<td>118 122</td>
<td>193</td>
<td>186 188</td>
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<tr>
<td>CD20</td>
<td>89 101</td>
<td>156 169</td>
<td>205 211 224</td>
<td>120 122</td>
<td>193 196</td>
<td>186 188 196</td>
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<td>CD26</td>
<td>210 217</td>
<td>89 99</td>
<td>163 169</td>
<td>205 211 214</td>
<td>118 122</td>
<td>184</td>
<td>183 186</td>
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<tr>
<td>CD30</td>
<td>217 219</td>
<td>89 99</td>
<td>156 169</td>
<td>208 217</td>
<td>118 122</td>
<td>184 193</td>
<td>188 196</td>
</tr>
<tr>
<td>CD5</td>
<td>212 217</td>
<td>89 99</td>
<td>156 163 169</td>
<td>208 211</td>
<td>120 122</td>
<td>184 193</td>
<td>183 186 196</td>
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<tr>
<td>CW1</td>
<td>217</td>
<td>89 99</td>
<td>169</td>
<td>214</td>
<td>122 137</td>
<td>184 193</td>
<td>186 196</td>
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<tr>
<td>CW12</td>
<td>204 217</td>
<td>89 99 115</td>
<td>156 169</td>
<td>202 214 230</td>
<td>118 122 128</td>
<td>193 196</td>
<td>183 188 196</td>
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<tr>
<td>CW24/14</td>
<td>212 217</td>
<td>89 99 115</td>
<td>156 163</td>
<td>205 211</td>
<td>120 122</td>
<td>184 193 196</td>
<td>186</td>
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<td>CW27</td>
<td>217 223</td>
<td>89 99</td>
<td>156 169</td>
<td>205 208 211</td>
<td>122</td>
<td>193 196</td>
<td>183 186</td>
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<tr>
<td>CW31</td>
<td>217 235</td>
<td>89 115</td>
<td>163 169 175</td>
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<td>89 99</td>
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<td>188 196</td>
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<td>210 212 219</td>
<td>89 115</td>
<td>169</td>
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<td>120 125</td>
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<td>183 186</td>
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Microsatellite analysis was completed for three individuals per putative genotype, and replicate analyses were run on roughly a third of the samples. In most cases, the three individual samples and replicates within each sample all agreed on allele presence or absence. Where discrepancies occurred, the most common combination of alleles was assigned. In no case did discrepancies among individuals or replicates call into question the uniqueness of individual genotypes, as clear differences were usually exhibited between genotypes at multiple loci. All individuals of genotype CW12 failed for the W-20 locus, and therefore these allele sizes are not available.

Several genotypes (CD20, CD26, CD5, CW12, CW24/14, CW27, CW31 and CW7) showed three distinct microsatellite alleles at one or more loci, suggesting putative triploidy or aneuploidy in these genotypes (Table A.1). In these cases, all three alleles were almost always at known allele sizes, and were unlikely to be caused by stutter in the replication process. Triploidy has been verified in Utah aspen stands (Every and Wiens 1971) and it is likely there are many triploid genotypes throughout Utah (Mock et al. 2008), although triploidy has not been verified in the particular genotypes planted in the common garden experiment.

Genotype CW12 showed four distinct peaks for locus P-2571, at sizes 89, 93, 99 and 115. The allele of size 93 was omitted from the table, as it was likely to be caused by stutter instead of a true allele, and was not an allele size found in any of the other samples. Genotyping analysis in this study was completed simply to verify that genotypes were unique, and I could already assign genotype CW12 as a unique genotype based on genotyping from other alleles. Therefore, I concluded that it was not necessary to re-run any microsatellite analyses.
APPENDIX B

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OBJECTIVE

Seeking a conservation scientist position leading ecological monitoring programs and working to develop innovative and practical solutions to conservation challenges.

EDUCATION

2004-2009 Utah State University, Logan, UT
Department of Biology / Ecology Center
PhD, Ecology. 2009
  Dissertation: Ecological effects of genotypic diversity on community and ecosystem function
  Courses: ecology, conservation biology, GIS, biogeography, plant physiological ecology, plant community ecology, environmental biophysics, soil classification & morphology, evolutionary genetics, linear regression, experimental design, multivariate statistics

1999-2003 Whitman College, Walla Walla, WA
BA, Biology–Environmental Studies. 2003
  Undergraduate thesis: Understory conifer survival in a mature alder forest of the Washington Cascades
  Related courses: biology, chemistry, environmental studies, resources & pollution, plant ecology, genetics, evolutionary biology, physiology, cell biology, geology

2002 Study abroad program. James Cook University, Townsville, Australia
  Related courses: biogeography of marine fishes, coral reef geomorphology

PROFESSIONAL EXPERIENCE

2006-2009 Graduate Teaching Assistant. Utah State University, Logan, UT
  Introductory Biology, fall 2009
  Human Physiology, spring 2008 and spring 2009
  Biological Discoveries, spring 2007
  Biodiversity in Utah, fall 2006

2009 Landscape Conservation Intern. The Nature Conservancy, Boise, ID
Used state-and-transition models to predict changes in vegetation communities, and completed documentation of the CEA Tool modeling program for the Landscape Toolbox website
Conducted field work characterizing plant communities and rangeland condition

2007-2009 GIS Assistant. Utah State University, Logan, UT
Intermittent work mapping freshwater mussel and aspen populations and analyzing spatial data in ArcGIS

2007 Graduate Research Assistant. Utah State University, Logan, UT
Used amplified fragment length polymorphism (AFLP) molecular analysis to investigate the role of local adaptation in resistance of trout populations to whirling disease

2004-2006 Graduate Research Fellow. College of Science and Engineering, Utah State University, Logan, UT
Independently designed and implemented field and experimental ecological research on the ecological effects of aspen genetic diversity
Developed proficiency in DNA extractions and PCR-based molecular genetic techniques

Used mist netting, telemetry and behavioral observation to monitor willow flycatcher populations of conservation concern in central Utah

2003-2004 Evolutionary Ecology Research Technician. Utah State University, Logan, UT
Implemented evolutionary experiments investigating genetic variation and phenotypic plasticity of *Daphnia* species in response to changing environmental conditions

2003 Limnology Research Technician. Utah State University, Logan, UT
Lab and field duties monitoring the biology and chemistry of a highly eutrophic section of the Great Salt Lake
Design and administration of a survey to address the scientific and human dimensions of odor emissions from the Great Salt Lake

2002 Environmental Studies Intern. US Army Corps of Engineers, Walla Walla, WA
Vegetation survey of three islands created as dam mitigation on the Columbia River

2001 Cedar River Habitat Conservation Plan Intern. City of Seattle Public Utilities, Seattle, WA
Assistance in the initial implementation of the Cedar River Watershed Habitat Conservation Plan, including field work, administrative support, assistance in website design and development of a public information brochure
GRANTS AND FELLOWSHIPS

2008 Utah State University (USU) Ecology Center fellowship. $8,000
2008-2009 USU Ecology Center research award. $4,000
2007-2008 USU Ecology Center research award. $4,500
2006-2007 USU Ecology Center research award. $2,200
2004-2006 USU Diversity Fellowship in Science and Engineering. $60,000
2004 USU Research Vice President Fellowship. $15,000 (declined)

PUBLICATIONS

Kanaga, M.K., K.E. Mock, R.J. Ryel and M.E. Pfrender. High variability among aspen genotypes alters the diversity-ecosystem function relationship. *In submission*.


PRESENTATIONS


VOLUNTEER WORK

2006-2007 USU Ecology Center seminar committee co-chair
2005-2006 USU Ecology Center seminar committee member
2004-2006 USU Biology Graduate Student Association officer