University of Alberta

Leaf and root functional traits, phylogeny, and the structure of plant populations and communities

by

Steven William Kembel

A thesis submitted to the Faculty of Graduate Studies and Research
in partial fulfillment of the requirements for the degree of

Doctor of Philosophy
in
Environmental Biology and Ecology

Department of Biological Sciences

Edmonton, Alberta
Spring 2007

Library and
Archives Canada

Published Heritage
Branch

395 Wellington Street
Ottawa ON K1A 0N4
Canada

Bibliothèque et
Archives Canada

Direction du
Patrimoine de l'édition

395, rue Wellington
Ottawa ON K1A 0N4
Canada

Your file Votre référence
ISBN: 978-0-494-29698-1
Our file Notre référence
ISBN: 978-0-494-29698-1

NOTICE:
The author has granted a non-exclusive license allowing Library and Archives Canada to reproduce, publish, archive, preserve, conserve, communicate to the public by telecommunication or on the Internet, loan, distribute and sell theses worldwide, for commercial or non-commercial purposes, in microform, paper, electronic and/or any other formats.

The author retains copyright ownership and moral rights in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

AVIS:
L'auteur a accordé une licence non exclusive permettant à la Bibliothèque et Archives Canada de reproduire, publier, archiver, sauvegarder, conserver, transmettre au public par télécommunication ou par l'Internet, prêter, distribuer et vendre des thèses partout dans le monde, à des fins commerciales ou autres, sur support microforme, papier, électronique et/ou autres formats.

L'auteur conserve la propriété du droit d'auteur et des droits moraux qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

In compliance with the Canadian Privacy Act some supporting forms may have been removed from this thesis.

While these forms may be included in the document page count, their removal does not represent any loss of content from the thesis.

Conformément à la loi canadienne sur la protection de la vie privée, quelques formulaires secondaires ont été enlevés de cette thèse.

Bien que ces formulaires aient inclus dans la pagination, il n'y aura aucun contenu manquant.

Canada

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Dedication

This thesis is dedicated to my family.

Abstract

I present a study of relationships among functional traits of plant leaves and roots, their evolutionary histories, and their influence on the ecology of populations and communities. My first objective was to describe how leaf and root functional traits vary and covary among and within plant species and communities. I measured the leaf and root traits, phylogenetic relationships and community structure of species at sites in mixedgrass and fescue grasslands in Alberta. Plants growing in mixedgrass communities, and at drier locations within each site, tended to have a suite of stress-tolerant traits, including smaller, thicker leaves with lower specific leaf area (area per unit mass) and dense roots with lower specific root length (length per unit mass). Phylogenetic comparative methods indicated that suites of leaf and root traits evolved independently in these grasslands. The traits of individual species varied more along environmental gradients than mean trait values did across entire communities, and trait variation among communities was much smaller than the magnitude of trait variation within communities. In a growth chamber experiment conducted with a single plant species (*Arabidopsis thaliana*), root traits such as root diameter and specific root length were more important for maintaining plant fitness when soil nutrients were limiting, but otherwise above- and belowground vegetative traits were highly integrated regardless of nutrient availability.

My second objective was to determine how patterns of trait evolution interact with ecological processes to determine the trait similarity and phylogenetic structure of ecological communities. In Alberta grasslands, I found consistent leaf and root trait clustering within communities, but variation in the pattern of trait evolution in different lineages and communities led to changes in patterns of phylogenetic relatedness at different scales. In a study of the relatedness of co-occurring trees in a neotropical forest, phylogenetic relatedness within communities differed among habitats, and measures of phylogenetic similarity were affected by null model choice. In general, communities in more stressful environments contained species that were more similar in terms of their traits and phylogenetic relatedness, indicating the potential importance of environmental filtering of traits during community assembly.

Acknowledgements

I would like to thank my supervisor JC Cahill for his encouragement and mentorship over the years. I am also grateful to my committee members Mark Dale, Mark Lewis, and John Spence for their advice and guidance.

Thanks to all the other members of the Cahill lab group, especially my office mates Eric Lamb, Gord McNickle, Matt Mitchell, and Bryon Shore for stimulating ideas and good times in the field and lab.

Kelley Dunfield and Steve Roehr provided good company and able assistance in the field and lab. Many other people assisted with data collection, including Esteban Acuña, Luc Barton, Maggie Czan, Lucia Daisog, Nadeesha Fernando, Colleen Stevens, Tara Stieglitz, and Agnes Wong.

Thanks to Alicia Hargrave, Darcy Henderson, and Nairn Howe for introducing me to my field sites and for sharing their wealth of knowledge of the amazing grasslands of Alberta.

I would also like to acknowledge my collaborators David Ackerly, Stephen Hubbell, and Campbell Webb. Thanks also to Kyle Harms and Richard Condit for providing some of the data used in Chapter 4.

For their funding and scholarship support, I would like to thank NSERC, the Faculty of Science, the Faculty of Graduate Studies and Research, the Department of Biological Sciences, the Province of Alberta, the McAfee Estate, and the Alberta Conservation Association Biodiversity Grants program.

Most importantly, thanks to my family and friends near and far who have supported me for so long. I am grateful to have had such a supporting and wonderful family, especially my parents Neil and Leslie, my sister Erin, my amma Thora McCaine, and my grandmother Evelyn Kembel. But most of all I want to thank Agnes and Maya, thank you for your constant love and support, I could not have done it without you.

Table of Contents

List of Tables

List of Figures

List of Nomenclature

Species nomenclature

Kartesz, J. T. 1994. A synonymized checklist of the vascular flora of the United States, Canada, and Greenland, 2nd edition. Timber Press, Portland, Oregon.

List of Abbreviations

DBH	Diameter at breast height
FDP	Forest Dynamics Plot
GxE	Genotype by environment interaction
LAR	Leaf area ratio
LWF	Leaf weight fraction
Ma	Millions of years
MNND	Mean nearest neighbour distance
MPD	Mean pairwise distance
NMS or NMDS	Non-metric multidimensional scaling
NRI	Net relatedness index
NTI	Nearest taxon index
PCA	Principal components analysis
PIC	Phylogenetically independent contrasts
RLR	Root length ratio
RWF	Root weight fraction
SAR	Simultaneous autoregression
SD	Standard deviation
SLA	Specific leaf area
SLV	Specific leaf volume
SRL	Specific root length
SRV	Specific root volume
UPGMA	Unweighted pair-group method with arithmetic mean

Chapter 1 General Introduction

Objectives

The goal of this dissertation is to understand relationships among plant leaf and root functional traits, phylogenetic relationships among species, and their influence on the ecology of populations and communities. I address two specific objectives. The first is to describe how leaf and root functional traits vary among and within plant species and communities, and to determine whether leaf and root traits covary as part of an integrated ecological strategy among and within species. The second objective is to understand what role the trait similarity and evolutionary relatedness of co-occurring species play in the assembly of ecological communities at different scales, and to determine how patterns of trait evolution interact with ecological processes to determine the phylogenetic structure of ecological communities.

Background

Leaf and root functional traits

Functional traits are broadly defined as any attribute of a species that affects its survival, establishment or fitness (Reich et al. 2003). Many researchers have suggested that a focus on understanding the functional traits of species provides a unifying framework that will improve the ability of ecologists to predict species effects on ecosystem processes (Grime et al. 1997) and their potential responses to processes such as climate change (Diaz et al. 2004, McGill et al. 2006), as well as improving our understanding of relationships between environment and organismal form and function (Southwood 1977, Westoby et al. 2002).

Leaves and roots are the organs responsible for resource uptake by plants. Roots perform a variety of important functions in addition to resource uptake, including anchorage, soil penetration, water uptake and interactions with a variety of soil microorganisms (Fitter 2002). These diverse functions may exert conflicting pressures on the evolution of leaf and root morphology and physiology. An understanding of root traits will be important if we wish to understand belowground interactions among species

(Callaway et al. 2003), the ecology and evolution of whole-plant ecological strategies, or the potential effects of plants on belowground processes such as nutrient cycling (Craine et al. 2002, Westoby and Wright 2006). While the fundamental functional traits and tradeoffs governing the ecology and evolution of leaves and other aboveground plant tissues are well known, our knowledge of corresponding belowground traits and their relationships with aboveground plant traits are poorly understood.

Leaf and root traits: variation among species and communities

A suite of correlated leaf traits known as the leaf economics spectrum explains most of the worldwide variation in leaf morphology and physiology (Wright et al. 2004). The spectrum separates 'fast' species that invest resources in short-lived leaves with a high expected rate of return on investment from 'slow' species with longer-lived leaves with a slower expected rate of return. 'Fast' species possess relatively large, fast growing leaves with short lifespan, high nitrogen concentration per unit mass, high specific leaf area (SLA; area per unit mass, caused by relatively thin leaves and/or low tissue density), and high instantaneous rates of respiration and photosynthesis (Reich et al. 1997), while 'slow' species possess the opposite set of traits. Because correlations among all of these leaf traits are extremely strong at spatial scales ranging from local communities to global databases of plant traits spanning many biomes, easily measured morphological traits such as specific leaf area have been suggested as useful surrogate measures of leaf ecophysiology, the resource uptake strategies and niches of species, and their effects on ecosystem processes (Westoby et al. 2002).

Several plant strategy theories have hypothesized that the leaf and root traits of species will be correlated, due to selection for a coordinated above- and belowground resource uptake strategy (Chapin et al. 1993, Diaz et al. 2004). Empirical evidence to support these hypotheses has been mixed. Fine roots follow some of the resource economic tradeoffs observed in leaves, with fast-growing species tending to possess thin, short-lived fine roots with high specific root length (SRL; length per unit mass), low tissue density, and high nitrogen concentrations and instantaneous rates of respiration and nutrient uptake (Ryser 1996, Eissenstat and Yanai 1997, Wahl and Ryser 2000, Comas and Eissenstat 2004). Some studies have found that corresponding leaf and root traits are correlated among species, with species possessing the 'fast' set of leaf traits also possessing the 'fast' set of root traits (Ryser and Lambers 1995, Reich et al. 1998, Wright and Westoby 1999, Craine et al. 2002), but others have found that leaf and root traits vary independently among species (Craine et al. 2005, Tjoelker et al. 2005).

Some of the earliest studies of comparative plant physiology noted that leaf and root traits vary predictably among communities along environmental gradients (Schimper 1903). Early comparative studies of leaf and root traits often focused on traits related to plant growth form such as canopy height or rooting depth (Coupland and Johnson 1965). More recently research on trait variation among communities has focused on the set of leaf and to a lesser extent root traits related to species resource uptake strategies, since these traits should be related to the trophic niches of plant species (Silvertown 2004), and have the potential to predict species interactions and effects on ecosystem processes (Diaz et al. 2004). Stress-tolerant species from habitats with lower water or nitrogen availability tend to possess a suite of traits including relatively small, thick leaves, thick roots, lower tissue density and nitrogen concentrations, longer tissue lifespans, lower instantaneous resource uptake and respiration rates, and higher resistance to mechanical damage and herbivory (Fonseca et al. 2000, Craine et al. 2001, Ackerly et al. 2002, McDonald et al. 2003, Diaz et al. 2004). Leaf and root traits vary a great deal among co-occurring species, but patterns and causes of trait variation within communites are not as well understood as patterns of among-species and among-community variation (Westoby et al. 2002).

Leaf and root traits: Intraspecific variation and plasticity

The state of knowledge with regards to variation in leaf and root traits within individual species is similar in many ways to our knowledge of among-species patterns. Due to the difficulty of working with roots, many studies of plasticity, selection and phenotypic integration within species have focused on aboveground traits including reproductive structures and leaves, their ecophysiology and response to environmental heterogeneity (Pigliucci 2001). Intraspecific variation and plasticity of root system architecture and root proliferation in response to nutrients can affect plant fitness in nutrient-limited environments (Fitter 2002), but the plasticity and fitness effects of other root morphological traits are not well understood.

Functional trait and phylogenetic similarity within communities

Numerous studies have used data on the trait similarity of co-occurring species in an attempt to test for the importance of ecological processes that can influence community assembly. One approach to this question has emphasized the role of biotic interactions, especially competitive exclusion and limiting similarity (Macarthur and Levins 1967, Pacala and Tilman 1994, Dayan and Simberloff 2005), which should create patterns of trait overdispersion or dissimilarity among co-occurring species. The other approach has emphasized the role of the abiotic environment as a filter which selects for species possessing particular traits (Southwood 1977, Grime 2001), leading to patterns of trait clustering or similarity in communities. These two approaches make contrasting predictions about the similarity of co-occurring species, and numerous studies have sought evidence for limiting similarity or environmental filtering by examining the similarity of traits of species occurring together in animal and plant communities (Ricklefs and Travis 1980, Cody 1991, Weiher and Keddy 1995, Weiher et al. 1998, Stubbs and Wilson 2004).

There has been an increasing call for ecologists to account for the historical factors that contribute to community structure (Ricklefs 2004, Wiens and Donoghue 2004), and more recently a number of studies have begun to take the evolutionary history of species into account when measuring trait similarity within communities, as well as asking the related questions of how patterns of trait evolution interact with ecological processes to determine the relatedness of species in communities (Webb et al. 2002). Based on the premise that species from the same genus are ecologically similar and will compete more intensely, leading to a pattern of close relatives not occurring together (Darwin 1859), early studies examined taxonomic ratios within communities in a search for evidence competitive exclusion (Elton 1946). With the increasing availability of phylogenetic data for large numbers of species, researchers have begun to use data on the phylogenetic structure of ecological communities to address similar questions (Losos 1996, Webb et al. 2002).

The phylogenetic structure of ecological communities may provide insights into the relative importance of different ecological processes, as ecological processes interact with the evolutionary history of plant traits and leave their signature on the phylogenetic structure of a community (Webb et al. 2002). Phylogenetic relatedness within communities will depend on patterns of trait evolution as well as the relative importance of different ecological processes (Table 1-1), but few studies have measured both trait and phylogenetic similarity within communities. Instead, based on evidence for widespread niche and trait conservatism among species (Wiens and Graham 2005), studies of community phylogenetic structure have often assumed that closely related species possess similar traits (phylogenetic trait conservatism), and have interpreted patterns of phylogenetic clustering in communities (close relatives co-occurring) as evidence for environmental trait filtering, and patterns of phylogenetic overdispersion (distant relatives co-occurring) as evidence for limiting similarity (Webb 2000).

Thesis outline

In Chapter 2, I examine patterns of variation in leaf and root traits of plants in Alberta grasslands. I ask how leaf and root traits vary among species, how they vary among major gradients of community composition, measure the relative magnitude of among- and within-community variation in traits, and compare evolutionary and non-evolutionary approaches to studies of trait correlations.

In Chapter 3, I ask whether species co-occurrence in Alberta grasslands can be predicted by the similarity of leaf and root traits or phylogenetic relatedness, and I ask what this tells us about the relative importance of limiting similarity and environmental filtering in these communities.

In Chapter 4, I use a large data set on the distribution of tree species in a neotropical forest to ask whether local communities contain species that are more closely or distantly related than expected by chance, describe how spatial scales and null model choice can affect studies of community phylogenetic structure, and ask what the observed patterns say about the assembly of tropical tree communities.

In Chapter 5, I present a study of intraspecific leaf and root trait variation and plasticity among genotypes of mouse-ear cress (*Arabidopsis thaliana)* growing in low and high nutrient availability environments. I ask whether leaf and root traits are phenotypically plastic, how correlations among leaf and root traits change with nutrient availability, and how traits and their plasticities are related to plant fitness in different environments.

In Chapter 6, I conclude with a general discussion of the findings of the thesis.

Literature cited

Ackerly, D. D., C. A. Knight, S. B. Weiss, K. Barton, and K. P. Starmer. 2002. Leaf size, specific leaf area and microhabitat distribution of chaparral woody plants: contrasting patterns in species level and community level analyses. Oecologia **130**:449-457.

Callaway, R. M., S. C. Pennings, and C. L. Richards. 2003. Phenotypic plasticity and interactions among plants. Ecology **84**:1115-1128.

Chapin, F. S., K. Autumn, and F. Pugnaire. 1993. Evolution of suites of traits in response to environmental stress. American Naturalist **142**:S78-S92.

Cody, M. L. 1991. Niche theory and plant growth form. Plant Ecology **97**:39-55.

Comas, L. H., and D. M. Eissenstat. 2004. Linking fine root traits to maximum potential growth rate among 11 mature temperate tree species. Functional Ecology **18**:388-397.

Coupland, R. T., and R. E. Johnson. 1965. Rooting characteristics of native grassland species in Saskatchewan. Journal of Ecology **53**:475-507.

Craine, J. M., J. Froehle, D. G. Tilman, D. A. Wedin, and F. S. Chapin. 2001. The relationships among root and leaf traits of 76 grassland species and relative abundance along fertility and disturbance gradients. Oikos **93**:274-285.

Craine, J. M., W. G. Lee, W. J. Bond, R. J. Williams, and L. C. Johnson. 2005. Environmental constraints on a global relationship among leaf and root traits of grasses. Ecology **86**:12-19.

Craine, J. M., D. Tilman, D. Wedin, P. Reich, M. Tjoelker, and J. Knops. 2002. Functional traits, productivity and effects on nitrogen cycling of 33 grassland species. Functional Ecology **16**:563-574.

Darwin, C. 1859. On the origin of species by natural selection. John Murray, London.

Dayan, T., and D. Simberloff. 2005. Ecological and community-wide character displacement: the next generation. Ecology Letters **8**:875-894.

Diaz, S., J. G. Hodgson, K. Thompson, M. Cabido, J. H. C. Cornelissen, A. Jalili, G. Montserrat-Marti, J. P. Grime, F. Zarrinkamar, Y. Asri, S. R. Band, S. Basconcelo, P. Castro-Diez, G. Funes, B. Hamzehee, M. Khoshnevi, N. Perez-Harguindeguy, M. C. Perez-Rontome, F. A. Shirvany, F. Vendramini, S. Yazdani, R. Abbas-Azimi, A. Bogaard, S. Boustani, M. Charles, M. Dehghan, L. de Torres-Espuny, V. Falczuk, J. Guerrero-Campo, A. Hynd, G. Jones, E. Kowsary, F. Kazemi-Saeed, M. Maestro-Martinez, A. Romo-Diez, S. Shaw, B. Siavash, P. Villar-Salvador, and M. R. Zak. 2004. The plant traits that drive ecosystems: Evidence from three continents. Journal of Vegetation Science **15**:295-304.

Eissenstat, D. M., and R. D. Yanai. 1997. The ecology of root lifespan. Advances in Ecological Research **27**:1-60.

Elton, C. 1946. Competition and the structure of ecological communities. Journal of Animal Ecology **15**:54-68.

Fitter, A. 2002. Characteristics and functions of root systems. Pages 15-32 *in* Y. Waishel, A. Eshel, and U. Kafkafi, editors. Plant roots: the hidden half. Marcel Dekker, New York, NY.

Fonseca, C. R., J. M. Overton, B. Collins, and M. Westoby. 2000. Shifts in trait-combinations along rainfall and phosphorus gradients. Journal of Ecology **88**:964-977.

Grime, J. P. 2001. Plant strategies, vegetation processes and ecosystem properties. Wiley, Chichester, U.K.

Grime, J. P., K. Thompson, R. Hunt, J. G. Hodgson, J. H. C. Cornelissen, I. H. Rorison, G. A. F. Hendry, T. W. Ashenden, A. P. Askew, S. R. Band, R. E. Booth, C. C. Bossard, B. D. Campbell, J. E. L. Cooper, A. W. Davison, P. L. Gupta, W. Hall, D. W. Hand, M. A. Hannah, S. H. Hillier, D. J. Hodkinson, A. Jalili, Z. Liu, J. M.

L. Mackey, N. Matthews, M. A. Mowforth, A. M. Neal, R. J. Reader, K. Reiling, W. RossFraser, R. E. Spencer, F. Sutton, D. E. Tasker, P. C. Thorpe, and J. Whitehouse. 1997. Integrated screening validates primary axes of specialisation in plants. Oikos **79**:259-281.

Losos, J. B. 1996. Phylogenetic perspectives on community ecology. Ecology **77**:1344-1354.

Macarthur, R., and R. Levins. 1967. The limiting similarity, convergence, and divergence of coexisting species. American Naturalist **101**:377-385.

McDonald, P. G., C. R. Fonseca, J. M. Overton, and M. Westoby. 2003. Leaf-size divergence along rainfall and soil-nutrient gradients: is the method of size reduction common among clades? Functional Ecology **17**:50-57.

McGill, B. J., B. J. Enquist, E. Weiher, and M. Westoby. 2006. Rebuilding community ecology from functional traits. Trends in Ecology and Evolution **21**:178-185.

Pacala, S. W., and D. Tilman. 1994. Limiting similarity in mechanistic and spatial models of plant competition in heterogeneous environments. American Naturalist **143**:222-257.

Pigliucci, M. 2001. Phenotypic plasticity: beyond nature and nurture. Johns Hopkins University Press, Baltimore, MD.

Reich, P. B., M. G. Tjoelker, M. B. Walters, D. W. Vanderklein, and C. Bushena. 1998. Close association of RGR, leaf and root morphology, seed mass and shade tolerance in seedlings of nine boreal tree species grown in high and low light. Functional Ecology **12**:327-338.

Reich, P. B., M. B. Walters, and D. S. Ellsworth. 1997. From tropics to tundra: Global convergence in plant functioning. Proceedings of the National Academy of Sciences of the United States of America **94**:13730-13734.

Reich, P. B., I. J. Wright, J. Cavender-Bares, J. M. Craine, J. Oleksyn, M. Westoby, and M. B. Walters. 2003. The evolution of plant functional variation: Traits, spectra, and strategies. International Journal of Plant Sciences **164**:S143-S164.

Ricklefs, R. E. 2004. A comprehensive framework for global patterns in biodiversity. Ecology Letters **7**:1-15.

Ricklefs, R. E., and J. Travis. 1980. A morphological approach to the study of avian community organization. Auk **97**:321-338.

Ryser, P. 1996. The importance of tissue density for growth and life span of leaves and roots: A comparison of five ecologically contrasting grasses. Functional Ecology **10**:717-723.

Ryser, P., and H. Lambers. 1995. Root and leaf attributes accounting for the performance of fast-growing and slow-growing grasses at different nutrient supply. Plant and Soil **170**:251-265.

Schimper, A. F. W. 1903. Plant geography on a physiological basis. Clarendon Press, Oxford, U.K.

Silvertown, J. 2004. Plant coexistence and the niche. Trends in Ecology and Evolution **19**:605-611.

Southwood, T. R. E. 1977. Habitat, the templet for ecological strategies? Journal of Animal Ecology **46**:336-365.

Stubbs, W. J., and B. Wilson. 2004. Evidence for limiting similarity in a sand dune community. Journal of Ecology **92**:557-567.

Tjoelker, M. G., J. M. Craine, D. Wedin, P. B. Reich, and D. Tilman. 2005. Linking leaf and root trait syndromes among 39 grassland and savannah species. New Phytologist **167**:493-508.

Wahl, S., and P. Ryser. 2000. Root tissue structure is linked to ecological strategies of grasses. New Phytologist **148**:459-471.

Webb, C. O. 2000. Exploring the phylogenetic structure of ecological communities: An example for rain forest trees. American Naturalist **156**:145-155.

Webb, C. O., D. D. Ackerly, M. A. McPeek, and M. J. Donoghue. 2002. Phylogenies and community ecology. Annual Review of Ecology and Systematics **33**:475-505.

Weiher, E., G. D. P. Clarke, and P. A. Keddy. 1998. Community assembly rules, morphological dispersion, and the coexistence of plant species. Oikos **81**:309-322.

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

Westoby, M., D. S. Falster, A. T. Moles, P. A. Vesk, and I. J. Wright. 2002. Plant ecological strategies: Some leading dimensions of variation between species. Annual Review of Ecology and Systematics **33**:125-159.

Westoby, M., and I. J. Wright. 2006. Land-plant ecology on the basis of functional traits. Trends in Ecology and Evolution **21**:261-268.

Wiens, J. J., and M. J. Donoghue. 2004. Historical biogeography, ecology and species richness. Trends in Ecology & Evolution **19**:639-644.

Wiens, J. J., and C. H. Graham. 2005. Niche conservatism: Integrating evolution, ecology, and conservation biology. Annual Review of Ecology Evolution and Systematics **36**:519-539.

Wright, I. J., P. B. Reich, M. Westoby, D. D. Ackerly, Z. Baruch, F. Bongers, J. Cavender-Bares, T. Chapin, J. H. C. Cornelissen, M. Diemer, J. Flexas, E. Garnier, P. K. Groom, J. Gulias, K. Hikosaka, B. B. Lamont, T. Lee, W. Lee, C. Lusk, J. J. Midgley, M. L. Navas, U. Niinemets, J. Oleksyn, N. Osada, H. Poorter, P. Poot, L. Prior, V. I. Pyankov, C. Roumet, S. C. Thomas, M. G. Tjoelker, E. J. Veneklaas, and R. Villar. 2004. The worldwide leaf economics spectrum. Nature **428**:821-827.

Wright, I. J., and M. Westoby. 1999. Differences in seedling growth behaviour among species: trait correlations across species, and trait shifts along nutrient compared to rainfall gradients. Journal of Ecology **87**:85-97.

Table 1-1. The predicted phylogenetic structure of an ecological community given different ecological processes and patterns of trait evolution (after Webb et al. 2002).

	Pattern of trait evolution	
Ecological process	Conserved	Convergent
Limiting similarity	Phylogenetic overdispersion	Random
Environmental filtering	Phylogenetic clustering	Phylogenetic overdispersion

Chapter 2 Leaf and root functional trait variation in Alberta grassland plant communities

Introduction

Functional traits are broadly defined as any attribute of a species that affects its survival, establishment or fitness (Reich et al. 2003). Many researchers have suggested that a focus on understanding the functional traits of species provides a unifying framework that could improve our ability to predict species effects on ecosystem processes (Grime et al. 1997) and their potential responses to processes such as climate change (Diaz et al. 2004, McGill et al. 2006). Such work will also improve understanding of relationships between environment and organismal form and function (Southwood 1977, Westoby et al. 2002). While the fundamental functional traits and tradeoffs governing the form and function of leaves and other aboveground plant tissues are well known, the corresponding root attributes and their relationships with aboveground plant traits are poorly understood.

In this study, I use data on leaf and root functional traits of Alberta grassland plants collected in the field and grown in a common garden to determine whether the leaf and root traits of these species are correlated as predicted by several plant ecological strategy hypotheses and studies in other grassland communities. I describe the relative contribution of among- and within-community variation in leaf and root traits to total trait variation at three Alberta grassland sites, use phylogenetic comparative methods to test for correlated evolution of leaf and root traits of plants growing in the field and in a common garden, and test whether leaf and root traits vary predictably along gradients of community composition in these grasslands,

Leaf and root functional traits and tradeoffs

A suite of correlated leaf traits known as the leaf economics spectrum explains most of the worldwide variation in leaf morphology and physiology (Wright et al. 2004). The spectrum separates 'fast' species that invest resources in short-lived leaves with a high expected rate of return on investment from 'slow' species with longer-lived leaves with a slower expected rate of return. 'Fast' species possess relatively large, fast growing leaves with short lifespan, high nitrogen concentration per unit mass, high specific leaf area (SLA; area per unit mass, caused by relatively thin leaves and/or low tissue density), and high instantaneous rates of respiration and photosynthesis (Reich et al. 1997). In contrast, 'slow' species possess the opposite set of traits. Because correlations among all of these leaf traits are extremely strong at spatial scales ranging from local communities to global databases of plant traits spanning many biomes, easily measured morphological traits such as specific leaf area have been suggested as useful surrogate measures of leaf ecophysiology, the resource uptake strategies of species, and their effects on ecosystem processes (Westoby et al. 2002).

Fine roots, generally defined as roots <2mm diameter, are the organs responsible for nutrient uptake by plants. Fine roots seem to follow some of the resource economic tradeoffs observed in leaves, with fast-growing species tending to possess thin, short-lived fine roots with high specific root length (SRL; length per unit mass), nitrogen concentrations and instantaneous rates of respiration and nutrient uptake, and low tissue density (Ryser 1996, Eissenstat and Yanai 1997, Wahl and Ryser 2000, Craine et al. 2001, Comas and Eissenstat 2004, Tjoelker et al. 2005). These tradeoffs are found both among species and within the root systems of individual plants (Pregitzer et al. 2002).

Roots perform a variety of important functions in addition to nutrient uptake, including anchorage, soil penetration, water uptake and interactions with a variety of soil microorganisms (Fitter 2002). These diverse functions may exert conflicting pressures on the evolution of the morphology and physiology of leaves versus roots, and due to the logistical difficulty of working with roots, relatively little is known about root functional traits or their relationships with leaf functional traits. Some studies of leaf and root traits of plants have found that corresponding leaf and root traits (e.g. leaf and root SLA and SRL, nitrogen concentrations, lifespans) are correlated (Ryser and Lambers 1995, Reich et al. 1998, Wright and Westoby 1999, Craine et al. 2002), but others found no relationship between some of these traits (e.g. SLA vs. SRL: Tjoelker et al. 2005).

It has been hypothesized that due to selection for a coordinated above- and belowground resource uptake strategy along gradients of water and nitrogen availability, the primary axis of leaf and root trait variation among temperate grassland species is a continuum from species with thin, dense leaf and root tissues to those with relatively thick, low-density leaf and root tissue (Craine et al. 2001, Craine et al. 2002). The generality of this pattern is unclear; among grasses from a variety of regions, leaves and roots each tend to fall along a spectrum of coordinated tissue density, nitrogen concentration, uptake rate and lifespan, but there were weak relationships between leaf and root trait syndromes (Craine and Lee 2003, Craine et al. 2005).

Many studies of plant functional traits have assumed that trait variation has arisen through adaptive evolution via selection for particular traits and combinations of traits in different environments (Westoby et al. 1995, Reich et al. 2003, Westoby and Wright 2006). However, treating species as independent data points when calculating correlations among traits may provide a misleading picture of the evolutionary correlations among traits (Felsenstein 1985). Phylogenetic comparative methods provide a way to test for correlated evolution of suites of functional traits (Harvey and Pagel 1991). If corresponding leaf and root traits of plants have evolved as part of an integrated ecological strategy, there should be evidence for correlated evolution of those traits taking phylogenetic relationships into account, but to date no study of leaf and root trait covariation in grassland plants has tested this idea directly.

Trait variation among and within communities

Some of the earliest studies of comparative plant physiology noted that leaf and root traits vary predictably among communities along environmental gradients (Schimper 1903). Early comparative studies of leaf and root traits often focused on traits related to plant growth form such as canopy height or rooting depth (Coupland and Johnson 1965). More recently research on trait variation among communities has focused on the set of leaf and to a lesser extent root traits related to species resource uptake strategies, since these traits also have the potential to influence species interactions and their effects on ecosystem processes (Diaz et al. 2004). Stress-tolerant species from habitats with lower water or nitrogen availability tend to possess a suite of traits including relatively small, thick leaves, thick roots, lower tissue density and nitrogen concentrations, longer tissue lifespans, lower instantaneous resource uptake and respiration rates, and higher resistance to mechanical damage and herbivory (Fonseca et al. 2000, Craine et al. 2001, Ackerly et al. 2002, McDonald et al. 2003, Diaz et al. 2004).

The magnitude of trait variation among co-occurring species is often similar to or greater than trait variation among communities (Wright et al. 2004), but very little is known about the patterns or causes of within-community trait variation. Following the terminology used in studies of species diversity (Whittaker 1960), the within- and among-community components of trait variation have been referred to as alpha and beta trait variation (Pickett and Bazzaz 1978, Wilson 1999, Silvertown 2004). While the concept of alpha and beta trait variation is inherently dependent on the scale used to define a local community, there is evidence that beta traits related to the habitat associations of species are broadly phylogenetically conserved in plants (Prinzing et al. 2001), while alpha traits related to the within-habitat niche position of species have been found to be phylogenetically conserved (Ackerly et al. 2006) or random (Silvertown et al. 2006). The relative magnitude of among- and within-community trait variation in grasslands, their potential effects on estimates of trait correlations among species and communities, and their evolutionary histories have not been studied.

Objectives

In this study, I use information on the abundances, leaf and root traits and phylogenetic relatedness of Alberta grassland plants growing in the field and in a common garden to address a set of hypotheses and questions. First, I measure the relative contributions of within- and among-community variation in leaf and root traits to overall patterns of trait variation in the field. Second, I describe patterns of trait covariation among species growing in the field and in a common garden, test for correlated evolution of leaf and root traits, and test for phylogenetic signal in the evolutionary history of these traits. Third, I describe variation of leaf and root traits along gradients of community composition and environment at three Alberta grassland sites.

Methods

Study sites

Temperate grasslands provide an ideal system in which to study variation in the leaf and root traits of plants, since the herbaceous nature of the dominant species would be expected to lead to differentiation of leaf and root traits related to resource uptake and stress tolerance (Reich et al. 2001, Craine et al. 2002, Tjoelker et al. 2005). Grassland plant communities at the northern fringe of the Great Plains in Alberta vary both along major gradients of climate and soil type among sites, as well as along local environmental gradients within sites (Coupland 1961). In general, precipitation increases and growing season evapotranspiration deficits decrease from south to north in the grassland and parkland natural regions, and soil types shift from brown to more productive black chernozems in the north (Chetner 2003). Grasslands in the south are dominated by dry mixedgrass plant communities, with grasses such as needle-and-thread (*Stipa comata)* and blue grama (*Bouteloua gracilis)* dominant (Coupland 1950), while the northern and western fringe of the grassland regions of the province are characterized by fescue-dominated plant communities with plains rough fescue (*Festuca hallii)*, porcupine grass (*Stipa spartea)* and bluegrass (*Poa pratensis)* the dominant grasses in the northern fescue and parkland regions (Moss and Campbell 1947, Coupland and Brayshaw 1953). Forb and shrub species make up a significant portion of the plant diversity in these grasslands, with the Asteraceae, Brassicaeae, and Fabaceae representing the major non-graminoid components of plant diversity.

I measured plant species abundances and leaf and root functional traits at three sites located in two of the major grassland community types in Alberta. The Kinsella site (53°05'N, 111°33'W) is a rough fescue-dominated native grassland in the central parkland natural region (Natural Regions Committee 2006). The Onefour (49°08'N, 110°31'W) and Hargrave sites (49°59'N, 110°02'W) are dry mixedgrass native grasslands, located in the dry mixedgrass natural region. The vegetation at all of these sites is representative of the native grassland communities described from the natural region including each site (Moss and Campbell 1947, Coupland 1950, Coupland and Brayshaw 1953, Coupland 1961). All sites are lightly to moderately grazed by cattle. Precipitation at these sites ranges from approximately 250-350mm/year at Onefour and Hargrave, to approximately 400-450mm/year at Kinsella (Chetner 2003). Growing season temperatures, wind speeds, and evapotranspiration deficits at the Onefour and Hargrave sites are also higher than at Kinsella (Natural Regions Committee 2006), leading to an overall trend of greater drought stress at mixedgrass sites.

Field sampling

During June and July of 2003 and 2004, I established from eight to ten 20m x 20m sampling plots at each site. Plots were distributed randomly along topographic gradients in order to sample the natural range of variation in plant community composition at each site. I recorded the identity of all angiosperm species present in ten 10 x 50cm quadrats scattered randomly within each plot. Species abundances within each plot were defined as the percentage of quadrats in that plot in which a species was present. Based on an examination of species accumulation curves, ten quadrats per plot and eight to ten plots per site was sufficient to capture the majority (>80%) of estimated total plant community diversity within plots and sites.

I recorded a qualitative estimate of the moisture regime in each plot (dry or mesic) relative to other plots at a site. The estimated moisture regime in a plot was based on my assessment of a number of factors, including the relative topographic position, slope, aspect and soil conditions in each plot. I used this estimate of moisture regime in place of direct measurements of variables such as slope and topographic position in order to provide a simple comparison of environmental conditions among the relatively small number of plots sampled at each site.

Field trait measurement

Species encountered in each plot were collected from the vicinity of the plot for leaf and root trait measurement. I collected at least one healthy mature plant of each species in each plot. Plants were collected in the morning, stored in plastic bags in a cooler and processed in the lab within 3 hours of collection. In order to allow measurement of fine root morphology, I excavated each plant with a portion of its root system intact by digging soil plugs measuring approximately 20cm diameter and 20cm deep, or deeper when necessary to obtain living fine root tissue from deep rooting or taprooted species.

Trait information was collected from each plant following published guidelines for leaf and root trait measurement (Cornelissen et al. 2003). For leaf traits, three mature leaves from each plant were scanned at 400dpi for image analysis of one-sided projected leaf area using WinFOLIA software (Regent Instruments Inc.), and the thickness of the lamina of each leaf was measured to the nearest 0.1mm using digital calipers. After measurement, leaves were dried for 72 hours at 70°C and weighed. Leaf trait information for two succulent species (*Opuntia polyacantha* and *Coryphantha vivipara*; Cactaceae) could not be collected, and these species were excluded from further trait analyses. These species were relatively rare (*Opuntia polyacantha*: 3 plots, *Coryphantha vivipara*: 1 plot) and never abundant when present.

For root traits, after washing each plant over a sieve to remove soil, I carefully extracted a sample of the fine root system of each plant, ensuring that roots of surrounding plants were removed during washing. I defined fine roots as living roots with diameter <2mm (Cornelissen et al. 2003). Fine roots were stored in a 70% ethanol solution and subsequently analyzed in a lab. Fine roots were floated in water in a plexiglass tray and scanned at 800dpi for image analysis of root length, volume, and average fine root diameter using WinRHIZO software (Regent Instruments Inc.). After scanning, fine roots were dried for 72 hours at 70°C and weighed.

In addition to direct measures of leaf area and leaf thickness for each leaf, I estimated specific leaf area (leaf area per unit biomass; cm^2/g) and specific leaf volume (1/leaf tissue density = leaf volume per unit biomass; mm^3/mg), with leaf volume calculated as the product of leaf thickness and area. Similarly, I used direct measures of fine root sample length, volume, and average diameter to estimate specific root length (root length per unit mass; m/g) and specific root volume (1/root tissue density = root volume per unit mass; mm^3/mg) for each plant.

Common garden growth conditions

I grew plants in a common garden in order to measure leaf and root traits for seedlings of plants grown under controlled conditions, and to obtain data on plant traits such as absolute growth rate and relative allocation to leaf and root tissue that could not be measured in the field. I obtained wild-collected seed for 50 Alberta grassland plant species, with 30 of these species also sampled in the field. Wild-collected seeds were obtained from a variety of sources, including collections at the three field sites (28 species) and a commercial seed provider (22 species; ALCLA Seeds Limited, Calgary). All seeds were cold stratified for two months at 4°C, followed by further stratification on moist filter paper for one week at 4°C prior to planting to promote synchronous germination. Seeds of species known to require seed coat scarification to promote germination were scarified prior to moist stratification using coarse grit sandpaper.

On June 7, 2004 I planted seeds of each species into ten 10cm wide pots containing a 3:1 sand:topsoil mix and 16 g/m^2 of Osmocote 14:14:14 NPK slow-release fertilizer. I thinned all pots to contain a single seedling within the first week of planting. Plants were grown outdoors in full sun at the University of Alberta Biotron, with daily watering to field capacity to ensure seedling survival.

Common garden trait measurement

All plants were harvested 75 days after planting. At harvest time, I measured the total leaf area, total fine root length, and total fine root volume for each plant using the methods described above for field-collected plants. Plants were separated into aboveground (leaf and stem) and belowground (root) tissue components, dried for three days at 70°C and weighed. I measured a subset of the derived variables estimated for field-collected plants, including specific leaf area (SLA), specific root length (SRL), specific root volume (SRV), and average fine root diameter. A number of other derived variables known to be related to the relative allocation of biomass to leaf and root tissues were estimated, including leaf area ratio (LAR; ratio of whole-plant leaf area to whole-plant biomass; cm^2/g), root length ratio (RLR; ratio of whole-plant fine root length to whole-plant biomass; m/g), leaf weight fraction (LWF; ratio of leaf biomass to whole-plant biomass), and root weight fraction (RWF; ratio of root biomass to whole-plant biomass).

Trait gradient analyses

I used the method of trait gradient analysis (Ackerly and Cornwell in press) to measure variation in leaf and roof functional traits among species growing in the field. Trait gradient analysis arranges communities along a gradient of mean community trait values, based on the abundance-weighted value of species' functional traits measured in each community. Following the terminology used in studies of species diversity (Whittaker 1960), the distribution of species along this trait gradient is partitioned into alpha (within-community) and beta (among-community) components of trait variation, This approach allowed us to evaluate the relative contribution of intraspecific trait variation to community-level shifts in functional trait values, and to estimate the relative contribution of within- versus among-community trait variation to the total variation in functional traits among species and communities.

Trait gradient analysis defines t_{sp} = the trait value of species s in plot p, a_{sp} = the abundance of species s in plot p, S = the total number of species, and P = the total number of plots. The abundance-weighted plot mean trait values and species mean trait values are defined, respectively, as:

$$\overline{t_p} = \sum_{s=1}^{S} a_{sp} t_{sp} \Big/ \sum_{s=1}^{S} a_{sp} \qquad (2.1)$$

$$\overline{t_s} = \sum_{p=1}^{P} a_{sp} t_{sp} \Big/ \sum_{p=1}^{P} a_{sp} \qquad (2.2)$$

The mean location of each species along the trait gradient is the abundance-weighted mean of $\overline{t_p}$ for plots containing the species:

$$\overline{t_{\beta s}} = \sum_{p=1}^{P} \overline{t_p} a_{sp} \Big/ \sum_{p=1}^{P} a_{sp} \qquad (2.3)$$

This is the beta trait value for the species, a measure of its position along the gradient of plot mean trait values.

A species' mean trait position relative to other species within plots is defined as:

$$\overline{t_{\alpha s}} = \overline{t_s} - \overline{t_{\beta s}} \qquad (2.4)$$

$\overline{t_{\alpha s}}$ is a measure of the alpha trait of the species (its trait value relative to that of co-occurring species). A species' mean trait value is equal to the sum of its alpha and beta trait values:

$$\overline{t_s} = \overline{t_{\beta s}} + \overline{t_{\alpha s}} \qquad (2.5)$$

I refer to mean trait values as 'total' trait values since they represent the combination of the among and within-community components of trait variation.

The breadth of species distributions along the community trait gradient are defined as R_s = the range of $\overline{t_p}$ values of plots occupied by each species. Intraspecific trait variation is then defined as b_s = the dimensionless slope of t_{sp} vs. $\overline{t_p}$ for species s based on an abundance-weighted regression. This slope expresses the relative shift in species traits relative to the shift in mean trait values among communities.

An example of a trait gradient analysis of leaf area in Alberta grassland plant communities is illustrated in Figure 2-1. The species *Galium boreale* (Rubiaceae) occurs in communities with a relatively high mean leaf area ($t_{\beta s} = 0.36$), but its leaves are small relative to the species it occurs with ($t_{\alpha s} = -0.71$), and its leaf area changed less among communities than the plot mean trait values did along the trait gradient ($b_s = 0.30$).

Field trait variation – alpha, beta and total components

I conducted trait gradient analyses for all leaf and roof functional traits measured in the field using published scripts (Ackerly and Cornwell in press) for the R statistical language (R Development Core Team 2006). The trait gradient analysis was based on the field-collected data on the abundances of species within sample plots, and the leaf and root traits of the species collected in each plot (leaf area, leaf thickness, specific leaf area, specific leaf volume, root diameter, specific root length, specific root volume). In a few cases, due to local rarity or extremely deep taproots, I was unable to collect trait information for a species encountered in a plot, in which case I substituted the mean trait values for that species based on collections from other plots at a site. All trait values were $\log_{10}$-transformed prior to analysis. Trait gradient analyses were based on abundance-weighted trait means. I repeated the trait analyses with non-abundance-weighted data and the results were extremely similar; I present only the results of the abundance-weighted analyses here.

Common garden trait variation

I calculated mean trait values for all common garden species with at least three plants surviving to harvest time (40 out of 50 planted species), and used these values for subsequent analyses of trait relationships among common garden species.

Phylogenetic data

I obtained a phylogenetic hypothesis for the 94 species included in this study based on a published phylogenetic supertree of angiosperm families (Davies et al. 2004), which was constructed using supertree analysis of data from 42 published studies of angiosperm phylogeny, along with data on molecular variation of three genes (*atpB*, *rbcL*, and 18S rDNA) among angiosperm families, with nonparametric rate smoothing of rates of *rbcL* change and fossil taxon age calibration used to estimate divergence dates for all nodes in the tree. While nonparametric rate smoothing is sensitive to the choice of calibration points and may overfit the data (Sanderson 2003), the Davies et al. (2004) tree was the most comprehensive angiosperm supertree for which branch length estimates were available. Species included in the present study were grafted onto the Davies et al. (2004) strict consensus supertree using Phylomatic (Webb and Donoghue 2005), a toolkit and database for automated phylogenetic supertree construction. Within-family phylogenetic relationships were resolved based on a variety of published phylogenetic trees (Asteraceae (Baldwin et al., Noyes and Rieseberg 1999, Beck et al. 2004), Brassicaeae (Al-Shehbaz et al. 2006), Fabaceae (Wojciechowski et al. 2000), Poaceae (Salamin 2002), Rosaceae (Eriksson et al. 2003)). Nodes in the resulting tree that were not dated directly (e.g. genera within families) were spaced evenly between dated nodes to minimize tree-wide variance in branch lengths. I did not assess the effects of uncertainty in the topology or branch lengths of the resulting tree directly, but analyses based on a different tree (Angiosperm Phylogeny Group 2003) and branch lengths (Wikstrom et al. 2001) were extremely similar to those obtained from analyses based on the Davies et al. (2004) tree. I therefore present only the results of analyses based on the Davies et al. supertree.

Ahistorical and PIC analyses of leaf and root trait covariation

I summarized patterns of trait covariation in the field and common garden using two approaches. The first involved calculation of pairwise correlations among species traits as measured in the field and common garden, and principal components analyses (PCA) of trait variation to identify suites of correlated traits. I refer to these analyses as ahistorical analyses, since they treat each species as an independent data point, ignoring the evolutionary relationships among species. The second approach involved testing for correlated evolution of traits, based on pairwise correlations and principal components analyses of phylogenetically independent contrasts (PIC) of traits at nodes on the phylogeny, taking into account the phylogenetic relationships among species. I refer to these analyses as PIC analyses.

The method of phylogenetically independent contrasts has been proposed as a solution to the problem of non-independence of species due to their shared evolutionary history (Felsenstein 1985). Assuming a Brownian motion model of trait evolution, contrasts in trait values among descendents of each node in a phylogeny are statistically independent, and correlations between standardized contrasts can be used to test for correlated evolution. Nodal contrasts for all traits were estimated as the divergence among the trait values of descendants of each node in a phylogeny, with raw contrasts standardized by the square root of the branch length separating each node from its daughters (Garland et al. 1992). Contrasts were calculated using the *aot* module of Phylocom version 3.40 software (Webb et al. 2006). I examined diagnostic plots of absolute contrasts versus standard deviations and found that they were generally unrelated, and were not improved by various transformations of branch lengths (branch lengths square root or log transformed, or all branch lengths set to equal length), supporting my use of estimated divergence dates as branch lengths in this study (Garland et al. 1992).

I tested separately for ahistorical and PIC relationships among the alpha, beta and total (mean) traits of species using pairwise correlations among traits and contrasts, along with principal components analyses (PCA) based on correlation matrices of scaled traits and contrasts to summarize multivariate relationships among traits and contrasts. I repeated these ahistorical and PIC analyses for the traits of species grown in the common garden. I also tested for intraspecific relationships between the alpha and beta components of variation in each trait. Finally, I measured the variance in ahistorical mean trait values in the field explained by the alpha and beta components of trait variation.

Comparisons between field and common garden trait measurements

To test whether trait values measured on mature plants in the field versus seedlings grown in a common garden were comparable, I calculated correlations between species trait values measured in the field (alpha, beta and total) and common garden for the four traits and 26 species included in both data sets.

Phylogenetic signal

I tested for non-random patterns in the evolution of traits by calculating a measure of phylogenetic signal for alpha, beta and total components of each trait measured in the field, and for trait means measured in the common garden. Phylogenetic signal was defined as a tendency for species to be more or less similar to their relatives than expected by chance, with phylogenetically conserved traits tending to have lower tree-wide average contrast values due to similarity of close relatives, and phylogenetically convergent traits having high tree-wide average contrast values, relative to contrasts calculated after random rearrangements of trait values across the tips of the phylogeny (Blomberg et al. 2003). I calculated mean contrasts and a two-tailed *P*-value to quantify the phylogenetic signal of each trait based on a comparison of observed mean contrasts with the distribution of mean contrasts calculated for 999 random reshufflings of species across the phylogeny, using the aot module of Phylocom version 3.40 software (Webb et al. 2006).

Community gradient analyses

I summarized variation in species composition among communities at all sites using a nonmetric multidimensional scaling (NMS) ordination of species abundances in plots using vegan version 1.8 software (Oksanen et al. 2006). Similarity among plots was measured using the Sørenson distance metric. I conducted a number of data standardizations as recommended for analysis of community data, including deleting species occurring in fewer than 3 plots prior to calculating distances, square root transformation of abundances, and principal components rotation of the resulting NMS solution (McCune and Grace 2002). The ordinations converged on a stable two-dimensional solution from ten random starting points (final stress = 8.2), with 98% of the variation in multivariate distances among plots explained by the distance separating plots on the two NMS axes.

I measured the variation in community composition among sites (Kinsella, Onefour, Hargrave) and plot moisture regimes (dry, dry-mesic, mesic) explained by the two NMS axes using the formula $R^2 = 1 -$ (Within-group NMS scores Sums of Squares / Among-group NMS scores Sums of Squares) (McCune and Grace 2002), with *P*-values calculated based on random shuffling of grouping labels among plots.

Relationships between community and trait gradients

To determine whether trait values varied predictably among grassland plant communities arranged based on their species composition, I tested for correlations between abundance-weighted community mean trait values ($\overline{t_p}$) versus plot scores on the

NMS axes. Similarly, I tested for correlations between species mean trait values ($\bar{t}_s$) and their scores on the NMS axes.

Results

Trait variation in the field – alpha and beta components

The magnitude of within-community variation in leaf and root traits was much higher than among-community variation, with the variation in alpha trait values of species ranging from approximately three to ten times greater than variation in beta trait values (Table 2-1). Alpha trait values explained from 90% to 98% of the overall variation in species mean trait values, while beta trait values explained from 1% to 17% of the variation in mean trait values (Table 2-2). The alpha and beta trait values of individual species were generally unrelated based on tests for ahistorical alpha versus beta trait correlations (all $r < 0.13$, $N = 76$, $P > 0.25$), except that species with relatively high root diameters within communities tended to occur in communities in which abundance weighted mean root diameter was higher (Ahistorical root diameter alpha vs. beta correlation: $r = 0.21$, $N=76$, $P = 0.04$).

Patterns of intraspecific trait variation generally mirrored patterns of interspecific trait variation among communities arranged along trait gradients (Figure 2-2). Average intraspecific trait slopes (b_s) for species occurring in at least 5 plots were not significantly different from 1 (t-tests, df=29, all $P > 0.4$), with the exception of leaf area, which varied less within species than among communities arranged along the trait gradient (leaf area b_s (± s.e.) = 0.17 ± 0.13; t = -6.5, df = 29, $P < 0.001$).

Phylogenetic signal

Alpha, beta and total components of trait variation in the field showed differing degrees of phylogenetic signal (Table 2-3). In general, beta traits did not exhibit phylogenetic signal, while most alpha traits and mean trait values were phylogenetically conserved, due to the presence of several large clades including the Poales (grasses and sedges), Asteraceae and Fabaceae each possessing characteristic suites of leaf and root traits (Figure 2-3). However, there was no phylogenetic signal in SLA variation, and all components of SRL variation showed patterns of phylogenetic convergence due to species with high and low SRL arising repeatedly across the angiosperm phylogeny. Phylogenetic signal for traits measured on species in the common garden were more consistent, with all measured traits except SRL showing patterns of phylogenetic conservatism (Table 2-3).

Trait tradeoffs – ahistorical correlations

Based on ahistorical analyses of species alpha trait values, the primary axis of leaf and root trait variation among co-occurring species in the field (Figure 2-4) separated species with large leaves, thick roots and low SLA and SRL from those with the opposite set of traits (PCA axis 1: 33% of variation). A second axis of variation among species separated species with thick leaves and low-density leaf and root tissue from those with the opposite set of traits (PCA axis 2: 27% of variation). Patterns of total trait variation in the field ($\bar{t}_s$) were very similar to relationships among alpha trait values of species due to the strong correlations between alpha and total trait values (Table 2-4).

The primary axis of among-community trait variation (beta traits; Figure 2-4) separated species growing in communities occupied by plants with high SRL and SRV, thin leaf and root tissue and low SLV from those growing in communities containing species with the opposite set of traits.(PCA axis 1: 36% of variation). The second major gradient of trait variation among communities was between communities with high mean leaf area, SLA and SLV and those containing plants with small leaves with low SLA and SLV (PCA axis 2: 28% of variation).

Trait tradeoffs – PIC correlations

PIC correlations among beta traits were very similar to ahistorical correlations (Table 2-4), but PIC correlations among alpha and total leaf and root traits differed from ahistorical correlations in a number of ways. The primary trend in the evolution of traits of co-occurring species (Figure 2-5) was a tradeoff between thick roots versus low density roots with high SRL (PCA axis 1: 30% of variation). The second major axis of trait evolution was a tradeoff between large, thick leaves with low SLA versus the opposite set of leaf traits (PCA axis 2: 25% of variation). A third major axis of variation was a pattern of correlated evolution of leaves with high SLA and SLV (PCA axis 3: 22%

of variation). As in the ahistorical analyses, patterns of correlated evolution were extremely similar between species alpha and total traits (Table 2-4).

Common garden trait variation

Traits of plants grown in a common garden (Table 2-5, Figure 2-6) varied primarily along an axis separating species with high absolute growth rates, low SRV, high total leaf area and root length, low relative allocation to leaf biomass (LWF) and low leaf and root absorptive area/length per unit plant biomass (LAR/RLR), from those with the opposite set of traits (PCA axis 1: 40% of variation). A second major axis of trait variation separated species with high SLA and SRL, high leaf and root absorptive area/length per unit plant biomass (LAR/RLR), high total leaf area and root length and thin roots with high SRV from those with the opposite set of traits (20% of variation).

The main trend in PIC correlations among traits measured in the common garden was very similar to the ahistorical pattern (Table 2-5, Figure 2-6), namely a correlation between high absolute growth rate, total leaf area and root length, and low LAR, RLR, LWF and SRV (PCA axis 1: 42% of variation). The second major axis involved the correlated evolution of high relative allocation to root length and biomass per unit whole plant biomass (RLR and RWF), high SLA and SRL, and thin roots (PCA axis 2: 18% of variation).

Comparisons between field and common garden traits

In a comparison of all measured traits of 26 species sampled in both the field and common garden, there was no relationship between the value of a species' trait measured in common garden versus beta traits measured in the field (Table 2-6). There was no relationship between SLA measured in the field and common garden, but alpha and total root traits measured in the field were positively correlated with traits measured in the common garden.

Community gradient analyses

The main gradient of variation in species composition among communities was among sites from different natural regions, with plots from sites in mixedgrass versus fescue grassland regions clearly separated along the first axis of the NMS ordination (Figures 2-7 and 2-8). The two dry mixedgrass region sites (Onefour and Hargrave) contained very similar plant communities, which were distinct from the communities found at the fescue region site (Kinsella). Within each site type, communities were further segregated along a gradient from relatively dry to relatively mesic plots, with estimated moisture regime shifting from mesic to dry with increasing plot scores on the second NMS axis. Site and moisture regime of plots explained 79% and 17% of the variation in plot scores on the NMS axes, respectively (Figure 2-6).

Relationships between community and trait gradients

The abundance weighted mean leaf area of communities in fescue grasslands was higher than mixedgrass grasslands, and community mean SRV and SRL decreased along the gradient from mesic to dry plots (Figure 2-9, Table 2-7). Abundance weighted mean leaf area in plots was positively correlated with plot scores on the first NMS axis, while plot mean SRV and to a lesser extent SRL decreased with increasing plot scores along the second NMS axis.

Species abundant in fescue grasslands had larger and thinner leaves with high SLA, and species mean SLA and SRV decreased along the mesic-dry plot gradient (Figure 2-10, Table 2-7). Species scores on the first NMS axis were positively correlated with species mean SLA and leaf area and negatively correlated with leaf thickness, while species scores on the second axis were negatively correlated with both SLA and SRV.

Discussion

Trait variation among and within communities

Most leaf and root trait variation in Alberta grasslands was explained by variation among species occurring together in local communities, rather than by variation among communities. This result is in contrast to other studies which have generally found that while traits vary a great deal at any given site, the magnitude and predictability of trait variation is greater among communities (Wright et al. 2004, Ackerly and Cornwell in press). However, these studies have primarily focused on communities spanning a much broader range of habitat types and environmental conditions than the present study. My results emphasize that alpha and beta trait variation will be highly dependent on the spatial and environmental scale and extent used to define communities, and that it may be premature to make generalizations about the causes of among and within community trait variation in different ecosystems.

Phenotypic plasticity or local adaptation contributed to variation in trait values among plots, with all traits except leaf area changing on average at the same rate within species and among plots arranged along a trait gradient (Figure 2-2), which suggests that differences in environmental conditions among plots have similar effects on within- and among-species trait variation. The causes of trait variation and covariation within communities are less clear. Alpha and beta trait variation may be the result of fundamentally different processes, with among-community beta trait variation generally thought to be caused by environmental filtering, adaptation or plasticity in response to predictably different habitats and environmental conditions, while within-community alpha trait variation may be caused by mechanisms such as niche partitioning of spatially and temporally heterogeneous resources, limiting similarity, or other frequency dependent processes that promote species coexistence (Chesson 2000, Hubbell 2001, Reich et al. 2003, Silvertown 2004).

Ahistorical and PIC correlations among traits

The main spectrum of trait variation among species in the field was similar to patterns reported in other temperate grasslands. Co-occurring species could be differentiated based on whether they possessed large, thick leaves and thick roots versus thin leaves and roots, and also based on whether they possessed high SLA and SRL roots with low tissue density, or the opposite set of traits (Table 2-4). Most leaf and root alpha traits were highly phylogenetically conserved. Relative to other species, grasses as a group tended to possess thin, high density roots and thin leaves, while many Asteraceae, Liliaceae and Fabaceae possessed relatively large, thick leaves and thick roots. These differences in the characteristic leaf and root morphology of different clades were largely responsible for the ahistorical pattern of correlations between suites of leaf and root traits (Kembel and Cahill 2005).

Conversely, PIC analyses of trait relationships indicated that the main trends of correlated trait evolution were a series of independent tradeoffs among leaf and root traits (Table 2-4). The evolution of thicker roots was accompanied by decreases in SRL. Increases in leaf thickness were accompanied by increases in leaf area and tissue density, while increases in specific leaf area were accompanied by decreases in leaf tissue density. Variation of root and leaf traits along these three axes of evolutionary change were largely independent, although the evolution of larger, thicker leaves was accompanied by increases in root diameter. Differential above- and belowground effects of environmental constraints such as soil freezing and drought (Craine et al. 2005), herbivory, or competition (Cahill 1999) may have imposed a different set of evolutionary pressures on leaf and root functional traits. This study provides a clear example of how incorporating information on the evolutionary relatedness of species can dramatically change the interpretation of the adaptive significance of trait correlations.

Functionally equivalent structures may arise in numerous ways in different lineages (Marks and Lechowicz 2006), and plants in Alberta grasslands have developed high SLA leaves in two evolutionarily unrelated ways. One is through decreased tissue density (higher SLV), the other is through decreased leaf thickness. There was also an unrelated evolutionary trend of larger leaves tending to be thicker and have lower tissue density. In general, species occurring in fescue grasslands had larger leaves (Figure 2-10), and it is possible that adaptation to broad climate gradients versus within-site environmental variation may exert different selection pressures on leaf and root functional traits.

It remains unclear whether the phylogenetic conservatism I observed has an adaptive basis due to direct or indirect selection on leaf and root traits, or is the result of biophysical, developmental, or phylogenetic constraints. For example, grasses as a group possess a number of shared derived characters in addition to thin leaves and high density roots, such as wind pollination and a sympodial habit (Linder and Rudall 2005). One could attribute the dominance of grasses in grasslands to these or any number of other traits, but the use of phylogenetic comparative methods allows hypotheses regarding the correlated evolution of these traits to be tested directly rather than assumed. Based on fossil and molecular evidence, the radiation and functional diversification of the grasses appears to have predated the origin of the northern temperate grassland biome (Linder and Rudall 2005, Stromberg 2005). Similar patterns of trait variation predating radiation into novel habitats are found across a wide range of clades (Ackerly 2004, Ackerly et al. 2006), which further suggests it would be erroneous to attribute all present-day differences in leaf and root traits among species in Alberta grasslands to coordinated leaf and root evolution.

Field versus common garden trait relationships and measurements

The primary axis of ahistorical and evolutionary trait variation among species grown in a common garden corresponds to the primary axes of variation among plant species found in many ecosystems, namely the stress-related continuum from a strategy of rapid growth and acquisition of resources to a strategy of slow growth and retention of acquired resources in well protected tissues (Cavender-Bares et al. 2004, Diaz et al. 2004). A second axis of correlated SLA and SRL evolution differed from the pattern of no evolutionary correlation between leaf and root traits observed in the field.

There are numerous explanations for the differences I observed between field and common garden trait syndromes. First, they may be due to differences in life stage between field and common garden. Seedlings and mature plants of the same species may possess a different suite of traits, due to conflicting selection pressures at different life stages (Grime et al. 1997). Second, I did not measure an identical suite of traits in the field and common garden. It was not possible to measure traits related to growth and relative allocation to leaf and root tissue on mature plants in the field, and variation in these traits among common garden plants was orthogonal to the variation in field-measured traits such as SLA, SRL and root diameter (Figure 2-6). Third, I did not measure exactly the same species in the field and common garden, although 26 species were studied in both locations. Finally, the lack of correlation between individual traits measured in the field and common garden may also be part of the explanation. Regardless of the cause of the difference, this finding has important implications for trait screening programmes that attempt to measure traits on greenhouse-grown seedlings and apply them to plants growing in natural ecosystems. While traits such as SLA and SRL have been suggested as valuable surrogate measures of plant ecological strategies, it appears that the variability of SLA and related traits makes it difficult to compare traits measured on plants in different locations or at different life stages (Dyer et al. 2001, Garnier et al. 2001).

Trait versus community gradients

Based on theoretical and empirical evidence, the predicted direction of trait changes between fescue sites and drier, less productive mixedgrass sites, and between mesic and dry communities at each site, would be a decrease in leaf area, increase in leaf and root thickness, increase in tissue density and resulting decrease in SRL and SLA (Craine et al. 2001, Westoby and Wright 2006). Observed patterns of trait variation among sites and along gradients of estimated moisture availability were generally in accordance with these predictions (Table 2-7), although relationships between trait values and community composition were fairly weak and inconsistent. It must be acknowledged that my measurements of within-site moisture availability were very coarse and subjective, and a number of unmeasured environmental factors such as grazing, nitrogen availability, or soil texture could also be responsible for the patterns I observed. Within-site variation in traits did appear to be driven more by differences between rough fescue dominated (*Festuca-Poa*) communities and porcupine grass dominated (*Stipa-Bouteloua*) communities at Kinsella, than by trait variation among communities at the two mixedgrass sites (Figure 2-10).

Despite differences in species abundances and overall community composition along environmental gradients in Alberta grasslands, there were relatively few relationships between community composition and the mean leaf and root traits of communities, but more consistent differences in the traits of individual species characteristic of different community types (Table 2-7). The highly phylogenetically conserved nature of most species' alpha traits, coupled with the relatively low amount of variation in beta traits and lack of phylogenetic signal in beta traits, could explain the small overall difference in mean trait values among communities that have been colonized by different species from the same few dominant clades.

Literature Cited

Ackerly, D. D. 2004. Adaptation, niche conservatism, and convergence: Comparative studies of leaf evolution in the California chaparral. American Naturalist **163**:654-671.

Ackerly, D. D., C. A. Knight, S. B. Weiss, K. Barton, and K. P. Starmer. 2002. Leaf size, specific leaf area and microhabitat distribution of chaparral woody plants: contrasting patterns in species level and community level analyses. Oecologia **130**:449-457.

Ackerly, D. D., D. W. Schwilk, and C. O. Webb. 2006. Niche evolution and adaptive radiation: testing the order of trait divergence. Ecology **87**:S50-61.

Al-Shehbaz, I. A., M. A. Beilstein, and E. A. Kellogg. 2006. Systematics and phylogeny of the Brassicaceae (Cruciferae): an overview. Plant Systematics and Evolution **259**:89-120.

Angiosperm Phylogeny Group. 2003. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. Botanical Journal of the Linnean Society **141**:399-436.

Baldwin, B. G., B. L. Wessa, and J. L. Panero. 2002. Nuclear rDNA Evidence for Major Lineages of helenioid Heliantheae (Compositae). Systematic Botany **27**:161-198.

Beck, J. B., G. L. Nesom, P. J. Calie, G. I. Baird, R. L. Small, and E. E. Schilling. 2004. Is subtribe Solidagininae (Asteraceae) monophyletic? Taxon **53**:691-698.

Blomberg, S. P., T. Garland, and A. R. Ives. 2003. Testing for phylogenetic signal in comparative data: Behavioral traits are more labile. Evolution **57**:717-745.

Cahill, J. F. 1999. Fertilization effects on interactions between above- and belowground competition in an old field. Ecology **80**:466-480.

Cavender-Bares, J., K. Kitajima, and F. A. Bazzaz. 2004. Multiple trait associations in relation to habitat differentiation among 17 Floridian oak species. Ecological Monographs **74**:635-662.

Chesson, P. 2000. Mechanisms of maintenance of species diversity. Annual Review of Ecology and Systematics **31**:343-366.

Chetner, S. 2003. Agroclimatic atlas of Alberta, 1971 - 2000. Alberta Agriculture, Food and Rural Development, Edmonton, AB.

Comas, L. H., and D. M. Eissenstat. 2004. Linking fine root traits to maximum potential growth rate among 11 mature temperate tree species. Functional Ecology **18**:388-397.

Cornelissen, J. H. C., S. Lavorel, E. Garnier, S. Diaz, N. Buchmann, D. E. Gurvich, P. B. Reich, H. ter Steege, H. D. Morgan, M. G. A. van der Heijden, J. G. Pausas, and H. Poorter. 2003. A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. Australian Journal of Botany **51**:335-380.

Coupland, R. T. 1950. Ecology of mixed prairie in Canada. Ecological Monographs **20**:273-315.

Coupland, R. T. 1961. A Reconsideration of grassland classification in the Northern Great Plains of North America. Journal of Ecology **49**:135-167.

Coupland, R. T., and T. C. Brayshaw. 1953. The fescue grassland in Saskatchewan. Ecology **34**:386-405.

Coupland, R. T., and R. E. Johnson. 1965. Rooting characteristics of native grassland species in Saskatchewan. Journal of Ecology **53**:475-507.

Craine, J. M., J. Froehle, D. G. Tilman, D. A. Wedin, and F. S. Chapin. 2001. The relationships among root and leaf traits of 76 grassland species and relative abundance along fertility and disturbance gradients. Oikos **93**:274-285.

Craine, J. M., and W. G. Lee. 2003. Covariation in leaf and root traits for native and non-native grasses along an altitudinal gradient in New Zealand. Oecologia **134**:471-478.

Craine, J. M., W. G. Lee, W. J. Bond, R. J. Williams, and L. C. Johnson. 2005. Environmental constraints on a global relationship among leaf and root traits of grasses. Ecology **86**:12-19.

Craine, J. M., D. Tilman, D. Wedin, P. Reich, M. Tjoelker, and J. Knops. 2002. Functional traits, productivity and effects on nitrogen cycling of 33 grassland species. Functional Ecology **16**:563-574.

Davies, T. J., T. G. Barraclough, M. W. Chase, P. S. Soltis, D. E. Soltis, and V. Savolainen. 2004. Darwin's abominable mystery: Insights from a supertree of the angiosperms. Proceedings of the National Academy of Sciences **101**:1904-1909.

Diaz, S., J. G. Hodgson, K. Thompson, M. Cabido, J. H. C. Cornelissen, A. Jalili, G. Montserrat-Marti, J. P. Grime, F. Zarrinkamar, Y. Asri, S. R. Band, S. Basconcelo, P. Castro-Diez, G. Funes, B. Hamzehee, M. Khoshnevi, N. Perez-

Harguindeguy, M. C. Perez-Rontome, F. A. Shirvany, F. Vendramini, S. Yazdani, R. Abbas-Azimi, A. Bogaard, S. Boustani, M. Charles, M. Dehghan, L. de Torres-Espuny, V. Falczuk, J. Guerrero-Campo, A. Hynd, G. Jones, E. Kowsary, F. Kazemi-Saeed, M. Maestro-Martinez, A. Romo-Diez, S. Shaw, B. Siavash, P. Villar-Salvador, and M. R. Zak. 2004. The plant traits that drive ecosystems: Evidence from three continents. Journal of Vegetation Science **15**:295-304.

Dyer, A. R., D. E. Goldberg, R. Turkington, and C. Sayre. 2001. Effects of growing conditions and source habitat on plant traits and functional group definition. Functional Ecology **15**:85-95.

Eissenstat, D. M., and R. D. Yanai. 1997. The ecology of root lifespan. Advances in Ecological Research **27**:1-60.

Eriksson, T., M. S. Hibbs, A. D. Yoder, C. F. Delwiche, and M. J. Donoghue. 2003. The phylogeny of Rosoideae (Rosaceae) based on sequences of the internal transcribed spacers (ITS) of nuclear ribosomal DNA and the *trnL/F* region of chloroplast DNA. International Journal of Plant Sciences **164**:197-211.

Felsenstein, J. 1985. Phylogenies and the comparative method. American Naturalist **125**:1-15.

Fitter, A. 2002. Characteristics and functions of root systems. Pages 15-32 *in* Y. Waishel, A. Eshel, and U. Kafkafi, editors. Plant roots: the hidden half. Marcel Dekker, New York, NY.

Fonseca, C. R., J. M. Overton, B. Collins, and M. Westoby. 2000. Shifts in trait-combinations along rainfall and phosphorus gradients. Journal of Ecology **88**:964-977.

Garland, T., P. H. Harvey, and A. R. Ives. 1992. Procedures for the analysis of comparative data using phylogenetically independent contrasts. Systematic Biology **41**:18-32.

Garnier, E., G. Laurent, A. Bellmann, S. Debain, P. Berthelier, B. Ducout, C. Roumet, and M. L. Navas. 2001. Consistency of species ranking based on functional leaf traits. New Phytologist **152**:69-83.

Grime, J. P., K. Thompson, R. Hunt, J. G. Hodgson, J. H. C. Cornelissen, I. H. Rorison, G. A. F. Hendry, T. W. Ashenden, A. P. Askew, S. R. Band, R. E. Booth, C. C.

Bossard, B. D. Campbell, J. E. L. Cooper, A. W. Davison, P. L. Gupta, W. Hall, D. W. Hand, M. A. Hannah, S. H. Hillier, D. J. Hodkinson, A. Jalili, Z. Liu, J. M. L. Mackey, N. Matthews, M. A. Mowforth, A. M. Neal, R. J. Reader, K. Reiling, W. RossFraser, R. E. Spencer, F. Sutton, D. E. Tasker, P. C. Thorpe, and J. Whitehouse. 1997. Integrated screening validates primary axes of specialisation in plants. Oikos **79**:259-281.

Harvey, P. H., and M. D. Pagel. 1991. The comparative method in evolutionary biology. Oxford University Press, Oxford, U.K.

Hubbell, S. P. 2001. The unified neutral theory of biogeography and biodiversity. Princeton University Press, Princeton, NJ.

Kembel, S. W., and J. F. Cahill. 2005. Plant phenotypic plasticity belowground: A phylogenetic perspective on root foraging trade-offs. American Naturalist **166**:216-230.

Linder, H. P., and P. J. Rudall. 2005. Evolutionary history of Poales. Annual Review of Ecology Evolution and Systematics **36**:107-124.

Marks, C. O., and M. J. Lechowicz. 2006. Alternative designs and the evolution of functional diversity. American Naturalist **167**:55-66.

McCune, B., and J. B. Grace. 2002. Analysis of ecological communities. MjM Software Design, Oregon.

McDonald, P. G., C. R. Fonseca, J. M. Overton, and M. Westoby. 2003. Leaf-size divergence along rainfall and soil-nutrient gradients: is the method of size reduction common among clades? Functional Ecology **17**:50-57.

McGill, B. J., B. J. Enquist, E. Weiher, and M. Westoby. 2006. Rebuilding community ecology from functional traits. Trends in Ecology and Evolution **21**:178-185.

Moss, E. H., and J. A. Campbell. 1947. The fescue grassland of Alberta. Canadian Journal of Research **25**:209–227.

Natural Regions Committee. 2006. Natural regions and subregions of Alberta. Publication Number T/852, Government of Alberta.

Noyes, R. D., and L. H. Rieseberg. 1999. ITS sequence data support a single origin for North American Astereae (Asteraceae) and reflect deep geographic divisions in Aster s.l. Am. J. Bot. **86**:398-412.

Oksanen, J., R. Kindt, P. Legendre, and R. B. O'Hara. 2006. vegan: Community ecology package for R. Version 1.8.

Pickett, S. T. A., and F. A. Bazzaz. 1978. Organization of an assemblage of early successional species on a soil moisture gradient. Ecology **59**:1248-1255.

Pregitzer, K. S., J. L. DeForest, A. J. Burton, M. F. Allen, R. W. Ruess, and R. L. Hendrick. 2002. Fine root architecture of nine North American trees. Ecological Monographs **72**:293-309.

Prinzing, A., W. Durka, S. Klotz, and R. Brandl. 2001. The niche of higher plants: evidence for phylogenetic conservatism. Proceedings of the Royal Society of London, Series B: Biological Sciences **268**:2383-2389.

R Development Core Team. 2006. R: A Language and Environment for Statistical Computing.

Reich, P. B., D. Tilman, J. Craine, D. Ellsworth, M. G. Tjoelker, J. Knops, D. Wedin, S. Naeem, D. Bahauddin, J. Goth, W. Bengtson, and T. D. Lee. 2001. Do species and functional groups differ in acquisition and use of C, N and water under varying atmospheric CO_2 and N availability regimes? A field test with 16 grassland species. New Phytologist **150**:435-448.

Reich, P. B., M. G. Tjoelker, M. B. Walters, D. W. Vanderklein, and C. Bushena. 1998. Close association of RGR, leaf and root morphology, seed mass and shade tolerance in seedlings of nine boreal tree species grown in high and low light. Functional Ecology **12**:327-338.

Reich, P. B., M. B. Walters, and D. S. Ellsworth. 1997. From tropics to tundra: Global convergence in plant functioning. Proceedings of the National Academy of Sciences of the United States of America **94**:13730-13734.

Reich, P. B., I. J. Wright, J. Cavender-Bares, J. M. Craine, J. Oleksyn, M. Westoby, and M. B. Walters. 2003. The evolution of plant functional variation: Traits, spectra, and strategies. International Journal of Plant Sciences **164**:S143-S164.

Ryser, P. 1996. The importance of tissue density for growth and life span of leaves and roots: A comparison of five ecologically contrasting grasses. Functional Ecology **10**:717-723.

Ryser, P., and H. Lambers. 1995. Root and leaf attributes accounting for the performance of fast-growing and slow-growing grasses at different nutrient supply. Plant and Soil **170**:251-265.

Salamin, N. 2002. Building supertrees: An empirical assessment using the grass family (Poaceae). Systematic Biology **51**:136-150.

Sanderson, M. J. 2003. Molecular data from 27 proteins do not support a Precambrian origin of land plants. American Journal of Botany **90**:954-956.

Schimper, A. F. W. 1903. Plant geography on a physiological basis. Clarendon Press, Oxford, U.K.

Silvertown, J. 2004. Plant coexistence and the niche. Trends in Ecology and Evolution **19**:605-611.

Silvertown, J., M. Dodd, D. Gowing, C. Lawson, and K. McConway. 2006. Phylogeny and the hierarchical organization of plant diversity. Ecology **87**:S39-S49.

Southwood, T. R. E. 1977. Habitat, the templet for ecological strategies? Journal of Animal Ecology **46**:336-365.

Stromberg, C. A. E. 2005. Decoupled taxonomic radiation and ecological expansion of open-habitat grasses in the Cenozoic of North America. Proceedings of the National Academy of Sciences **102**:11980-11984.

Tjoelker, M. G., J. M. Craine, D. Wedin, P. B. Reich, and D. Tilman. 2005. Linking leaf and root trait syndromes among 39 grassland and savannah species. New Phytologist **167**:493-508.

Wahl, S., and P. Ryser. 2000. Root tissue structure is linked to ecological strategies of grasses. New Phytologist **148**:459-471.

Webb, C. O., D. D. Ackerly, and S. W. Kembel. 2006. Phylocom: software for the analysis of community phylogenetic structure and character evolution. Version 3.40. http://www.phylodiversity.net/phylocom/.

Webb, C. O., and M. J. Donoghue. 2005. Phylomatic: tree assembly for applied phylogenetics. Molecular Ecology Notes **5**:181-183.

Westoby, M., D. S. Falster, A. T. Moles, P. A. Vesk, and I. J. Wright. 2002. Plant ecological strategies: Some leading dimensions of variation between species. Annual Review of Ecology and Systematics **33**:125-159.

Westoby, M., M. R. Leishman, and J. M. Lord. 1995. On misinterpreting the phylogenetic correction. Journal of Ecology **83**:531-534.

Westoby, M., and I. J. Wright. 2006. Land-plant ecology on the basis of functional traits. Trends in Ecology and Evolution **21**:261-268.

Whittaker, R. H. 1960. Vegetation of the Siskiyou Mountains, Oregon and California. Ecological Monographs **30**:279-338.

Wikstrom, N., V. Savolainen, and M. W. Chase. 2001. Evolution of the angiosperms: calibrating the family tree. Proceedings of the Royal Society of London, Series B: Biological Sciences **268**:2211-2220.

Wilson, J. B. 1999. Guilds, functional types and ecological groups. Oikos **86**:507-522.

Wojciechowski, M. F., M. J. Sanderson, K. P. Steele, and A. Liston. 2000. Molecular phylogeny of the temperate herbaceous tribes of Papilionoid legumes: a supertree approach. Pages 277-298 *in* P. S. Herendeen and A. Bruneau, editors. Advances in Legume Systematics. Royal Botanic Gardens, Kew.

Wright, I. J., P. B. Reich, M. Westoby, D. D. Ackerly, Z. Baruch, F. Bongers, J. Cavender-Bares, T. Chapin, J. H. C. Cornelissen, M. Diemer, J. Flexas, E. Garnier, P. K. Groom, J. Gulias, K. Hikosaka, B. B. Lamont, T. Lee, W. Lee, C. Lusk, J. J. Midgley, M. L. Navas, U. Niinemets, J. Oleksyn, N. Osada, H. Poorter, P. Poot, L. Prior, V. I. Pyankov, C. Roumet, S. C. Thomas, M. G. Tjoelker, E. J. Veneklaas, and R. Villar. 2004. The worldwide leaf economics spectrum. Nature **428**:821-827.

Wright, I. J., and M. Westoby. 1999. Differences in seedling growth behaviour among species: trait correlations across species, and trait shifts along nutrient compared to rainfall gradients. Journal of Ecology **87**:85-97.

Table 2-1. Summary of trait gradient analyses for seven leaf and root traits measured on 76 plant species in Alberta grasslands. All reported trait values are log10-transformed. See methods section for an explanation of terminology.

Species trait	Statistic	Specific leaf area	Leaf area	Leaf thickness	Specific leaf volume	Speecific root length	Specific root volume	Root diameter
		(mm^2/mg)	(cm^2)	(mm)	(mm^3/mg)	(m/g)	(mm^3/mg)	(mm)
Total (t_s)	Mean	2.25	0.29	-0.53	1.72	1.86	0.54	-0.60
	SD	0.19	0.54	0.15	0.21	0.30	0.19	0.15
	Minimum	1.94	-1.08	-0.91	1.34	1.19	0.19	-0.93
	Maximum	2.96	1.39	-0.02	2.39	2.67	1.00	-0.30
Alpha ($t_{\alpha s}$)	Minimum	-0.25	-0.94	-0.31	-0.33	-0.70	-0.21	-0.29
	Maximum	1.28	1.25	0.50	1.34	0.81	0.62	0.34
	Range	1.54	2.19	0.82	1.67	1.51	0.83	0.63
Beta ($t_{\beta s}$)	Minimum	2.07	-0.19	-0.63	1.47	1.77	0.36	-0.70
	Maximum	2.24	0.43	-0.47	1.75	2.03	0.61	-0.60
	Range	0.17	0.61	0.16	0.28	0.26	0.25	0.10
Range (R_s)	Mean	0.08	0.25	0.08	0.10	0.18	0.12	0.06
	SD	0.07	0.19	0.06	0.09	0.12	0.10	0.04
	Minimum	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Maximum	0.26	0.61	0.18	0.34	0.37	0.27	0.13
Slope (b_s)	Mean	0.66	0.17	0.78	0.67	1.01	0.86	0.73
	SD	1.87	0.70	0.93	1.58	0.97	1.17	1.76
	Minimum	-4.86	-2.25	-0.58	-4.64	-1.70	-0.91	-3.43
	Maximum	4.27	1.09	3.49	3.62	3.52	4.04	6.17

Table 2-2. Proportion of the total variation in the mean value of seven leaf and root traits explained by alpha (within-community) and beta (among-community) components of trait variation for 76 plant species in Alberta grasslands. See methods section for an explanation of terminology.

	Total vs. alpha trait		Total vs. beta trait	
Total trait	R^2	*P*-value	R^2	*P*-value
SLA	0.98	<0.001	0.01	0.429
Leaf area	0.90	<0.001	0.12	0.002
Leaf thickness	0.96	<0.001	0.17	<0.001
SLV	0.96	<0.001	0.00	0.993
SRL	0.96	<0.001	0.03	0.166
SRV	0.93	<0.001	0.06	0.029
Root diameter	0.99	<0.001	0.07	0.019

Table 2-3. Measures of phylogenetic signal for a) alpha, beta, and total components of variation in seven traits measured on 76 plant species in Alberta grasslands and b) 12 traits measured on 40 grassland species grown in a common garden. Mean contrast is the average value of phylogenetically independent contrasts at all nodes in the phylogeny for each trait. Observed contrast rank indicates the relative position of observed mean contrast values versus 999 random reshufflings of species labels on the phylogeny, with low rank indicating phylogenetic conservatism (contrast lower than expected), and high rank indicating phylogenetic convergence (contrast higher than expected), and two-tailed *P*-values calculated based on ranks.

a) Field traits (N = 76 species)

Trait type	Trait	Mean contrast	Observed contrast rank vs. randomizations (/1000)	*P*-value
Alpha	SLA	0.106	548	0.906
	Leaf area	0.278	1	0.002
	Leaf thickness	0.083	99	0.198
	SLV	0.106	4	0.008
	SRL	0.198	839	0.324
	SRV	0.09	1	0.002
	Root diameter	0.088	11	0.022
Beta	SLA	0.017	346	0.692
	Leaf area	0.115	542	0.918
	Leaf thickness	0.018	382	0.764
	SLV	0.024	398	0.796
	SRL	0.043	967	0.068
	SRV	0.033	472	0.944
	Root diameter	0.01	51	0.102
Total	SLA	0.104	396	0.792
	Leaf area	0.304	4	0.008
	Leaf thickness	0.084	20	0.040
	SLV	0.107	7	0.014
	SRL	0.205	925	0.152
	SRV	0.093	1	0.002
	Root diameter	0.09	5	0.010

Table 2-3 continued.

b) Common garden traits (N = 40 species)

Trait	Mean contrast	Observed contrast rank vs. randomizations (/1000)	*P*-value
Aboveground biomass	0.251	1	0.002
Belowground biomass	0.259	7	0.014
SLA	0.07	43	0.086
Total leaf area	0.213	2	0.004
Total root length	0.264	1	0.002
SRL	0.122	510	0.982
SRV	0.077	1	0.002
LAR	0.103	3	0.006
LWF	0.079	21	0.042
RLR	0.09	2	0.004
RWF	0.059	3	0.006
Root diameter	0.053	8	0.016

Table 2-4. Summary of ahistorical and PIC correlations among alpha, beta and total components of trait variation for 76 plant species (68 contrasts) in Alberta grasslands. Cell contents are correlation coefficients. Above-diagonal values are ahistorical correlations, below-diagonal values are PIC correlations. Bold cells indicate *P*-value < 0.05.

a) Alpha traits

	SLA	Leaf area	Leaf thickness	SLV	SRL	SRV	Root diameter
SLA		-0.13	**-0.36**	**0.54**	0.21	0.06	-0.15
Leaf area	-0.11		**0.29**	0.13	**-0.33**	0.16	**0.49**
Leaf thickness	**-0.41**	**0.32**		**0.51**	-0.08	**0.25**	**0.26**
SLV	**0.55**	0.20	**0.45**		0.13	**0.32**	0.07
SRL	0.06	-0.19	0.09	0.13		**0.29**	**-0.73**
SRV	-0.10	0.13	0.21	0.10	**0.56**		**0.33**
Root diameter	-0.10	**0.27**	0.00	-0.08	**-0.83**	-0.07	

b) Beta traits

	SLA	Leaf area	Leaf thickness	SLV	SRL	SRV	Root diameter
SLA		**0.32**	-0.10	**0.57**	0.14	0.11	0.00
Leaf area	**0.38**		**-0.37**	-0.01	-0.02	0.09	-0.04
Leaf thickness	-0.06	**-0.31**		**0.68**	**-0.39**	**-0.27**	**0.44**
SLV	**0.61**	0.09	**0.65**		**-0.30**	**-0.32**	**0.26**
SRL	0.08	-0.07	**-0.36**	**-0.30**		**0.73**	**-0.57**
SRV	0.08	0.00	**-0.27**	-0.22	**0.84**		-0.01
Root diameter	0.05	0.12	**0.34**	**0.33**	**-0.49**	-0.11	

c) Total traits

	SLA	Leaf area	Leaf thickness	SLV	SRL	SRV	Root diameter
SLA		-0.12	**-0.41**	**0.51**	0.16	0.03	-0.18
Leaf area	-0.06		0.16	0.02	**-0.35**	0.14	**0.44**
Leaf thickness	**-0.47**	0.17		**0.49**	-0.08	**0.24**	**0.24**
SLV	**0.49**	0.08	**0.44**		0.09	**0.30**	0.06
SRL	0.09	**-0.25**	0.12	0.15		**0.26**	**-0.75**
SRV	-0.04	0.02	0.16	0.04	**0.55**		**0.34**
Root diameter	-0.13	**0.29**	-0.03	-0.13	**-0.85**	-0.09	

Table 2-5. Summary of ahistorical and PIC correlations among traits for 40 grassland plant species (33 contrasts) grown in a common garden. Cell contents are correlation coefficients. Above-diagonal values are ahistorical correlations, below-diagonal values are PIC correlations. Bold cells indicate *P*-value < 0.05. See methods section for an explanation of abbreviations.

	Aboveground mass	Belowground mass	Total leaf area	Total root length	LAR	RLR	LWF	RWF	SLA	SRL	SRV	Root diameter
Aboveground mass		**0.87**	**0.92**	**0.87**	**-0.45**	**-0.50**	**-0.43**	-0.26	-0.21	-0.02	**-0.43**	**-0.40**
Belowground mass	**0.93**		**0.88**	**0.89**	**-0.50**	-0.21	**-0.54**	0.14	-0.16	-0.03	-0.30	-0.21
Total leaf area	**0.91**	**0.81**		**0.93**	-0.23	-0.27	**-0.33**	-0.13	0.04	0.13	-0.27	**-0.37**
Total root length	**0.87**	**0.87**	**0.82**		**-0.31**	-0.15	**-0.39**	0.01	-0.03	0.26	-0.19	**-0.44**
LAR	**-0.44**	**-0.54**	-0.17	**-0.34**		**0.32**	**0.80**	-0.12	**0.59**	0.30	0.29	-0.05
RLR	**-0.51**	**-0.42**	**-0.38**	-0.29	0.32		0.12	**0.61**	**0.52**	**0.31**	**0.73**	**0.40**
LWF	**-0.42**	**-0.47**	**-0.37**	**-0.49**	**0.62**	0.22		**-0.31**	0.12	0.16	0.28	-0.01
RWF	0.16	**0.39**	0.13	**0.35**	-0.15	0.31	-0.25		0.22	0.11	0.13	0.15
SLA	-0.21	-0.23	0.02	-0.08	**0.38**	0.31	-0.19	0.09		**0.34**	0.27	-0.05
SRL	-0.14	-0.17	-0.11	0.18	0.26	**0.35**	0.07	0.06	0.24		**0.42**	**-0.45**
SRV	**-0.63**	**-0.63**	**-0.57**	**-0.46**	0.23	**0.74**	0.25	-0.19	0.14	**0.47**		**0.45**
Root diameter	-0.32	**-0.36**	-0.27	**-0.52**	-0.01	0.20	0.11	-0.32	-0.11	**-0.63**	0.28	

Table 2-6. Correlations between intraspecific values for traits measured on plants growing in a common garden (CG) versus in the field (alpha, beta and total components of trait variation; see Methods) for 26 species with traits measured in both locations. See Methods section for an explanation of terminology.

	CG vs. alpha		CG vs. beta		CG vs. total	
Trait	Correlation (r)	*P*	Correlation (r)	*P*	Correlation (r)	*P*
SLA	0.09	0.677	0.02	0.906	0.16	0.424
SRL	0.37	0.064	-0.11	0.608	0.35	0.083
SRV	0.48	0.013	0.08	0.701	0.47	0.015
Root diameter	0.85	0.000	0.29	0.147	0.86	0.000

Table 2-7. Correlations between plot mean trait values (t_p) and species mean trait values (t_s) measured in a trait gradient analysis of 76 plant species in Alberta grasslands versus their respective scores on first two axes of NMS ordination of community composition in those grasslands. See Methods section for an explanation of terminology.

a) Plot mean trait values (t_p)

	NMS Axis 1		NMS Axis 2	
Trait	Correlation (r)	*P*-value	Correlation (r)	*P*-value
SLA	0.28	0.159	-0.30	0.127
Leaf area	0.80	0.000	-0.14	0.479
Leaf thickness	0.11	0.587	0.10	0.624
SLV	0.29	0.139	-0.08	0.690
SRL	-0.22	0.272	-0.37	0.057
SRV	0.24	0.224	-0.60	0.001
Root diameter	0.13	0.526	-0.17	0.392

b) Species mean trait values (t_s)

	NMS Axis 1		NMS Axis 2	
Trait	Correlation (r)	*P*-value	Correlation (r)	*P*-value
SLA	0.30	0.038	-0.28	0.056
Leaf area	0.29	0.051	0.00	0.998
Leaf thickness	-0.32	0.031	0.14	0.332
SLV	-0.07	0.650	-0.16	0.292
SRL	-0.13	0.381	-0.17	0.263
SRV	-0.07	0.659	-0.48	0.001
Root diameter	0.06	0.698	-0.07	0.659

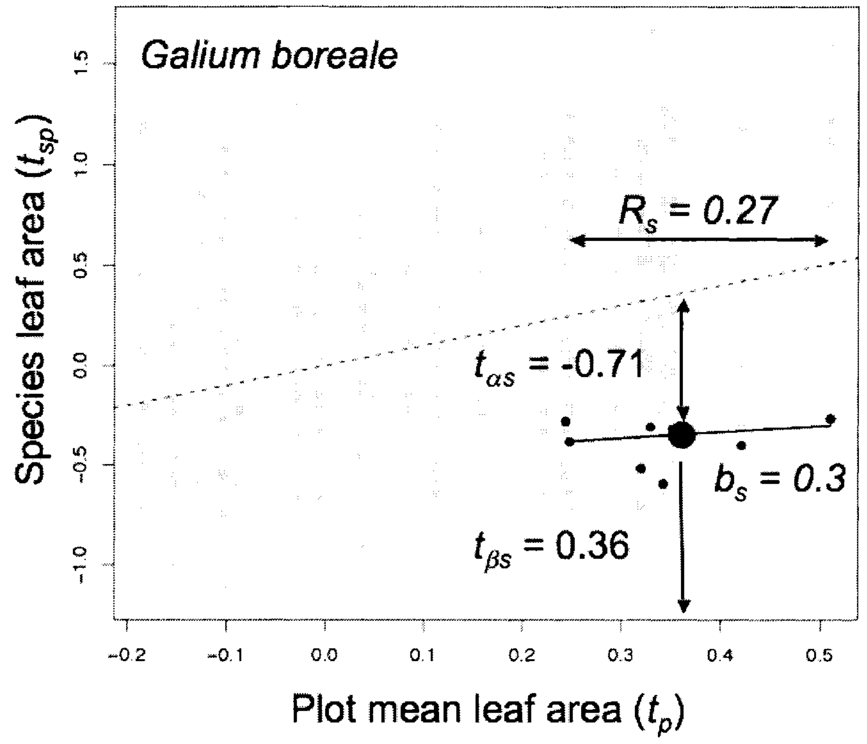

Figure 2-1. Results from a trait gradient analysis of $\log_{10}$-transformed leaf area measured on 76 plant species in Alberta grasslands. The large black circle indicates the mean alpha ($\overline{t_{\alpha s}}$) and beta ($\overline{t_{\beta s}}$) trait value for *Galium boreale* (Rubiaceae), while the smaller circles indicate trait values of individual plants of *Galium boreale* (black circles) and all other species (gray circles) measured in each plot (t_{sp}). Dashed lines indicate the regression line of t_{sp} vs. $\overline{t_p}$. The solid line indicate the intraspecific slope (b_s). See methods section for an explanation of terminology.

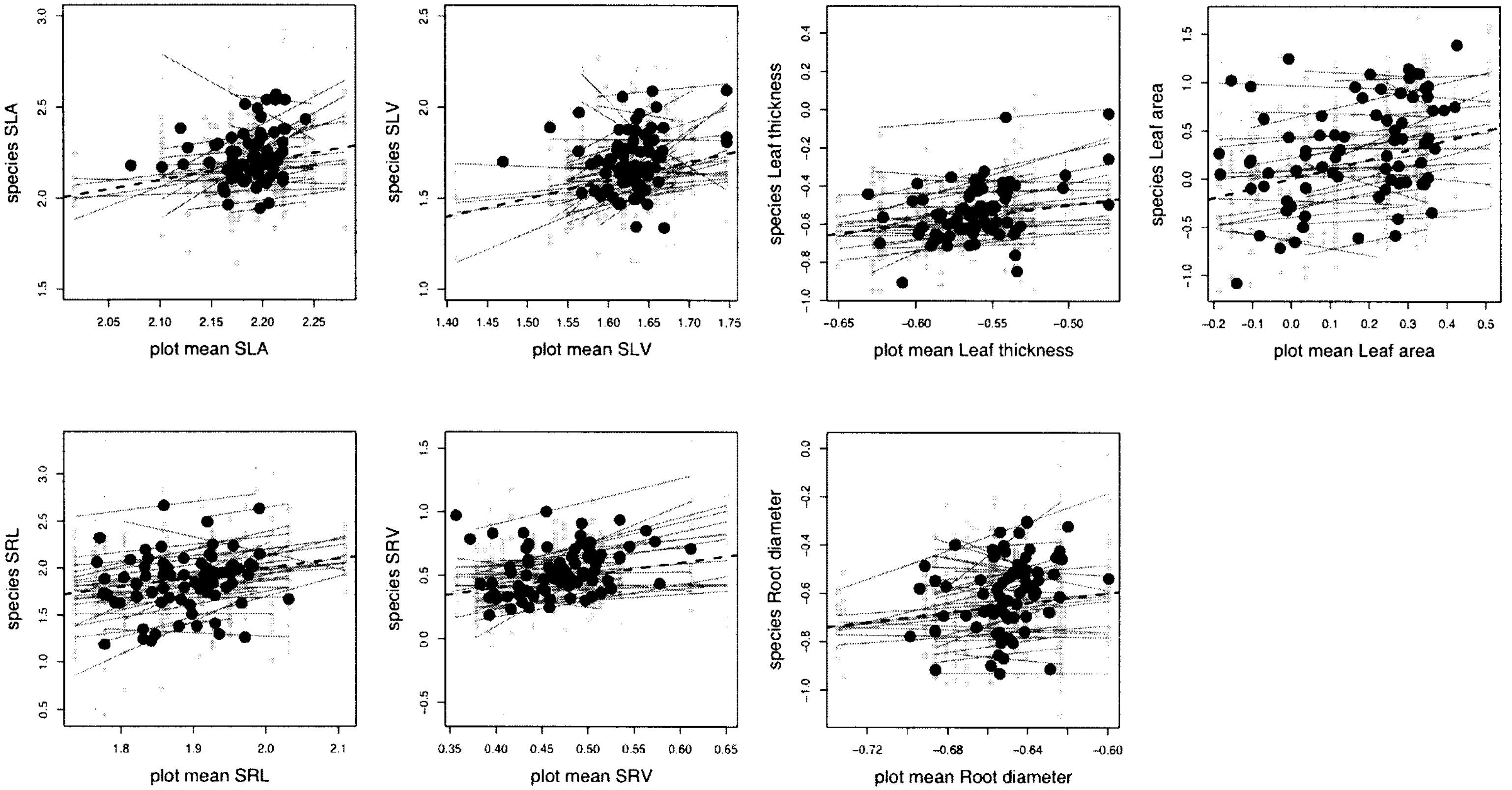

Figure 2-2. Results from a trait gradient analysis for seven leaf and root traits measured on 76 plant species in Alberta grasslands. All reported trait values are $\log_{10}$-transformed. Black circles indicate species mean alpha ($\overline{t_{\alpha s}}$) and beta ($\overline{t_{\beta s}}$) values, gray circles indicate trait values of individual plants measured in each plot (t_{sp}). Dashed lines indicate the regression line of t_{sp} vs. $\overline{t_p}$. Solid lines indicate intraspecific slopes (b_s). See methods section for an explanation of terminology.

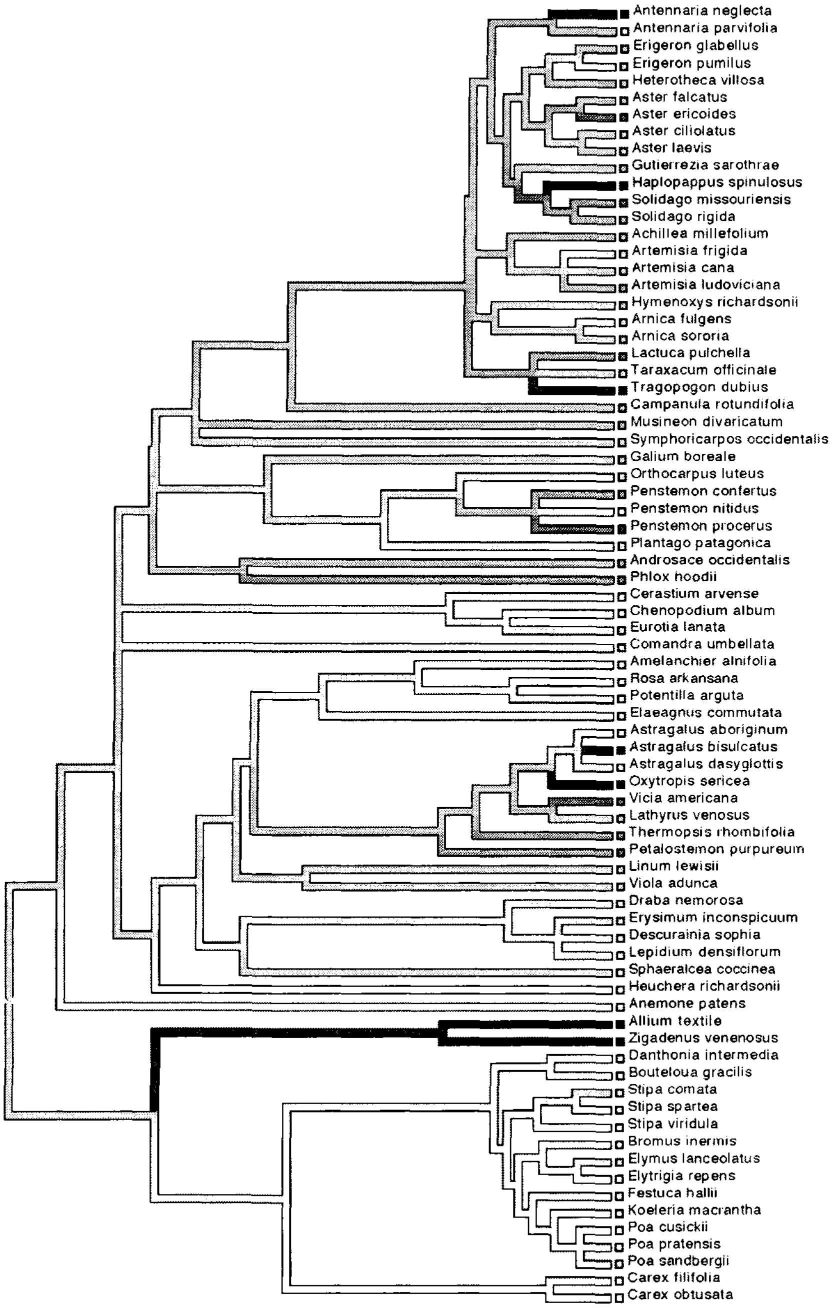

Figure 2-3. Hypothesized phylogenetic tree for 76 plant species growing in Alberta grasslands. See Methods section for an explanation of data sources and methods used to construct the tree. Branches are scaled proportional to estimated divergence times, with the root node (monocot – eudicot divergence) estimated at 139 million years ago. Branch shading indicates squared-change parsimony reconstruction of the alpha component of SRV (specific root volume = 1/root tissue density), a highly phylogenetically conserved trait, with lighter shades indicating lower SRV (higher root tissue density).

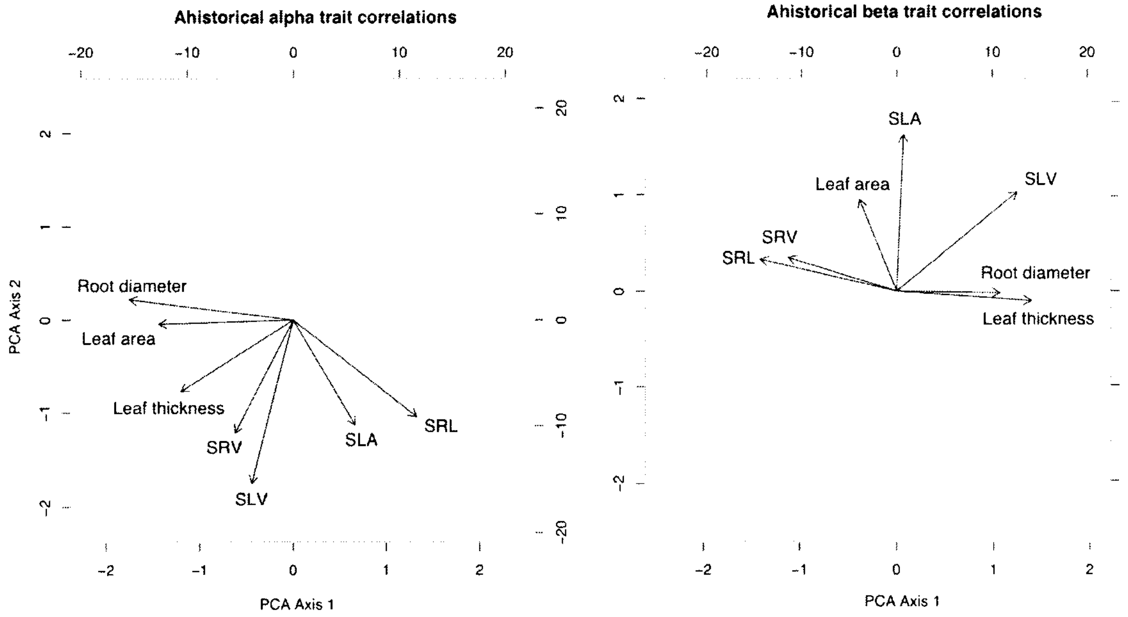

Figure 2-4. Principal components analysis plot indicating strength of multivariate ahistorical correlations among alpha and beta components of trait variation among 76 plant species growing in Alberta grasslands. The absolute position of arrow heads relative to each axis indicates the strength of the relationship between that trait and the axis.

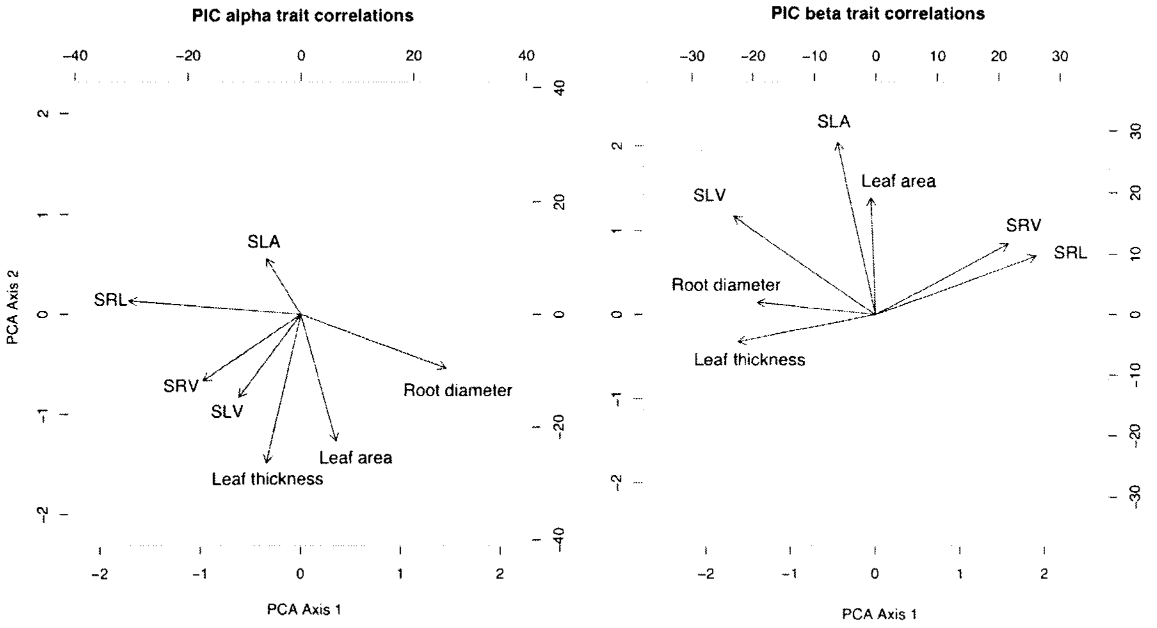

Figure 2-5. Principal components analysis plot indicating strength of multivariate PIC correlations among 68 phylogenetically independent contrasts for alpha and beta components of trait variation among 76 plant species growing in Alberta grasslands. The absolute position of arrow heads relative to each axis indicates the strength of the relationship between that trait and the axis.

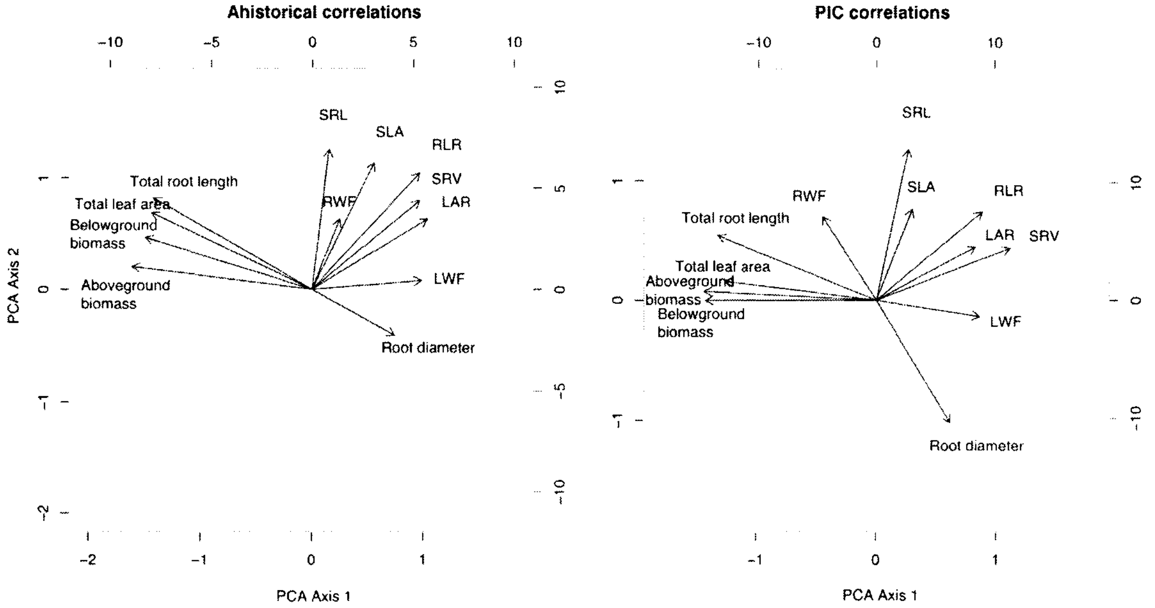

Figure 2-6. Principal components analysis plot indicating strength of multivariate ahistorical and PIC correlations among traits measured on 40 grassland species (33 phylogenetically independent contrasts) growing in a common garden. The absolute position of arrow heads relative to each axis indicates the strength of the relationship between that trait and the axis.

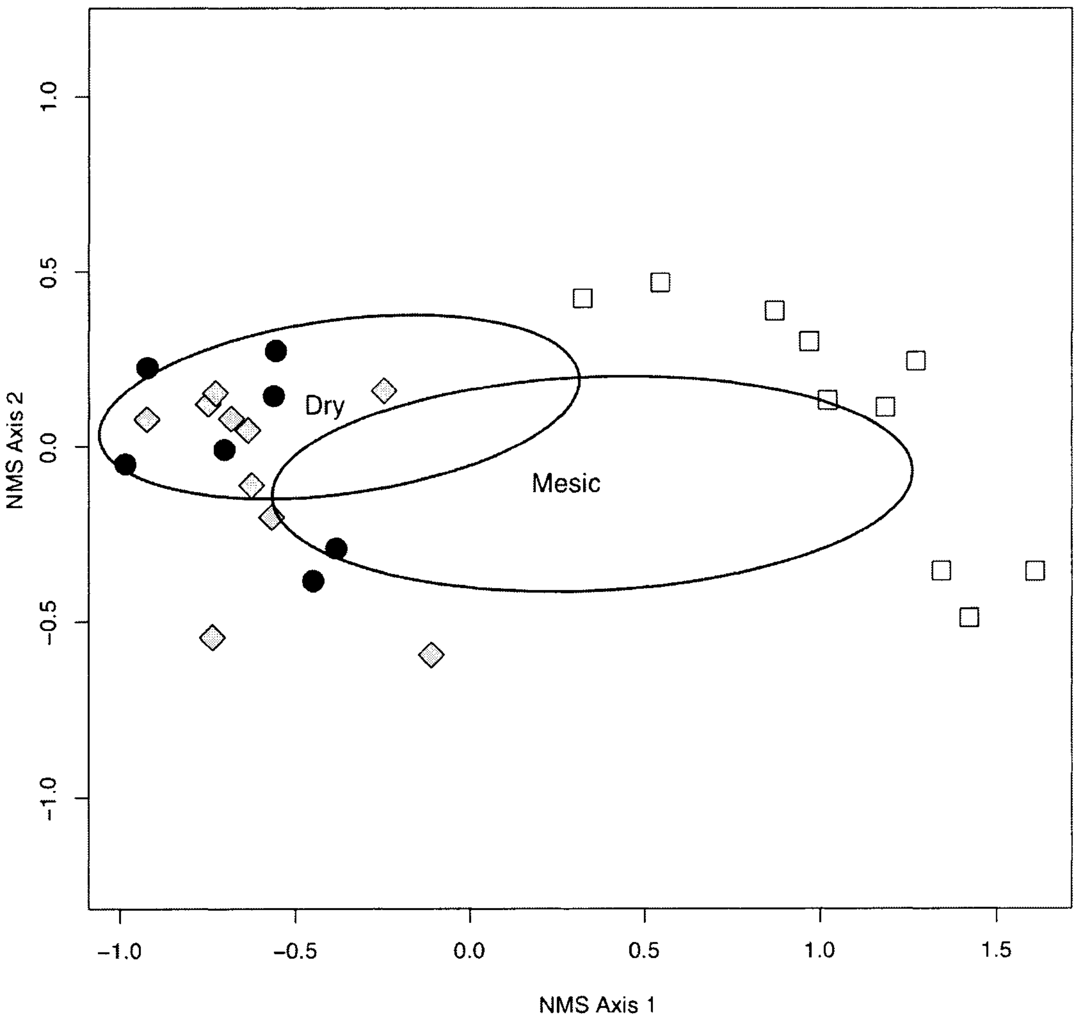

Figure 2-7. Results of a NMS ordination of community composition data for 28 plots located in three sites in Alberta grasslands (white boxes: Kinsella, black circles: Onefour, gray diamonds: Hargrave). Ellipses indicate 68% confidence ellipses around plots classified as dry or mesic relative to other plots at each site.

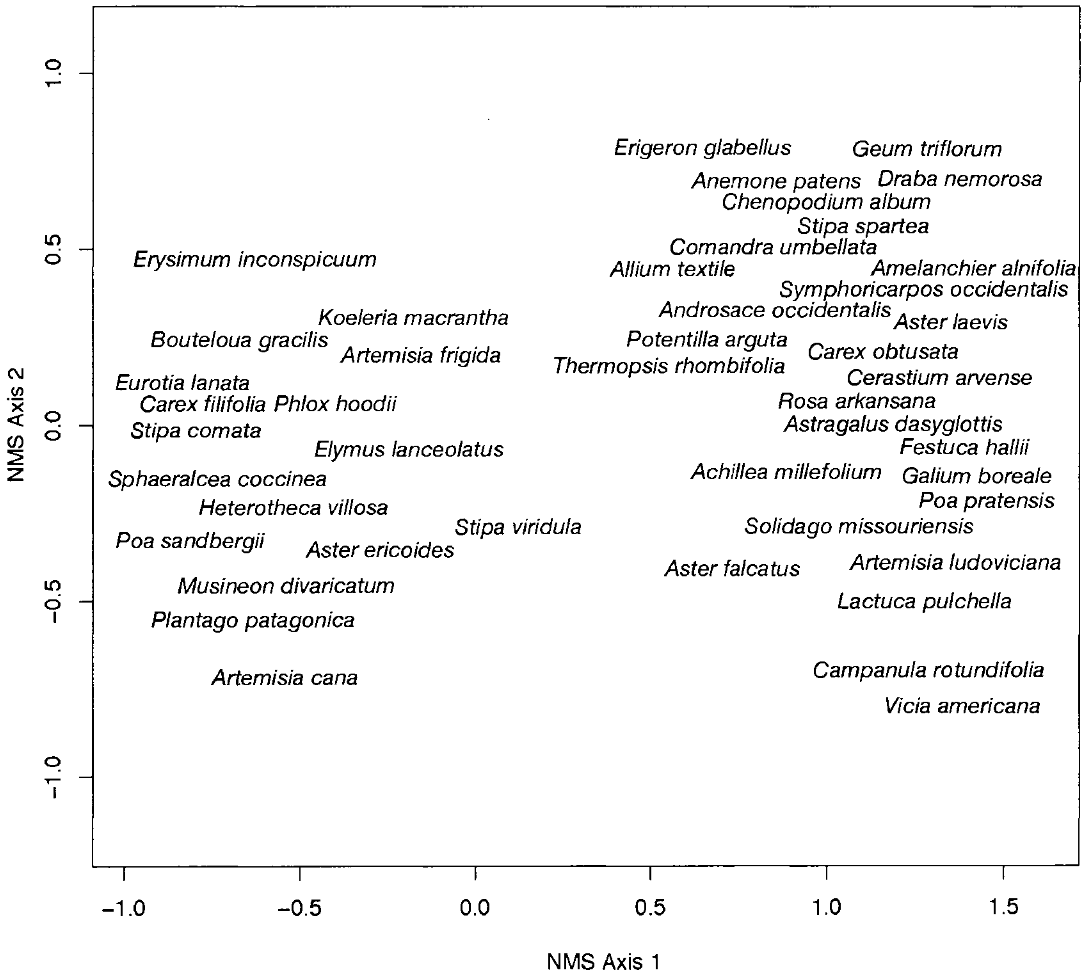

Figure 2-8. Species scores from a NMS ordination of community composition data for 28 plots located in three sites in Alberta grasslands.

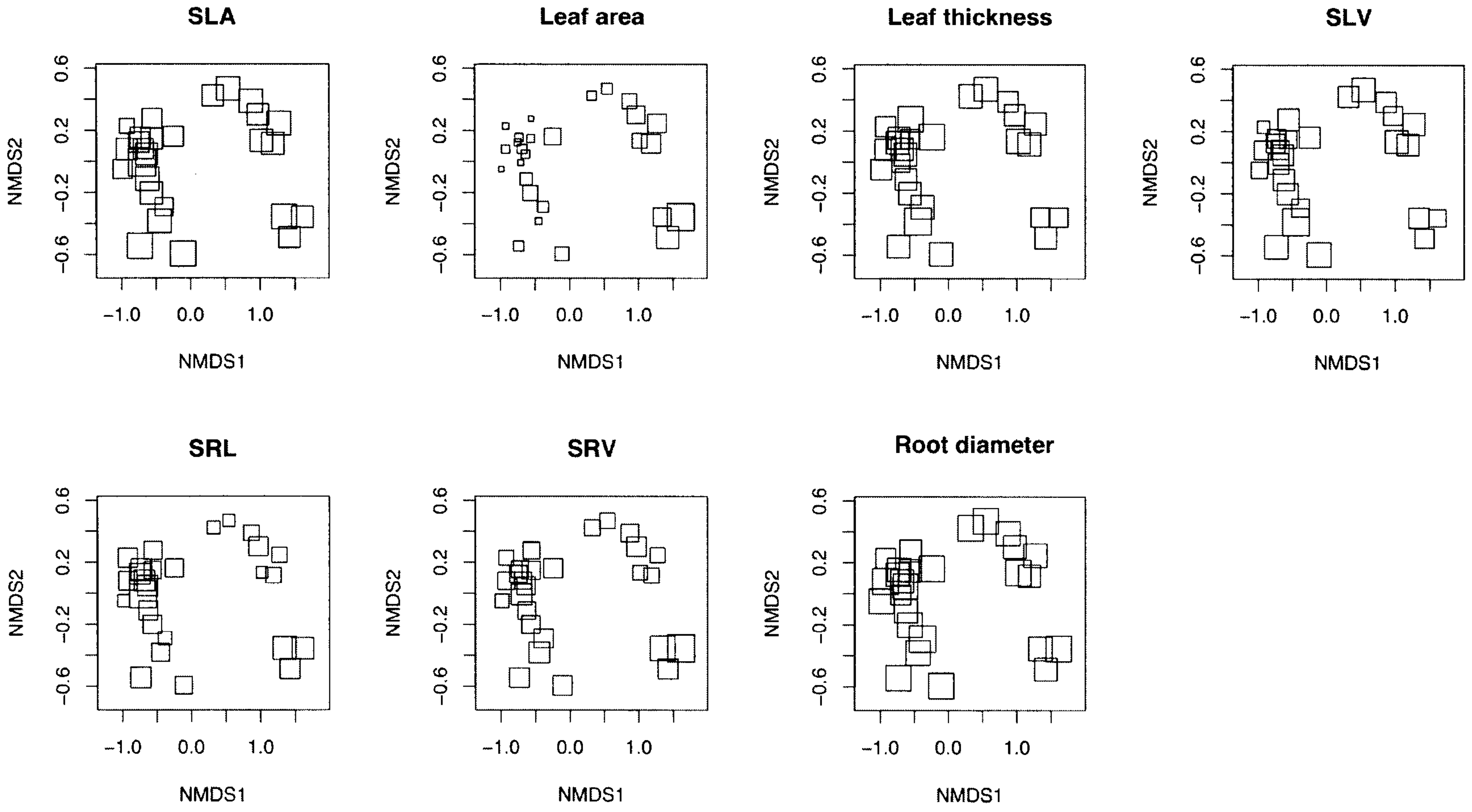

Figure 2-9. Relative plot mean trait values ($\overline{t}_p$) from a trait gradient analysis of seven traits versus plot scores from a NMS ordination of community composition data for 28 plots located in three sites in Alberta grasslands. Larger box size indicates higher relative plot mean trait value for a given trait.

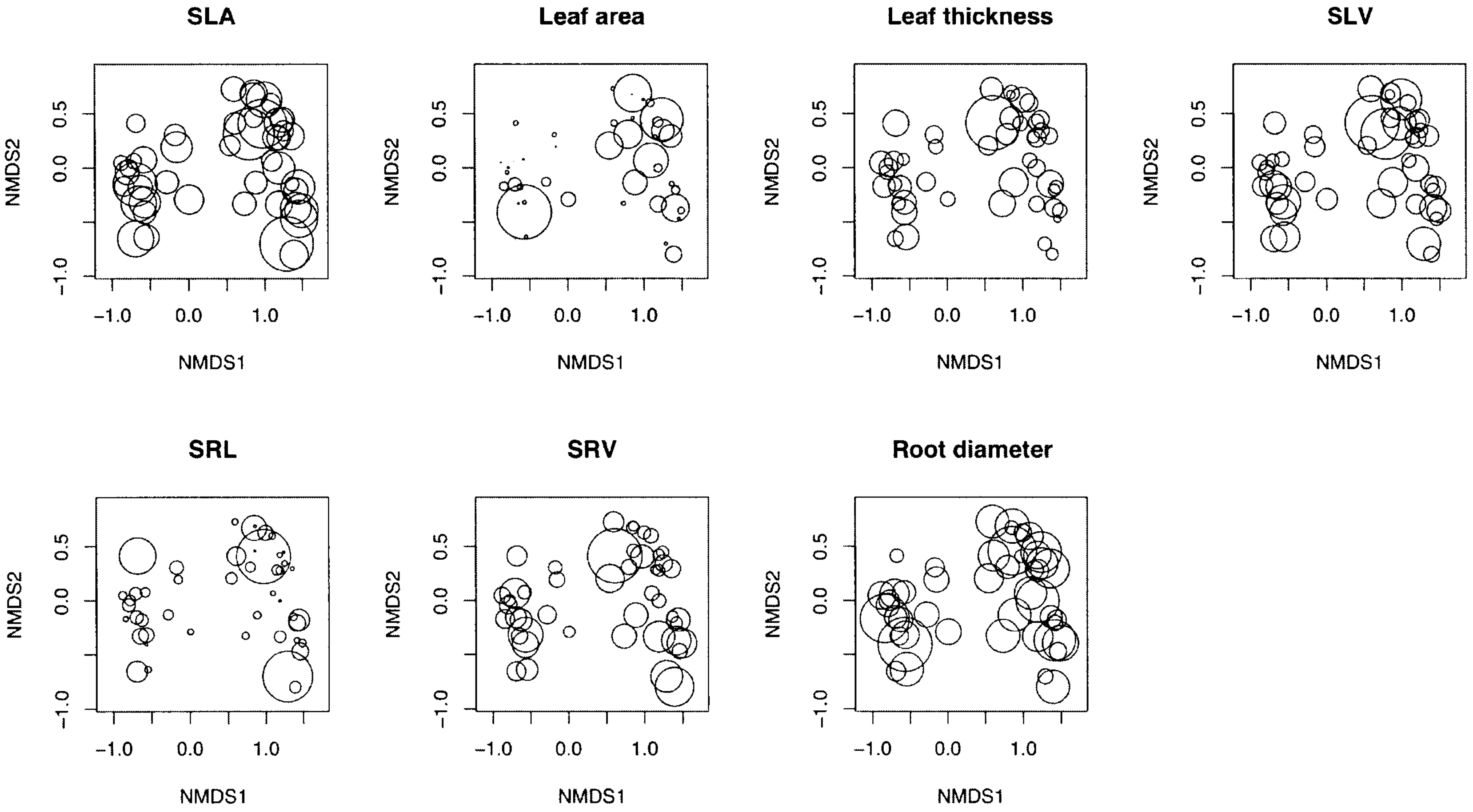

Figure 2-10. Relative species mean trait values ($\overline{t_s}$) from a trait gradient analysis of seven traits versus species scores from a NMS ordination of community composition data for 28 plots located in three sites in Alberta grasslands. Larger circle size indicates higher relative species mean trait value for a given trait.

Chapter 3 Phylogenetic and functional trait similarity in Alberta grasslands: environmental filtering of leaf and root traits at multiple scales

Introduction

Contemporary ecological processes as well as the evolutionary history of species niches and traits may all interact to determine the structure of ecological communities (Webb et al. 2002), and there has been an increasing call for ecologists to account for the historical factors that structure ecological communities (Losos 1996, Ricklefs 2004, Wiens and Donoghue 2004). In this chapter, I use data on the co-occurrence, phylogenetic relationships and leaf and root functional traits of co-occurring species at three sites in Alberta grasslands to test whether plant communities in these grasslands are more or less similar than expected with regards to their evolutionary relatedness and leaf and root functional traits, and I ask what these patterns can tell us about the relative importance of different ecological and evolutionary processes structuring these communities.

Numerous studies have used data on the similarity of co-occuring species to test for the importance of two general types of processes than can influence community assembly. One approach has emphasized the role of biotic interactions, especially competitive exclusion and limiting similarity (Macarthur and Levins 1967, Pacala and Tilman 1994, Dayan and Simberloff 2005). The other approach has emphasized the role of the abiotic environment as a filter which selects for species possessing particular traits (Southwood 1977, Grime 2001). These two approaches make contrasting predictions about the similarity of co-occurring species, and numerous studies have sought evidence for limiting similarity or environmental filtering by examining the similarity of traits of species occurring together in ecological communities (Ricklefs and Travis 1980, Cody 1991, Weiher and Keddy 1995, Weiher et al. 1998, Stubbs and Wilson 2004). If competitive exclusion and limiting similarity are important, co-occurring species are predicted to exhibit trait overdispersion (dissimilarity), while if environmental filtering is important co-occurring species should exhibit trait clustering (similarity).

In addition to numerous studies measuring the trait similarity of species directly, others have used the observation that closely related species tend to possess similar niches and traits (phylogenetic niche and trait conservatism (Harvey and Pagel 1991, Wiens and Graham 2005)), along with information on the relatedness of species, to test for the importance of these two different processes during community assembly. This approach has ranged from early studies of taxonomic ratios (Elton 1946), to more recent studies of phylogenetic similarity in ecological communities (Webb et al. 2002).

The phylogenetic similarity of co-occurring species will depend on the interaction between ecological processes and the evolutionary history of traits and niches (e.g. Table 3-1-1; Webb et al. 2002). If traits are evolutionarily conserved, limiting similarity would lead to a pattern of phylogenetic overdispersion (distant relatives co-ocurring), and environmental filtering would lead to a pattern of phylogenetic clustering (close relatives co-occurring). If traits are evolutionarily convergent, environmental filtering of those traits would lead to a pattern of phylogenetic overdispersion, while limiting similarity would result in a random pattern of phylogenetic relatedness.

In the absence of detailed information on the traits and niches of co-occurring species, and based on the observation that species traits and niches are broadly evolutionarily conserved (Prinzing et al. 2001, Wiens and Graham 2005), many studies of community phylogenetic structure and have interpreted patterns of phylogenetic clustering in communities as evidence for environmental filtering, and patterns of phylogenetic overdispersion as evidence for limiting similarity (Webb 2000, Horner-Devine and Bohannan 2006, Kembel and Hubbell 2006, Lovette and Hochachka 2006).

Very few studies have directly evaluated the influence of the evolutionary history of different traits on measures of community phylogenetic and functional trait similarity. One such study (Cavender-Bares et al. 2006) found that patterns of phylogenetic and functional trait similarity in Florida plant communities were consistent with the effects of environmental filtering of traits, but that patterns of community phylogenetic similarity varied from clustered to overdispersed depending on the spatial and phylogenetic scale used to define communities. However, due to the small number of studies that have simultaneously considered community structure, trait similarity and phylogenetic relatedness, the generality of these results is unclear.

Measures of community phylogenetic and trait similarity are sensitive to the spatial scale, spatial extent and phylogenetic scale used to define communities (Levin 1992, Webb et al. 2002). Spatial scale is the size or area of samples used to define communities. Spatial extent is the geographic and environmental distribution of samples used to define a community. Phylogenetic scale refers to the diversity of lineages included when defining communities. Several authors have hypothesized that limiting similarity will be important at small spatial scales and extents (Weiher et al. 1998, Webb et al. 2002), as this is the scale at which individual organisms interact within habitats, and at small phylogenetic scales, as competition among closely related species may lead to phylogenetic and trait overdispersion within narrowly defined lineages (Losos et al. 2003, Cavender-Bares et al. 2004, Slingsby and Verboom 2006).

In this study I quantify the phylogenetic relatedness and functional trait similarity of plant species growing in Alberta grasslands to test for evidence of limiting similarity and environmental filtering in these communities at a range of spatial and phylogenetic scales. I test for relationships between co-occurrence and phylogenetic and functional similarity among species at sites in mixedgrass and fescue grasslands, describe variation in patterns of trait evolution among species in these grasslands, and measure the effects of spatial scale, spatial extent and phylogenetic scale on patterns of community phylogenetic and functional trait similarity and trait evolution in these grasslands.

Methods

Study system

Grassland plant communities at the northern fringe of the Great Plains in Alberta vary both along major gradients of climate and soil type among sites, as well as along local environmental gradients within sites (Coupland 1961). In general, precipitation increases and growing season evapotranspiration deficits decrease from south to north in the grassland and parkland natural regions, and soil types shift from brown to more productive black chernozems in the north (Chetner 2003). Grasslands in the south are dominated by dry mixedgrass plant communities, with grasses such as needle-and-thread (*Stipa comata)* and blue grama (*Bouteloua gracilis)* dominant (Coupland 1950), while the northern and western fringe of the grassland regions of the province are characterized by fescue-dominated plant communities with plains rough fescue (*Festuca hallii)*, porcupine grass (*Stipa spartea)* and bluegrass (*Poa pratensis)* the dominant grasses in the northern fescue and parkland regions (Moss and Campbell 1947, Coupland and Brayshaw 1953). Forb and shrub species make up a significant portion of the plant diversity in these grasslands, with the Asteraceae, Brassicaceae, and Fabaceae representing some of the major non-graminoid components of plant diversity in both community types.

I measured plant species abundances and leaf and root functional traits at three sites located in two of the major grassland community types in Alberta. The Kinsella site (53°05'N, 111°33'W) is a rough fescue-dominated native grassland in the central parkland natural region (Natural Regions Committee 2006). The Onefour (49°08'N, 110°31'W) and Hargrave sites (49°59'N, 110°02'W) are dry mixedgrass native grasslands, located in the dry mixedgrass natural region. The vegetation at all of these sites is representative of the native grassland communities described from each site's natural region (Moss and Campbell 1947, Coupland 1950, Coupland and Brayshaw 1953, Coupland 1961). All sites are lightly to moderately grazed by cattle. Precipitation at these sites ranges from approximately 250-350mm/year at Onefour and Hargrave, to approximately 400-450mm/year at Kinsella (Chetner 2003). Growing season temperatures, wind speeds, and evapotranspiration deficits at the Onefour and Hargrave sites are also higher than at Kinsella (Natural Regions Committee 2006), leading to an overall trend of greater drought stress at mixedgrass sites.

Plant community measurements

During June and July of 2003 and 2004, I established from eight to ten 20m x 20m sampling plots at each site. Plots were distributed randomly along topographic gradients in order to sample the natural range of variation in plant community composition at each site. I recorded the identity of all angiosperm species present in ten 0.2m x 0.5m quadrats scattered randomly within each plot. Based on an examination of species accumulation curves, ten quadrats per plot and eight to ten plots per site was sufficient to capture the majority (>80%) of estimated total plant community diversity within plots and sites. I defined communities as the species co-occurring in each plot and quadrat.

Trait measurements

Species encountered in each plot were collected from the vicinity of the plot for leaf and root trait measurement. I collected a healthy mature plant of each species in each plot. Additionally, I opportunistically collected species at each site in order to obtain as many trait measurements per species as possible. I based all analyses on the mean trait values for each species. While this approach ignores intraspecific trait variation, in a related study (Chapter 2) I found that the majority of leaf and root trait variation in these grasslands was among species. The number of plants collected per species ranged from one to 16 (median = 5). Of 76 species collected, trait means for 16 rare species encountered in a single plot were necessarily based only on measurements on plants from that plot.

Leaf and root trait information was measured on field-collected plants following published guidelines for trait measurement (Cornelissen et al. 2003). Trait measurement methods are described in greater detail in Chapter 2. I measured a number of leaf and root functional traits, all of which have been linked to the resource uptake and stress tolerance strategies of species (Grime et al. 1997). Leaf traits included specific leaf area (SLA; leaf area per unit biomass; cm^2/g), specific leaf volume (SLV; 1/leaf tissue density = leaf volume per unit biomass; mm^3/mg), leaf area (cm^2), and leaf thickness (mm). Root traits included specific root length (SRL; root length per unit mass; m/g), specific root volume (SRV: 1/root tissue density = root volume per unit mass; mm^3/mg), and root diameter (mm). In general, species with high specific leaf area or specific root length, high specific leaf or root volume (low tissue density), and thin leaf or root tissues tend to possess a fast, acquisitive resource uptake strategy, with high instantaneous rates of resource uptake, short tissue lifespans and less resistance to water and nutrient stress and herbivory than species with the opposite set of leaf or root traits (Westoby et al. 2002, Diaz et al. 2004).

Phylogenetic data

I obtained a phylogenetic hypothesis for the 76 species included in this study (Figure 3-1) based on a published phylogenetic supertree of angiosperm families (Davies et al. 2004), which was constructed using supertree analysis of data from 42 published studies of angiosperm phylogeny, along with data on molecular variation of three genes (*atpB*, *rbcL*, and 18S rDNA), with nonparametric rate smoothing of rates of molecular change and fossil taxon age calibration used to estimate divergence dates for all nodes in the tree. Species included in the present study were grafted onto the Davies et al. (2004) strict consensus supertree using Phylomatic (Webb and Donoghue 2005), a toolkit and database for automated phylogenetic supertree construction. Within-family phylogenetic relationships were resolved based on a variety of published phylogenetic trees (Asteraceae (Baldwin et al. 2002, Noyes and Rieseberg 1999, Beck et al. 2004), Brassicaeae (Al-Shehbaz et al. 2006), Fabaceae (Wojciechowski et al. 2000), Poaceae (Salamin 2002), Rosaceae (Eriksson et al. 2003)).

As a result of a lack of within-family branch length information, all within-family phylogenetic distance measurements were based on equal spacing of nodes between the deepest node in the family (the estimated family age from the supertree) and the present. While the choice of branch lengths can potentially affect any comparative study, within-family molecular branch length estimates were not available for the species included in this study, and in any case molecular branch lengths may not always reflect actual ecological differences among species (Mooers et al. 1999, Bromham et al. 2002). To address the uncertainty in within-family branch lengths, I repeated all within-family analyses using an alternate set of branch lengths by setting all branch lengths to equal length for each analysis, resulting in non-ultrametric within-family trees. However, this did not have a major effect on results, and so I present only the results of analyses based on the single ultrametric tree.

Interspecific co-occurrence, phylogenetic and trait distances

To determine whether plant co-occurrence in Alberta grasslands is influenced by the phylogenetic or functional trait similarity of species, I compared a measure of species co-occurrence with measures of the phylogenetic distance and trait distances among species. The co-occurrence C_{ij} of each pair of species (i,j) was measured using Jaccard's coefficient (Legendre and Legendre 1998):

$$C_{ij} = \frac{a}{a+b+c} \tag{3.1}$$

where a is the number of samples in which both species are present, and b and c are the number of samples in which only species i or j are present, respectively. This measure of co-occurrence ranges from 0 (species never co-occur) to 1 (species always found together when present).

Interspecific phylogenetic distances were measured as the total estimated branch length separating species, in units of millions of years (Ma). Trait distances were measured as the absolute difference in $\log_{10}$-transformed trait values between species.

Co-occurrence versus phylogenetic and functional similarity

I tested for relationships among interspecific co-occurrence, phylogenetic distance and trait distances using a randomization method similar to a Mantel test (Mantel 1967, Legendre and Legendre 1998), in order to account for the non-normality and non-independence of co-occurrence and distance measures among species. All analyses were performed using Phylocom version 3.40 software (Webb et al. 2006) and scripts written in the R statistical language (R Development Core Team 2006). For a given pair of interspecific co-occurrence and distance matrices, I calculated the Pearson correlation between the corresponding lower-diagonal elements of the two matrices. I then created 999 randomized versions of the distance matrices using one of several null models (see below), and recalculated the correlations between the randomized matrices.

I calculated a two-tailed P-value based on the randomization results for each test, defined as twice the proportion of correlations between randomized matrices whose value was more extreme than the observed correlation, in the direction of the observed correlation (Manly 1997). Low P-values indicate that the strength of the observed relationship between matrices is greater than expected by chance. I considered positive correlations between co-occurrence and phylogenetic or functional trait distances to be evidence for phylogenetic or trait overdispersion in communities, and negative correlations to be evidence for phylogenetic or trait clustering within communities. Given the exploratory nature of this study, and a desire to detect potentially weak patterns of phylogenetic and trait similarity, I considered tests with $P < 0.05$ to be evidence for clustering or overdispersion, and tests with $P < 0.10$ to be evidence for weak phylogenetic or functional clustering or overdispersion.

Null models

I compared observed relationships among co-occurrence, phylogenetic and trait similarity to the patterns expected by chance using three different null models to randomize the raw data. Each of these null models has been used in previous studies of community phylogenetic structure (e.g. Cavender-Bares et al. 2004), although the statistical properties of these null models when applied to phylogenetic and trait data have not been explored extensively as they have been for species co-occurrence data (Gotelli 2001).

Null 1 involved shuffling species labels across the tips of the phylogeny or the list of trait values. This null model tests whether, given patterns of species co-occurrence and a set of trait values or phylogenetic relationships, phylogenetic or trait similarity have a stronger relationship with co-occurrence than expected by chance.

Null 2 involved shuffling species occurrences across samples while maintaining species frequencies. This null model tests whether, given species phylogenetic relationships or trait values, the relationship between co-occurrence and phylogeny or traits is stronger than expected than if species co-occurrence was random. This null model preserves the relative frequency of species but does not preserve the species richness of samples.

Null 3 involved randomizing species co-occurrences while maintaining both sample richnesses and species frequencies using the independent swap algorithm with 2,000 checkerboard swaps per randomization (Roberts and Stone 1990, Gotelli and Entsminger 2003). This null model preserves all features of the raw data except for patterns of species co-occurrence within samples, and asks whether given species' frequencies, sample richnesses and a set of phylogenetic relationships or trait values, co-occurring species are more or less similar than expected. This null model is explained in greater detail in Chapter 4.

Effects of spatial and phylogenetic scale and spatial extent

To test the hypothesis that community phylogenetic and trait similarity will vary with spatial scale, extent, and phylogenetic scale, I repeated all analyses at several scales. Effects of spatial scale were examined by comparing patterns for species co-occurrences calculated based on the presence of species in 0.2m x 0.5m quadrats and in 20m x 20m plots. To examine the influence of the spatial extent used to define a community, I compared patterns for species in samples at all three sites, as well as separately for samples from mixedgrass (Hargrave and Onefour) and fescue (Kinsella) sites, for both spatial scales. I tested for variation in community phylogenetic and functional similarity at a smaller phylogenetic scale by repeating these analyses including all 76 angiosperm species, and separately for species from each of the two most speciose families encountered at the study sites, the Asteraceae (23 species) and Poaceae (13 species).

Trait evolution at different scales

I tested for relationships between the phylogenetic distance and trait distance separating species using the matrix correlation methods described above. Observed correlations between species phylogenetic and trait distances were compared to the correlations expected if species labels were shuffled across the phylogeny and trait list (equivalent to a Mantel test, and to Null 1, described above). Positive correlations between phylogenetic and trait distance were interpreted as evidence for phylogenetic trait conservatism (close relatives possess similar trait values), and negative correlations were interpreted as phylogenetic trait convergence (distant relatives possess similar trait values). I repeated these analyses for all combinations of spatial scale, spatial extent, and phylogenetic scale.

Trait similarity versus trait evolution

In order to determine whether traits that evolve in a conservative fashion are more likely to be overdispersed within communities (Cavender-Bares et al. 2006), I compared the correlation between co-occurrence and trait similarity with the correlation between trait similarity and phylogenetic distances, using simple linear regression. A negative relationship between these two correlation scores for each trait would indicate that phylogenetically conserved traits are more clustered within communities. A positive relationship indicates that phylogenetically convergent traits are more clustered within communities.

Results

Phylogenetic similarity within communities

Relationships between co-occurrence and phylogenetic distance were uncommon, and generally weak when present (Table 3-1). Angiosperm species occurring together in mixedgrass sites were phylogenetically clustered (Table 3-1, Figure 3-2), but otherwise angiosperm species co-occurrence across all sites and at the fescue site was unrelated to phylogenetic similarity. Within the Poaceae, patterns of co-occurrence were random with respect to phylogeny at most scales, except in quadrats at mixedgrass sites where co-occurring grasses were phylogenetically overdispersed. Within the Asteraceae, species occurring together in plots at mixedgrass sites were weakly phylogenetically clustered, while Asteraceae species occurring together in plots and quadrats at fescue sites were weakly phylogenetically overdispsersed (Table 3-1).

Trait similarity within communities

Many leaf and root traits were clustered within communities, although this pattern varied depending on the spatial extent and scale used to define communities, and the null model used to generate random communities. Across all sites and at mixedgrass sites, co-occurring angiosperm species generally had similar leaf and root tissue density, SLA and leaf thickness (Table 3-2). At fescue sites, co-occurring species generally had similar leaf area and root tissue density.

Across all sites and at mixedgrass sites, co-occurring Asteraceae had similar SRV and root diameters, but dissimilar leaf thicknesses (Table 3-3), while at fescue sites they were clustered with respect to SRL and root diameter and overdispersed with respect to SLA and leaf thickness (Figure 3-3). Co-occurring Poacaeae were clustered with respect to most measured leaf and root traits (Table 3-4).

Trait evolution

Across angiosperm species at all sites and at the fescue site, most traits were phylogenetically conserved, while among species occurring at the mixedgrass sites only SLV, SRV and root diameter were evolutionarily conserved (Table 3-5). Patterns of trait evolution among Asteraceae species at all sites and mixedgrass sites were predominantly random, except at the fescue site where SLA was phylogenetically conserved and SRL and root diameter were phylogenetically convergent. Many traits were phylogenetically conserved within the Poaceae, while SLA was phylogenetically convergent among grasses occurring at mixedgrass sites (Table 3-5).

Trait similarity versus trait evolution

Across all angiosperms at all spatial scales and extents, traits that were more strongly phylogenetically conserved were more clustered within communities (Figure 3-4). Within the Asteraceae, traits that were more convergent were more similar within communities at the fescue site. Within the Poaceae, across species at all sites and at the fescue site, traits that were more conserved were more clustered within communities.

Discussion

Phylogenetic and trait similarity in Alberta grasslands

Effects of spatial scale

Although it has been suggested that limiting similarity will be more important at smaller spatial scales due to interactions at the scale of individual plant neighbourhoods (Weiher et al. 1998), I did not find evidence for consistently stronger trait clustering or phylogenetic structure at smaller spatial scales (Tables 3-1 – 3-4). *P*-values were sometimes smaller at smaller spatial scales, but this may have been caused by the larger number of samples used to calculate co-occurrence at the quadrat scale.

Effects of spatial extent

Calculating species co-occurrence across sites containing distinct habitat types obscured the stronger relationships between co-occurrence, phylogenetic and trait similarity found within each natural region (Tables 3-1 & 3-2). Studies of community phylogenetic and trait similarity must be careful to account for the presence of distinct habitat types containing species with different traits and evolutionary histories when defining communities (Kembel and Hubbell 2006), and it is possible that habitat heterogeneity within natural regions may have contributed to the patterns I observed.

Environmental conditions at the relatively dry mixedgrass sites were likely to be more stressful compared to conditions at the fescue site (Natural Regions Committee

2006), and I found stronger patterns of trait and phylogenetic clustering at the mixedgrass sites (Tables 3-1 – 3-4) despite the smaller sample sizes (fewer species pairs) at these sites. This finding supports the hypothesis that conditions in stressful environments may act as a stronger environmental filter, leading to stronger patterns of trait clustering (Grime 2006), and to phylogenetic clustering when traits have evolved in a conservative fashion (Warwick and Clarke 1998, Webb et al. 2002).

Effects of phylogenetic scale

Several authors have suggested that competition among close relatives will lead to a pattern of phylogenetic overdispersion among species in single lineage communities (Losos et al. 2003, Cavender-Bares et al. 2004, Cavender-Bares et al. 2006, Slingsby and Verboom 2006). I found both phylogenetic overdispersion and clustering within the single-lineage communities I examined (Table 3-1), suggesting that the idiosyncratic details of trait and niche evolution in different lineages and communities may make it difficult to make generalizations about the effects of phylogenetic scale on community structure. I did not find any evidence for increased limiting similarity or competition within single lineage communities, with most traits being clustered among co-occurring Asteraceae and Poaceae.

Trait evolution and community structure in Alberta grasslands

Many studies of community phylogenetic structure are based on the assumption that phylogenetic distances are a useful surrogate measure of the niche and trait similarity of species (Webb et al. 2002). In this study, and in other recent studies which have measured traits directly in addition to measuring phylogenetic similarity (Cavender-Bares et al. 2004, Cavender-Bares et al. 2006), patterns of trait evolution were more complicated than a simple dichotomy of conserved versus convergent evolution (e.g. Table 3-1-1). At broad phylogenetic scales, most traits were evolutionarily conserved, but within the two most speciose families at the study sites all possible patterns of trait evolution were observed (Table 3-5). Relationships between trait evolution and trait similarity in communities varied from positive to negative (Figure 3-4), in contrast with previous studies (Cavender-Bares et al. 2004, Cavender-Bares et al. 2006) which have found that traits which are more evolutionarily conserved tend to be overdispersed within single-lineage communities.

Phylogenetic similarity within communities will be highly dependent on the evolutionary history of traits and the relationship between trait evolution and trait similarity within communities at different spatial and phylogenetic scales, which seem to vary among lineages with different biogeographic histories. Mixedgrass and fescue grasslands contain species with different biogeographic affinities, with mixedgrass species generally having southern temperate distributions, while fescue grasslands contain predominantly species with northern temperate and boreal affinities (Leopold and Denton 1987), which may also explain some of the present-day variation in species and traits characteristic of these two grassland types (Ackerly 2003).

Competition versus environmental filtering in Alberta grasslands

The trait clustering found in this study (Tables 3-2 – 3-4) provides support for the assertion that environmental filtering of traits has a stronger influence on plant community structure than limiting similarity (Grime 2006). Although close relatives might be expected to compete more intensely and co-occur less often, the effect of phylogenetic relatedness on the strength of competition between species is not well understood, and subtle effects of limiting similarity among close relatives might easily be swamped by the strength of environmental filtering, especially if different sets of traits affect competition among species and the ability to colonize novel habitats (Losos et al. 2003), or if there is a lack of phylogenetic signal for traits related to within-habitat coexistence (Silvertown et al. 2006).

While most studies have assumed that competition will lead to limiting similarity and trait or phylogenetic overdispersion, diffuse competition could actually lead to a pattern of trait or phylogenetic clustering as a result of environmental filtering of traits that confer the ability to compete for resources (Grime 2006). This may explain why I found so little evidence for competitive exclusion, despite the fact that competition for resources is intense in these communities (Cahill 2003). Even if competitive exclusion has occurred at some point in the assembly of Alberta grassland communities, I could only sample species and traits from a post-competition pool from which species that are too similar to coexist may have already been excluded (Colwell and Winkler 1984).

Species characteristic of fescue and mixedgrass grasslands, and communities with composition similar to present-day communities in these grasslands have been widespread in the Great Plains region of North America since as long as 13 million years ago (Leopold and Denton 1987). If competitive exclusion within these broad habitat types were important it would surely have happened by now.

Filtering of leaf and root traits seems to play a role in the assembly of Alberta grassland communities, but the majority of variation in species co-occurrence could not be explained by trait or phylogenetic similarity. Numerous processes unrelated to leaf and root trait similarity such as dispersal from local and regional species pools (Ricklefs 2004), or other mechanisms that promote coexistence in the absence of niche differentiation (Chesson 2000) are also likely to contribute to contemporary patterns of co-occurrence. A portion of the unexplained variation in species co-occurrences may also be due to the non-linear (triangular) form of most co-occurrence versus similarity relationships (e.g. Figure 3-3), which are not well described by a linear correlation between matrices.

A lack of informative within-family phylogenetic branch lengths may have also contributed to the weak relationships between phylogenetic distances and co-occurrence. A relationship between co-occurrence and phylogenetic distances among species would only be expected if phylogenetic branch lengths correspond to the ecological similarity of species. Previous studies of community phylogenetic structure have generally used branch lengths based on molecular distances (e.g. Cavender-Bares et al. 2004, Slingsby and Verboom 2006), estimated divergence dates (e.g. Cavender-Bares et al. 2006, Kembel and Hubbell 2006), or uninformative branch lengths (Webb 2000, Cavender-Bares et al. 2006). There have been no quantitative comparisons of the relative merits of different branch lengths in studies of community phylogenetic structure (Mooers et al. 1999, Bromham et al. 2002), and more research on this subject is needed.

Effects of null model choice

I found evidence for non-random patterns of trait and phylogenetic similarity when using nulls 1 and 2, which randomize phylogenetic and trait patterns across species, or randomize species occurrences across samples. However, most patterns that were non-random according to nulls 1 and 2 were random according to null 3, which generated random communities while maintaining both species frequencies and sample richnesses. This difference was likely a result of the low species richness of most samples and the dominance of a few species at each site. For example, individual quadrats at mixedgrass sites contained an average of six species, and the six most frequent species at mixedgrass sites (*Stipa comata, Bouteloua gracilis, Carex filifolia, Koeleria macrantha, Sphaeralcea coccinea, Poa sandbergii*) were present in 40-70% of quadrats at those sites. Because null model 3 maintains both sample richness and species frequencies, the random communities generated by null 3 would tend to be almost identical to the observed communities, making it very difficult to find even the strongest relationship between co-occurrence and species similarity to be different from random. This idea is further supported by the fact that the data set in which communities were not dominated by a few common species (the quadrat-scale Asteraceae data) also tended to find similar patterns using nulls 1, 2, and 3.

By comparing co-occurrences with phylogenetic and trait similarity across species, instead of patterns of within-sample similarity, I avoided some of the problems with null models encountered by studies of phylogenetic and functional trait similarity within samples, which are highly sensitive to both sample richness and species frequencies (Kembel and Hubbell 2006). Future research on this topic should focus on developing and testing models that explicitly predict the effects of different ecological and evolutionary processes on community structure (e.g. Colwell and Winkler 1984), as opposed to current null models which have been designed to randomize species co-occurrences but do not necessarily correspond to any particular model of trait evolution or community assembly from a species pool (Gotelli 2001).

Conclusions

Several main conclusions may be drawn from this study. I found evidence suggesting that the environmental filtering of several leaf and root traits has led to widespread patterns of trait clustering within Alberta grassland communities, resulting in weak patterns of phylogenetic clustering or overdispersion depending on the evolutionary history of traits in different lineages and communities. Patterns of phylogenetic and trait clustering within communities were more common at mixedgrass sites, consistent with the idea that the more stressful conditions at these sites act as a stronger environmental filter. This study suggests that local communities must be viewed as the product both of contemporary ecological interactions, as well as historical factors including hierarchical patterns of trait evolution and the biogeographic history of species lineages.

Literature Cited

Ackerly, D. D. 2003. Community assembly, niche conservatism, and adaptive evolution in changing environments. International Journal of Plant Sciences **164**:S165-S184.

Al-Shehbaz, I. A., M. A. Beilstein, and E. A. Kellogg. 2006. Systematics and phylogeny of the Brassicaceae (Cruciferae): an overview. Plant Systematics and Evolution **259**:89-120.

Baldwin, B. G., B. L. Wessa, and J. L. Panero. 2002. Nuclear rDNA evidence for major lineages of helenioid Heliantheae (Compositae). Systematic Botany **27**:161-198.

Beck, J. B., G. L. Nesom, P. J. Calie, G. I. Baird, R. L. Small, and E. E. Schilling. 2004. Is subtribe Solidagininae (Asteraceae) monophyletic? Taxon **53**:691-698.

Bromham, L., M. Woolfit, M. S. Y. Lee, and A. Rambaut. 2002. Testing the relationship between molecular and morphological rates of change along phylogenies. Evolution **56**:1921-1930.

Cahill, J. F. 2003. Neighbourhood-scale diversity, composition and root crowding do not alter competition during drought in a native grassland. Ecology Letters **6**:599-603.

Cavender-Bares, J., D. D. Ackerly, D. A. Baum, and F. A. Bazzaz. 2004. Phylogenetic overdispersion in Floridian oak communities. American Naturalist **163**:823-843.

Cavender-Bares, J., A. Keen, and B. Miles. 2006. Phylogenetic structure of Floridian plant communities depends on taxonomic and spatial scale. Ecology **87**:S109-122.

Chesson, P. 2000. Mechanisms of maintenance of species diversity. Annual Review of Ecology and Systematics **31**:343-366.

Chetner, S. 2003. Agroclimatic atlas of Alberta, 1971 - 2000. Alberta Agriculture, Food and Rural Development, Edmonton, AB.

Cody, M. L. 1991. Niche theory and plant growth form. Plant Ecology **97**:39-55.

Colwell, R. K., and D. W. Winkler. 1984. A null model for null models in biogeography. Pages 344-359 *in* D. R. Strong, D. Simberloff, L. G. Abele, and A. B. Thistle, editors. Ecological communities: Conceptual issues and the evidence. Princeton University Press, Princeton, NJ.

Cornelissen, J. H. C., S. Lavorel, E. Garnier, S. Diaz, N. Buchmann, D. E. Gurvich, P. B. Reich, H. ter Steege, H. D. Morgan, M. G. A. van der Heijden, J. G. Pausas, and H. Poorter. 2003. A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. Australian Journal of Botany **51**:335-380.

Coupland, R. T. 1950. Ecology of mixed prairie in Canada. Ecological Monographs **20**:273-315.

Coupland, R. T. 1961. A reconsideration of grassland classification in the Northern Great Plains of North America. Journal of Ecology **49**:135-167.

Coupland, R. T., and T. C. Brayshaw. 1953. The fescue grassland in Saskatchewan. Ecology **34**:386-405.

Davies, T. J., T. G. Barraclough, M. W. Chase, P. S. Soltis, D. E. Soltis, and V. Savolainen. 2004. Darwin's abominable mystery: Insights from a supertree of the angiosperms. Proceedings of the National Academy of Sciences **101**:1904-1909.

Dayan, T., and D. Simberloff. 2005. Ecological and community-wide character displacement: the next generation. Ecology Letters **8**:875-894.

Diaz, S., J. G. Hodgson, K. Thompson, M. Cabido, J. H. C. Cornelissen, A. Jalili, G. Montserrat-Marti, J. P. Grime, F. Zarrinkamar, Y. Asri, S. R. Band, S. Basconcelo, P. Castro-Diez, G. Funes, B. Hamzehee, M. Khoshnevi, N. Perez-Harguindeguy, M. C. Perez-Rontome, F. A. Shirvany, F. Vendramini, S. Yazdani, R. Abbas-Azimi, A. Bogaard, S. Boustani, M. Charles, M. Dehghan, L. de Torres-Espuny, V. Falczuk, J. Guerrero-Campo, A. Hynd, G. Jones, E. Kowsary, F. Kazemi-Saeed, M. Maestro-Martinez, A. Romo-Diez, S. Shaw, B. Siavash, P. Villar-Salvador, and M. R. Zak. 2004. The plant traits that drive ecosystems: Evidence from three continents. Journal of Vegetation Science **15**:295-304.

Elton, C. 1946. Competition and the structure of ecological communities. Journal of Animal Ecology **15**:54-68.

Eriksson, T., M. S. Hibbs, A. D. Yoder, C. F. Delwiche, and M. J. Donoghue. 2003. The phylogeny of Rosoideae (Rosaceae) based on sequences of the internal transcribed spacers (ITS) of nuclear ribosomal DNA and the *trnL/F* region of chloroplast DNA. Int. J. Plant Sci **164**:197-211.

Gotelli, N. J. 2001. Research frontiers in null model analysis. Global Ecology and Biogeography **10**:337-343.

Gotelli, N. J., and G. L. Entsminger. 2003. Swap algorithms in null model analysis. Ecology **84**:532-535.

Grime, J. P. 2001. Plant strategies, vegetation processes and ecosystem properties. Wiley, Chichester, U.K.

Grime, J. P. 2006. Trait convergence and trait divergence in herbaceous plant communities: Mechanisms and consequences. Journal of Vegetation Science **17**:255-260.

Grime, J. P., K. Thompson, R. Hunt, J. G. Hodgson, J. H. C. Cornelissen, I. H. Rorison, G. A. F. Hendry, T. W. Ashenden, A. P. Askew, S. R. Band, R. E. Booth, C. C. Bossard, B. D. Campbell, J. E. L. Cooper, A. W. Davison, P. L. Gupta, W. Hall, D. W. Hand, M. A. Hannah, S. H. Hillier, D. J. Hodkinson, A. Jalili, Z. Liu, J. M. L. Mackey, N. Matthews, M. A. Mowforth, A. M. Neal, R. J. Reader, K. Reiling, W. RossFraser, R. E. Spencer, F. Sutton, D. E. Tasker, P. C. Thorpe, and J. Whitehouse. 1997. Integrated screening validates primary axes of specialisation in plants. Oikos **79**:259-281.

Harvey, P. H., and M. D. Pagel. 1991. The comparative method in evolutionary biology. Oxford University Press, Oxford, U.K.

Horner-Devine, M. C., and B. J. Bohannan. 2006. Phylogenetic clustering and overdispersion in bacterial communities. Ecology **87**:S100-108.

Kembel, S. W., and S. P. Hubbell. 2006. The phylogenetic structure of a Neotropical forest tree community. Ecology **87**:S86-S99.

Legendre, P., and L. Legendre. 1998. Numerical ecology, 2nd English edition. Elsevier, New York.

Leopold, E. B., and M. F. Denton. 1987. Comparative age of grassland and steppe east and west of the Northern Rocky Mountains. Annals of the Missouri Botanical Garden **74**:841-867.

Levin, S. A. 1992. The problem of pattern and scale in ecology. Ecology **73**:1943-1967.

Losos, J. B. 1996. Phylogenetic perspectives on community ecology. Ecology **77**:1344-1354.

Losos, J. B., M. Leal, R. E. Glor, K. de Queiroz, P. E. Hertz, L. R. Schettino, A. C. Lara, T. R. Jackman, and A. Larson. 2003. Niche lability in the evolution of a Caribbean lizard community. Nature **424**:542-545.

Lovette, I. J., and W. M. Hochachka. 2006. Simultaneous effects of phylogenetic niche conservatism and competition on avian community structure. Ecology **87**:S14-28.

Macarthur, R., and R. Levins. 1967. The limiting similarity, convergence, and divergence of coexisting species. American Naturalist **101**:377-385.

Manly, B. F. J. 1997. Randomization, bootstrap and Monte Carlo methods in biology. Chapman & Hall, London, U.K.

Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. Cancer Research **27**:209-220.

Mooers, A. O., S. M. Vamosi, and D. Schluter. 1999. Using phylogenies to test macroevolutionary hypotheses of trait evolution in Cranes (Gruinae). American Naturalist **154**:249-259.

Moss, E. H., and J. A. Campbell. 1947. The fescue grassland of Alberta. Canadian Journal of Research **25**:209–227.

Natural Regions Committee. 2006. Natural regions and subregions of Alberta. Publication Nmber T/852, Government of Alberta.

Noyes, R. D., and L. H. Rieseberg. 1999. ITS sequence data support a single origin for North American Astereae (Asteraceae) and reflect deep geographic divisions in *Aster* s.l. American Journal of Botany **86**:398-412.

Pacala, S. W., and D. Tilman. 1994. Limiting similarity in mechanistic and spatial models of plant competition in heterogeneous environments. American Naturalist **143**:222-257.

Prinzing, A., W. Durka, S. Klotz, and R. Brandl. 2001. The niche of higher plants: evidence for phylogenetic conservatism. Proceedings of the Royal Society of London, Series B: Biological Sciences **268**:2383-2389.

R Development Core Team. 2006. R: A Language and Environment for Statistical Computing.

Ricklefs, R. E. 2004. A comprehensive framework for global patterns in biodiversity. Ecology Letters **7**:1-15.

Ricklefs, R. E., and J. Travis. 1980. A morphological approach to the study of avian community organization. Auk **97**:321-338.

Roberts, A., and L. Stone. 1990. Island-sharing by archipelago species. Oecologia **83**:560-567.

Salamin, N. 2002. Building supertrees: An empirical assessment using the grass family (Poaceae). Systematic Biology **51**:136-150.

Silvertown, J., K. McConway, D. Gowing, M. Dodd, M. F. Fay, J. A. Joseph, and K. Dolphin. 2006. Absence of phylogenetic signal in the niche structure of meadow plant communities. Proceedings of the Royal Society, Series B: Biological Sciences **273**:39-44.

Slingsby, J. A., and G. A. Verboom. 2006. Phylogenetic relatedness limits co-occurrence at fine spatial scales: Evidence from the schoenoid sedges (Cyperaceae: Schoeneae) of the Cape Floristic Region, South Africa. Am Nat **168**:14-27.

Southwood, T. R. E. 1977. Habitat, the templet for ecological strategies? Journal of Animal Ecology **46**:336-365.

Stubbs, W. J., and B. Wilson. 2004. Evidence for limiting similarity in a sand dune community. Journal of Ecology **92**:557-567.

Warwick, R. M., and K. R. Clarke. 1998. Taxonomic distinctness and environmental assessment. Journal of Applied Ecology **35**:532-543.

Webb, C. O. 2000. Exploring the phylogenetic structure of ecological communities: An example for rain forest trees. American Naturalist **156**:145-155.

Webb, C. O., D. D. Ackerly, and S. W. Kembel. 2006. Phylocom: software for the analysis of community phylogenetic structure and character evolution. Version 3.40. http://www.phylodiversity.net/phylocom/.

Webb, C. O., D. D. Ackerly, M. A. McPeek, and M. J. Donoghue. 2002. Phylogenies and community ecology. Annual Review of Ecology and Systematics **33**:475-505.

Webb, C. O., and M. J. Donoghue. 2005. Phylomatic: tree assembly for applied phylogenetics. Molecular Ecology Notes **5**:181-183.

Weiher, E., G. D. P. Clarke, and P. A. Keddy. 1998. Community assembly rules, morphological dispersion, and the coexistence of plant species. Oikos **81**:309-322.

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

Westoby, M., D. S. Falster, A. T. Moles, P. A. Vesk, and I. J. Wright. 2002. Plant ecological strategies: Some leading dimensions of variation between species. Annual Review of Ecology and Systematics **33**:125-159.

Wiens, J. J., and M. J. Donoghue. 2004. Historical biogeography, ecology and species richness. Trends in Ecology & Evolution **19**:639-644.

Wiens, J. J., and C. H. Graham. 2005. Niche conservatism: Integrating evolution, ecology, and conservation biology. Annual Review of Ecology Evolution and Systematics **36**:519-539.

Wojciechowski, M. F., M. J. Sanderson, K. P. Steele, and A. Liston. 2000. Molecular phylogeny of the temperate herbaceous tribes of Papilionoid legumes: a supertree approach. Pages 277-298 *in* P. S. Herendeen and A. Bruneau, editors. Advances in legume systematics. Royal Botanic Gardens, Kew.

Table 3-1. Phylogenetic similarity (correlation between species co-occurrences and phylogenetic distances) within samples at a range of spatial scales, extents and phylogenetic scales in Alberta grasslands. Positive correlations indicate phylogenetic overdispersion, negative correlations indicate phylogenetic clustering. See methods section for a description of null models used to generate *P*-values.

						P-values		
Phylogenetic scale	Spatial extent	Spatial scale	Species	Samples	Correlation	Null 1	Null 2	Null 3
Angiosperms	All sites	Plots	76	27	0.02	0.250	0.218	0.497
Angiosperms	All sites	Quadrats	76	270	0.00	0.459	0.448	0.425
Angiosperms	Fescue	Plots	52	10	0.03	0.249	0.188	0.765
Angiosperms	Fescue	Quadrats	52	100	0.01	0.435	0.392	0.877
Angiosperms	Mixedgrass	Plots	42	17	-0.06	0.121	0.146	***0.069***
Angiosperms	Mixedgrass	Quadrats	42	170	-0.10	**0.024**	**0.006**	**0.038**
Asteraceae	All sites	Plots	23	27	-0.05	0.261	0.258	0.937
Asteraceae	All sites	Quadrats	23	270	-0.01	0.288	0.422	0.999
Asteraceae	Fescue	Plots	13	10	0.10	0.218	0.218	***0.099***
Asteraceae	Fescue	Quadrats	13	100	0.11	0.248	0.214	***0.065***
Asteraceae	Mixedgrass	Plots	15	17	-0.15	***0.062***	***0.096***	0.608
Asteraceae	Mixedgrass	Quadrats	15	170	-0.02	0.222	0.394	0.736
Poaceae	All sites	Plots	13	27	0.06	0.367	0.318	***0.097***
Poaceae	All sites	Quadrats	13	270	0.06	0.325	0.316	0.369
Poaceae	Fescue	Plots	11	10	-0.16	0.181	0.156	0.486
Poaceae	Fescue	Quadrats	11	100	-0.13	0.214	0.210	0.437
Poaceae	Mixedgrass	Plots	7	17	0.16	0.345	0.340	0.349
Poaceae	Mixedgrass	Quadrats	7	170	0.35	***0.100***	***0.080***	0.149

Table 3-2. Trait similarity among angiosperms (correlation between species co-occurrences and trait distances) within samples at a range of spatial scales and extents in Alberta grasslands. See methods section for a description of null models. Positive correlations indicate trait overdispersion, negative correlations indicate trait clustering.

				P-values		
Spatial extent	Trait	Spatial scale	Correlation	Null 1	Null 2	Null 3
All sites	Leaf area	Plots	-0.03	0.218	0.210	0.229
All sites	Leaf area	Quadrats	0.00	0.477	0.408	0.328
All sites	Leaf thickness	Plots	-0.03	0.244	0.190	0.518
All sites	Leaf thickness	Quadrats	-0.06	***0.102***	**0.022**	0.918
All sites	Root diameter	Plots	0.01	0.422	0.418	0.288
All sites	Root diameter	Quadrats	0.00	0.481	0.460	0.837
All sites	SLA	Plots	-0.07	***0.093***	***0.078***	0.545
All sites	SLA	Quadrats	-0.04	0.197	***0.094***	0.959
All sites	SLV	Plots	-0.07	***0.083***	**0.048**	0.806
All sites	SLV	Quadrats	-0.07	***0.061***	**0.008**	0.968
All sites	SRL	Plots	0.03	0.279	0.234	0.968
All sites	SRL	Quadrats	0.02	0.325	0.292	0.989
All sites	SRV	Plots	-0.08	**0.022**	**0.014**	0.418
All sites	SRV	Quadrats	-0.03	0.156	***0.092***	0.977
Fescue	Leaf area	Plots	-0.03	0.247	0.260	0.326
Fescue	Leaf area	Quadrats	-0.06	0.134	***0.070***	0.378
Fescue	Leaf thickness	Plots	0.06	0.254	0.158	0.695
Fescue	Leaf thickness	Quadrats	0.02	0.354	0.288	0.686
Fescue	Root diameter	Plots	0.00	0.466	0.444	0.496
Fescue	Root diameter	Quadrats	0.02	0.347	0.254	0.896
Fescue	SLA	Plots	-0.04	0.306	0.278	0.448
Fescue	SLA	Quadrats	0.00	0.502	0.474	0.865
Fescue	SLV	Plots	-0.02	0.368	0.354	0.458
Fescue	SLV	Quadrats	-0.04	0.248	0.146	0.916
Fescue	SRL	Plots	0.02	0.392	0.354	0.510
Fescue	SRL	Quadrats	0.00	0.487	0.528	0.747
Fescue	SRV	Plots	-0.07	0.122	0.084	***0.076***
Fescue	SRV	Quadrats	-0.09	***0.055***	***0.018***	***0.078***
Mixedgrass	Leaf area	Plots	0.01	0.412	0.424	0.419
Mixedgrass	Leaf area	Quadrats	-0.02	0.350	0.300	0.786
Mixedgrass	Leaf thickness	Plots	-0.12	**0.027**	**0.026**	0.568
Mixedgrass	Leaf thickness	Quadrats	-0.09	**0.039**	**0.010**	0.977
Mixedgrass	Root diameter	Plots	-0.05	0.212	0.210	0.217
Mixedgrass	Root diameter	Quadrats	-0.05	0.200	0.102	0.739
Mixedgrass	SLA	Plots	-0.11	**0.078**	***0.082***	0.459
Mixedgrass	SLA	Quadrats	-0.11	**0.020**	**0.010**	0.459
Mixedgrass	SLV	Plots	-0.13	**0.037**	***0.060***	0.598
Mixedgrass	SLV	Quadrats	-0.15	**0.000**	**0.004**	0.826
Mixedgrass	SRL	Plots	-0.05	0.231	0.214	0.769
Mixedgrass	SRL	Quadrats	-0.07	0.139	**0.048**	0.986
Mixedgrass	SRV	Plots	-0.16	**0.005**	**0.010**	0.368
Mixedgrass	SRV	Quadrats	-0.16	**0.000**	**0.002**	0.440

Table 3-3. Trait similarity among Asteraceae (correlation between species co-occurrences and trait distances) within samples at a range of spatial scales and extents in Alberta grasslands. See methods section for a description of null models. Positive correlations indicate trait overdispersion, negative correlations indicate trait clustering.

				P-values		
Spatial extent	Trait	Spatial scale	Correlation (r)	Null 1	Null 2	Null 3
All sites	Leaf area	Plots	-0.02	0.374	0.362	0.178
All sites	Leaf area	Quadrats	-0.06	0.193	0.198	**0.038**
All sites	Leaf thickness	Plots	0.06	0.268	0.230	***0.099***
All sites	Leaf thickness	Quadrats	0.12	***0.054***	**0.034**	**0.008**
All sites	Root diameter	Plots	-0.18	**0.011**	**0.028**	0.130
All sites	Root diameter	Quadrats	-0.13	**0.017**	**0.028**	0.197
All sites	SLA	Plots	0.00	0.498	0.444	0.478
All sites	SLA	Quadrats	0.05	0.220	0.224	***0.070***
All sites	SLV	Plots	-0.05	0.285	0.268	0.839
All sites	SLV	Quadrats	0.01	0.450	0.418	0.027
All sites	SRL	Plots	-0.07	0.207	0.214	0.490
All sites	SRL	Quadrats	-0.04	0.335	0.320	0.729
All sites	SRV	Plots	-0.15	**0.031**	**0.034**	0.127
All sites	SRV	Quadrats	-0.16	**0.002**	**0.010**	**0.027**
Fescue	Leaf area	Plots	-0.13	0.161	0.158	0.289
Fescue	Leaf area	Quadrats	-0.19	0.127	***0.090***	**0.006**
Fescue	Leaf thickness	Plots	0.28	**0.001**	**0.002**	**0.010**
Fescue	Leaf thickness	Quadrats	0.22	***0.078***	***0.064***	0.109
Fescue	Root diameter	Plots	-0.33	**0.016**	**0.024**	0.256
Fescue	Root diameter	Quadrats	-0.39	**0.023**	**0.004**	0.267
Fescue	SLA	Plots	0.14	0.130	0.152	0.399
Fescue	SLA	Quadrats	0.21	***0.089***	***0.084***	***0.079***
Fescue	SLV	Plots	0.09	0.277	0.274	0.140
Fescue	SLV	Quadrats	-0.18	0.169	0.116	0.639
Fescue	SRL	Plots	-0.19	***0.091***	0.110	0.366
Fescue	SRL	Quadrats	-0.11	0.289	0.222	0.659
Fescue	SRV	Plots	-0.13	0.146	0.178	0.239
Fescue	SRV	Quadrats	-0.38	**0.006**	**0.010**	**0.016**
Mixedgrass	Leaf area	Plots	0.05	0.357	0.332	0.478
Mixedgrass	Leaf area	Quadrats	-0.06	0.259	0.306	0.969
Mixedgrass	Leaf thickness	Plots	0.07	0.310	0.304	0.155
Mixedgrass	Leaf thickness	Quadrats	0.18	***0.056***	**0.030**	***0.080***
Mixedgrass	Root diameter	Plots	-0.14	0.145	0.162	0.115
Mixedgrass	Root diameter	Quadrats	-0.12	***0.096***	0.122	0.999
Mixedgrass	SLA	Plots	-0.01	0.416	0.460	0.408
Mixedgrass	SLA	Quadrats	0.06	0.265	0.278	0.827
Mixedgrass	SLV	Plots	-0.01	0.425	0.440	0.749
Mixedgrass	SLV	Quadrats	0.06	0.252	0.248	0.420
Mixedgrass	SRL	Plots	-0.01	0.442	0.464	0.460
Mixedgrass	SRL	Quadrats	-0.04	0.388	0.338	**0.027**
Mixedgrass	SRV	Plots	-0.28	**0.020**	**0.020**	**0.006**
Mixedgrass	SRV	Quadrats	-0.22	**0.000**	**0.018**	***0.069***

Table 3-4. Trait similarity among Poaceae (correlation between species co-occurrences and trait distances) within samples at a range of spatial scales and extents in Alberta grasslands. See methods section for a description of null models. Positive correlations indicate trait overdispersion, negative correlations indicate trait clustering.

				P-values		
Spatial extent	Trait	Spatial scale	Correlation (r)	Null 1	Null 2	Null 3
All sites	Leaf area	Plots	-0.25	**0.047**	**0.032**	0.177
All sites	Leaf area	Quadrats	-0.26	**0.032**	**0.016**	0.469
All sites	Leaf thickness	Plots	-0.04	0.371	0.392	0.820
All sites	Leaf thickness	Quadrats	-0.03	0.390	0.352	0.995
All sites	Root diameter	Plots	-0.23	***0.082***	***0.054***	0.866
All sites	Root diameter	Quadrats	-0.19	0.116	**0.038**	0.996
All sites	SLA	Plots	-0.01	0.440	0.498	0.689
All sites	SLA	Quadrats	-0.08	0.305	0.254	0.798
All sites	SLV	Plots	-0.28	**0.036**	**0.026**	0.896
All sites	SLV	Quadrats	-0.26	**0.023**	**0.026**	0.997
All sites	SRL	Plots	-0.35	**0.020**	**0.028**	0.777
All sites	SRL	Quadrats	-0.26	**0.043**	**0.010**	0.997
All sites	SRV	Plots	-0.25	**0.036**	**0.022**	**0.018**
All sites	SRV	Quadrats	-0.16	0.114	***0.088***	**0.020**
Fescue	Leaf area	Plots	-0.42	**0.029**	**0.018**	0.286
Fescue	Leaf area	Quadrats	-0.32	**0.035**	**0.014**	0.968
Fescue	Leaf thickness	Plots	-0.09	0.324	0.312	0.128
Fescue	Leaf thickness	Quadrats	0.02	0.449	0.454	0.820
Fescue	Root diameter	Plots	-0.31	0.108	**0.048**	0.255
Fescue	Root diameter	Quadrats	-0.07	0.422	0.332	0.770
Fescue	SLA	Plots	-0.02	0.439	0.428	0.125
Fescue	SLA	Quadrats	-0.03	0.425	0.410	0.928
Fescue	SLV	Plots	-0.49	**0.004**	**0.004**	0.319
Fescue	SLV	Quadrats	-0.28	**0.062**	**0.028**	0.979
Fescue	SRL	Plots	-0.44	**0.045**	**0.024**	0.336
Fescue	SRL	Quadrats	-0.17	0.236	0.130	0.928
Fescue	SRV	Plots	-0.17	0.245	0.182	0.550
Fescue	SRV	Quadrats	-0.09	0.296	0.248	0.959
Mixedgrass	Leaf area	Plots	-0.46	**0.036**	**0.046**	0.179
Mixedgrass	Leaf area	Quadrats	-0.59	**0.000**	**0.018**	**0.029**
Mixedgrass	Leaf thickness	Plots	0.33	0.154	0.134	0.256
Mixedgrass	Leaf thickness	Quadrats	0.21	0.263	0.256	0.816
Mixedgrass	Root diameter	Plots	-0.10	0.399	0.322	0.509
Mixedgrass	Root diameter	Quadrats	-0.31	0.116	0.112	0.246
Mixedgrass	SLA	Plots	0.02	0.602	0.484	0.426
Mixedgrass	SLA	Quadrats	-0.41	0.139	***0.072***	0.226
Mixedgrass	SLV	Plots	-0.05	0.327	0.406	0.257
Mixedgrass	SLV	Quadrats	-0.30	0.111	0.130	**0.008**
Mixedgrass	SRL	Plots	-0.69	***0.099***	**0.008**	0.226
Mixedgrass	SRL	Quadrats	-0.57	**0.039**	**0.024**	0.989
Mixedgrass	SRV	Plots	-0.43	***0.081***	**0.048**	0.238
Mixedgrass	SRV	Quadrats	-0.08	0.395	0.426	0.998

Table 3-5. Trait evolution (correlation between phylogenetic distances and trait distances) within samples at a range of spatial scales, extents and phylogenetic scales in Alberta grasslands. Positive correlations indicate trait conservatism, negative correlations indicate trait convergence.

		Angiosperms		Asteraceae		Poaceae	
Spatial extent	Trait	Correlation (r)	P-value	Correlation (r)	P-value	Correlation (r)	P-value
All sites	SLA	0.06	0.101	-0.05	0.217	-0.12	0.228
All sites	Leaf area	0.06	**0.042**	0.05	0.182	0.13	0.187
All sites	Leaf thickness	0.06	***0.072***	-0.01	0.324	-0.01	0.492
All sites	SLV	0.11	**0.005**	0.06	0.185	0.24	**0.038**
All sites	SRL	0.04	0.118	0.03	0.326	0.33	**0.026**
All sites	SRV	0.20	**0.001**	0.02	0.394	0.02	0.390
All sites	Root diameter	0.11	**0.001**	-0.03	0.318	0.33	**0.016**
Mixedgrass	SLA	0.07	0.210	-0.05	0.267	-0.50	**0.017**
Mixedgrass	Leaf area	0.05	0.214	0.00	0.485	-0.03	0.415
Mixedgrass	Leaf thickness	0.03	0.323	-0.07	0.191	0.24	0.206
Mixedgrass	SLV	0.12	***0.061***	0.02	0.484	0.14	0.316
Mixedgrass	SRL	0.04	0.294	0.09	0.143	-0.05	0.507
Mixedgrass	SRV	0.23	**0.001**	-0.01	0.417	-0.24	0.209
Mixedgrass	Root diameter	0.12	**0.047**	-0.01	0.385	0.12	0.350
Fescue	SLA	0.08	**0.028**	0.30	**0.014**	-0.17	0.183
Fescue	Leaf area	0.05	***0.071***	0.02	0.420	0.28	0.039
Fescue	Leaf thickness	0.09	**0.021**	0.09	0.211	-0.01	0.502
Fescue	SLV	0.15	**0.001**	0.20	0.102	0.35	**0.027**
Fescue	SRL	0.05	0.103	-0.26	**0.017**	0.30	***0.051***
Fescue	SRV	0.18	**0.001**	-0.07	0.321	-0.05	0.392
Fescue	Root diameter	0.10	**0.003**	-0.21	***0.071***	0.29	***0.054***

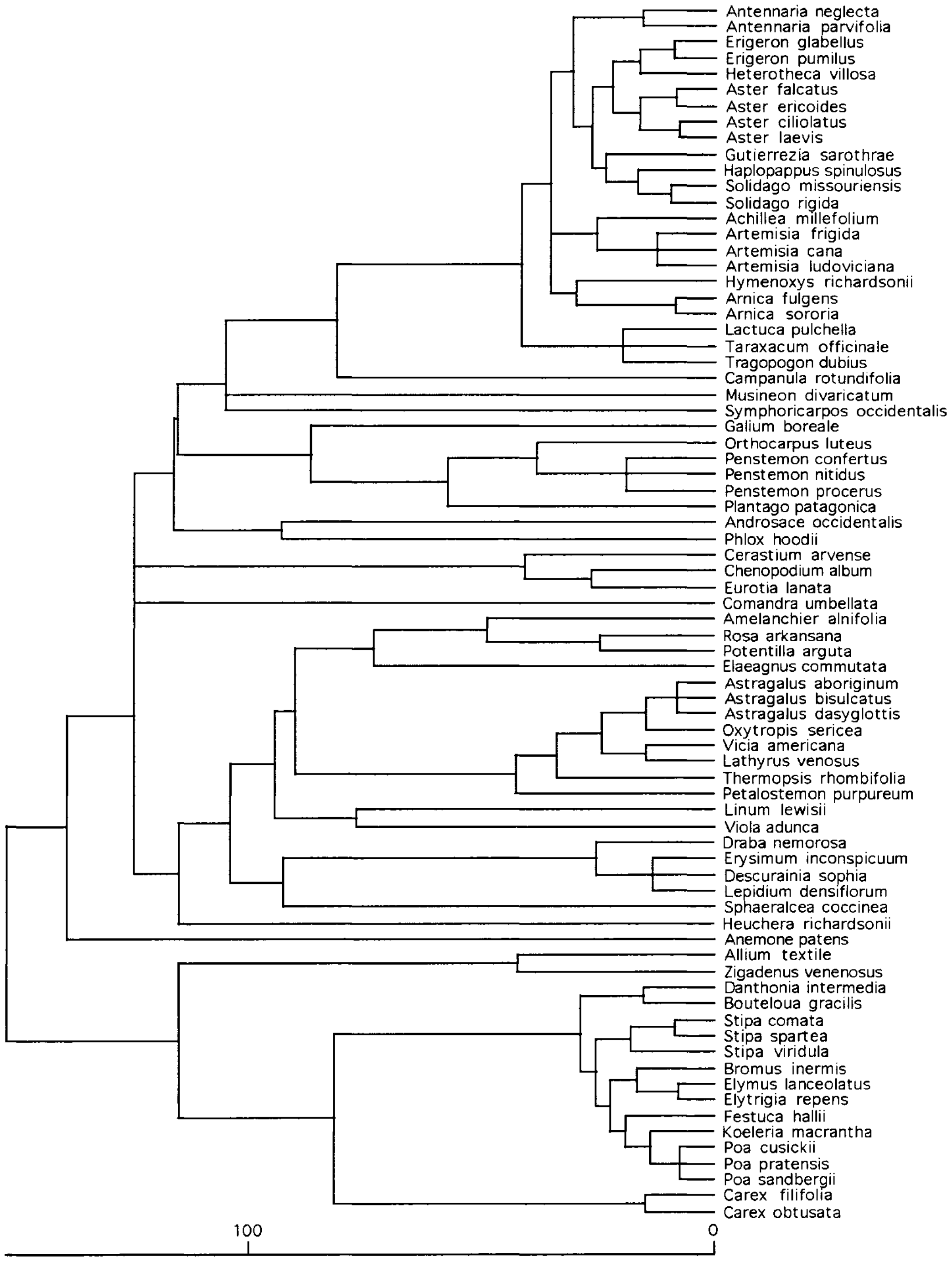

Figure 3-1. Hypothesized phylogenetic relationships among 76 angiosperm species sampled at sites in mixedgrass and fescue grasslands in Alberta. Branch lengths are in millions of years (Ma). The backbone of the tree is based on the Davies et al. (2004) angiosperm supertree; see text for further details and sources of phylogenetic data.

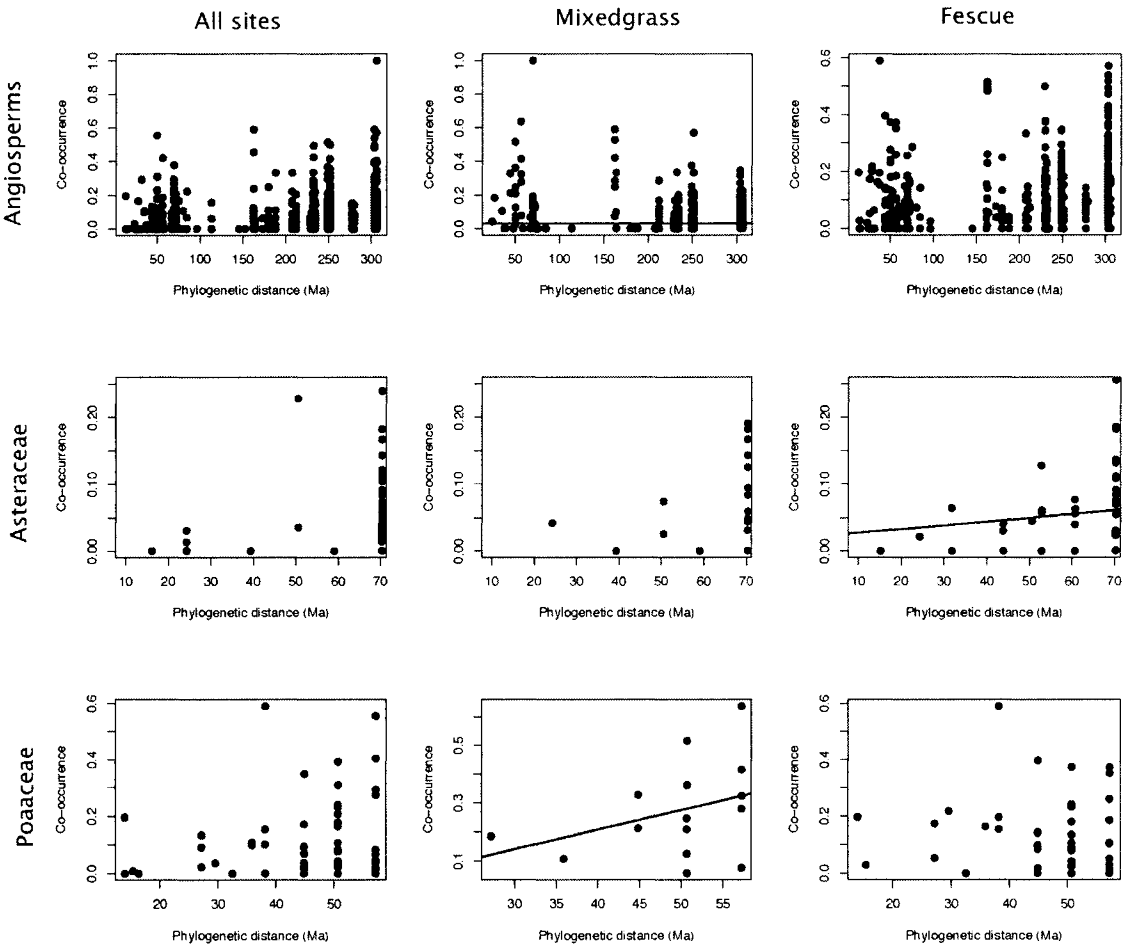

Figure 3-2. Co-occurrence in quadrats versus phylogenetic distance (Ma) for angiosperm, Asteraceae and Poaceae species occurring in all sites and separately by natural region in Alberta grasslands. A line of best fit is indicated for relationships that were significant according to randomization tests (*P* < 0.10; Table 3-1). Positive relationships indicate phylogenetic overdispersion, negative relationships indicate phylogenetic clustering.

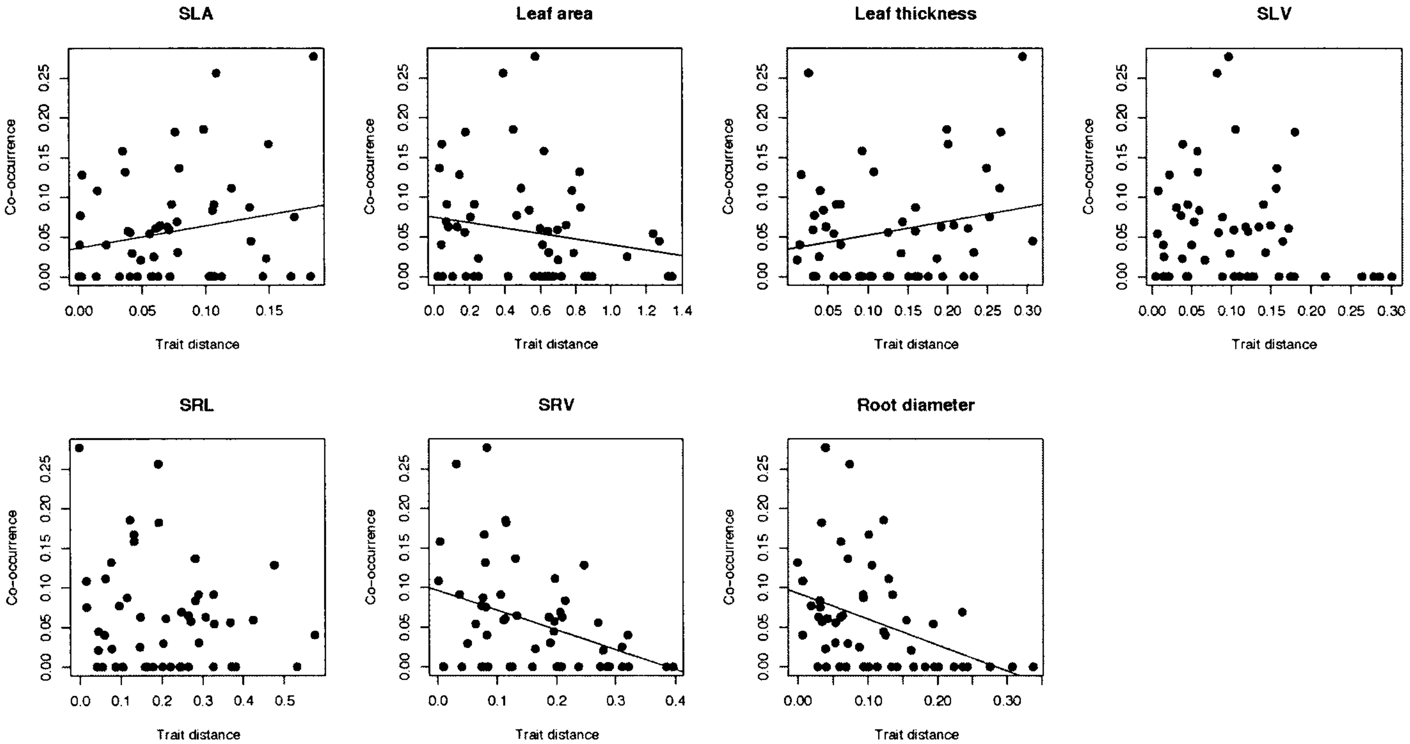

Figure 3-3. Co-occurrence in quadrats versus trait distance for seven leaf and root traits of Asteraceae species occurring at a fescue grassland in Alberta. A line of best fit is indicated for relationships that were significant according to randomization tests ($P < 0.10$; Table 3-3). Positive relationships indicate trait overdispersion, negative relationships indicate trait clustering.

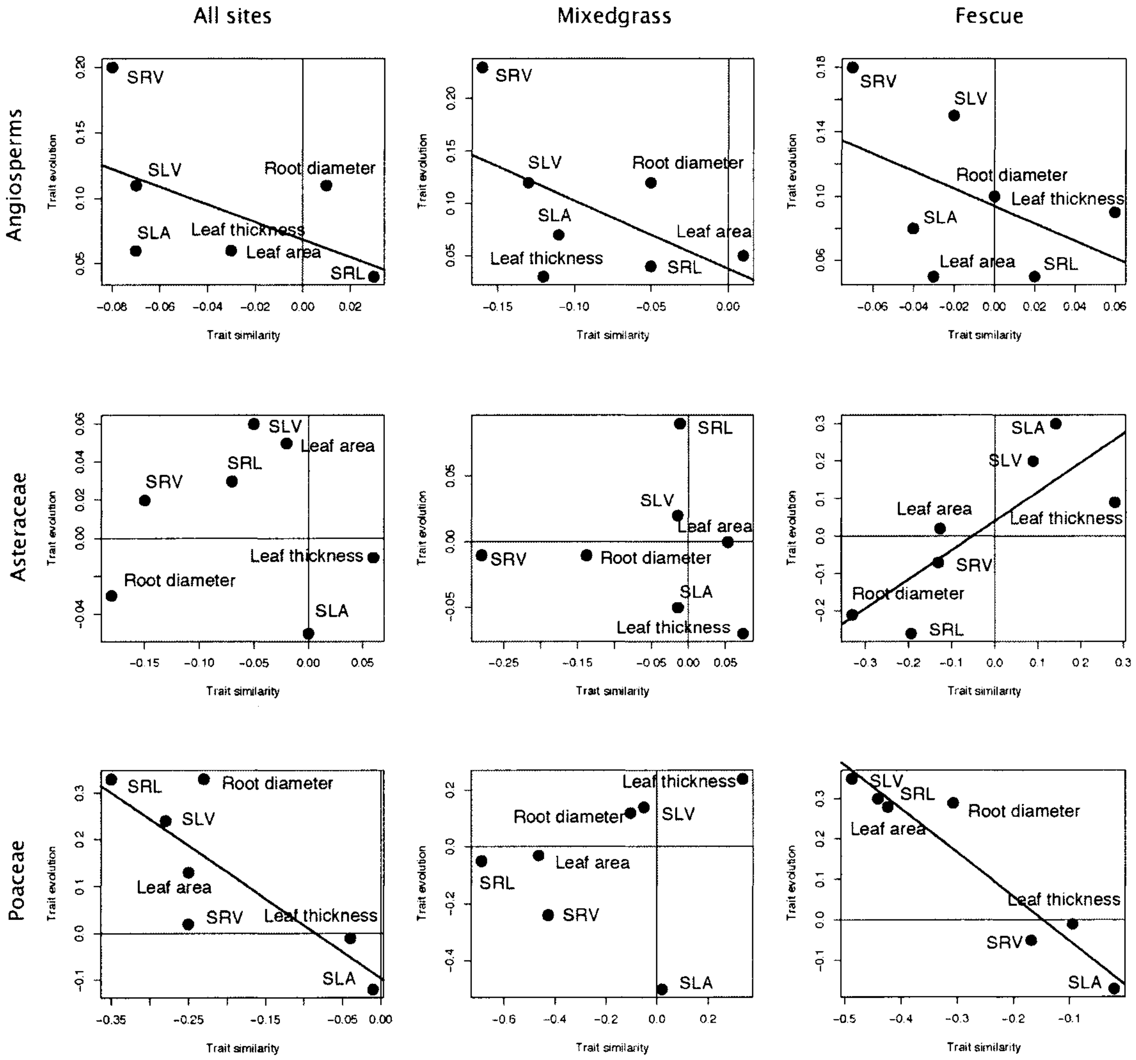

Figure 3-4. Relationships between trait similarity in plots and trait evolution for seven leaf and root traits of angiosperm, Asteraceae and Poaceae species occurring in all sites and separately by natural region in Alberta grasslands. Trait similarity is the correlation between species co-occurrence in plots and trait distances, with positive correlations indicating overdispersion and negative correlations indicating clustering. Trait evolution is the correlation between species phylogenetic distances and trait distances, with positive correlations indicating conservatism and negative correlations indicating convergence. A line of best fit is indicated for relationships that were significant according to a linear regression ($P < 0.05$). Positive relationships indicate that traits that are phylogenetically conserved are more overdispersed within communities. Negative relationships indicate that traits that are phylogeneticically conserved are more clustered within communities.

Chapter 4 The phylogenetic structure of a neotropical forest tree community[1]

Introduction

Why are there so many species of trees in the tropics? Tropical forests are incredibly biologically diverse, and numerous ecological and evolutionary processes such as niche differentiation, herbivory, dispersal, competition, parasitism, and disease may interact to play a role in maintaining the high species diversity of tropical tree communities at a range of spatial scales (Wright 2002). Although all of these processes have been demonstrated to occur, their relative importance in structuring ecological communities is not well understood. Numerous studies have demonstrated niche differentiation and habitat specialization in tropical tree species (Hubbell and Foster 1983, Condit et al. 1996, Clark et al. 1998, Webb and Peart 2000, Harms et al. 2001), but the strength of habitat specialization is often not sufficient to explain observed levels of species richness in tropical forests (Webb and Peart 2000, Harms et al. 2001). Similarly, finding niche differentiation among species does not mean that niche differences are more important than species-neutral processes in structuring a community (Hubbell 2001). The phylogenetic structure of ecological communities may provide insights into the relative importance of different ecological processes, as these processes interact with the evolutionary history of plant traits and leave their signature on the phylogenetic structure of a community (Webb et al. 2002).

Previous studies have identified two general types of processes that can interact with phylogenetic patterns of niche and trait evolution to give rise to non-random phylogenetic community structure, namely competitive exclusion and environmental filtering processes (Webb 2000, Webb et al. 2002, Cavender-Bares et al. 2004).

Competitive exclusion and other negative density-dependent interactions among ecologically similar species can create non-random community phylogenetic structures. Many processes such as herbivory (Novotny et al. 2002) and competition (Uriarte et al.

---

[1] A version of this chapter has been published:
Kembel, S. W., and S. P. Hubbell. 2006. The phylogenetic structure of a Neotropical forest tree community. Ecology **87**:S86-S99.

2004) can have negative density-dependent effects not only on conspecific individuals, but on close phylogenetic relatives as well. Density-dependent processes that negatively affect close phylogenetic relatives could give rise to phylogenetic overdispersion of co-occurring species, meaning that co-occurring species are more distantly phylogenetically related than expected by chance. Direct competitive exclusion as well as indirect interactions among relatives mediated by herbivores, parasites or pathogens could all give rise to the same pattern of community phylogenetic overdispersion (Webb et al. 2002).

Environmental filters (Weiher and Keddy 1995, Webb et al. 2002, Cavender-Bares et al. 2004) or assembly rules (Weiher and Keddy 1999) restricting community membership to individuals possessing certain traits may also affect community phylogenetic structure. If environmental conditions in a habitat act as a filter selecting for species possessing certain traits, I would expect to find either phylogenetic clustering or overdispersion, depending on the evolutionary history of ecologically important traits and species niches (Cavender-Bares et al. 2004, Ackerly et al. 2006, Cavender-Bares et al. 2006, Silvertown et al. 2006).

The phylogenetic structure of a community may also be random. If species-neutral interactions (Hubbell 2001) structure a community, if the strength of density-dependent and environmental filtering processes are balanced or weak, or if species niches or traits are phylogenetically random, local communities could exhibit phylogenetic structures indistinguishable from random.

Given the variation of measures of community structure such as taxonomic and functional diversity with spatial scale and extent (Levin 1992), and changes in the phylogenetic signal of niches and traits with spatial scale (Cavender-Bares et al. 2006, Silvertown et al. 2006), I would predict that the phylogenetic structure of a community will be highly dependent on the spatial scale and extent used to define the community.

The choice of an appropriate null model to use when measuring the structure of ecological communities has been very contentious (Gotelli and Graves 1996, Gotelli 2004), since analyses of the same data set with different null models can lead to very different conclusions. Simulation studies have been used to directly assess the performance of different null models when measuring species co-occurrence patterns (e.g. Gotelli 2000), and a number of different null models have been used to measure community phylogenetic structure, but the effects of different null models on estimates of community phylogenetic structure have not been evaluated quantitatively.

In this study, I used large data sets on the phylogenetic relationships and co-occurrence patterns of neotropical forest tree species in communities at a range of local spatial scales (10x10m to 100x100m) to address several questions. First, I compared the performance of different null models used to study the phylogenetic structure of ecological communities. Second, I asked whether trees occurring together in communities at a range of local spatial scales are phylogenetically clustered or overdispersed, in order to test the relative importance of different ecological processes in structuring these communities, and to test whether community phylogenetic structure changes with spatial scale. Third, I asked whether the phylogenetic structure of tree communities differs among habitats characterized by different environmental conditions.

Methods

Ecological data

I estimated the phylogenetic structure of a neotropical forest tree community using data from the 50-hectare Forest Dynamics Plot (FDP) on Barro Colorado Island in the Republic of Panama. The moist lowland forests in the 1000x500m FDP receive approximately 2600mm of rain each year, with a dry season from January to April, and average annual temperatures of 27°C (Dietrich et al. 1982). Within the FDP, a variety of habitats have been identified (Harms et al. 2001), including (in approximate order of decreasing water availability during the dry season) swamp, stream, slope, and upland plateaus. The majority of the FDP contains old-growth primary forests, although some relatively young secondary forest habitat is found within the plot (Harms et al. 2001).

Within the 1000x500m FDP, all tree and shrub stems with diameter at breast height (DBH) $\geq$ 1cm have been mapped and identified to species in repeated censuses conducted since 1982 (Condit 1998). I measured the phylogenetic structure of the forests on Barro Colorado Island using data on occurrence of tree and shrub species in the 50-hectare Forest Dynamics Plot. Mapped tree locations from the 1982 census of the FDP were divided into square non-overlapping quadrats of four different spatial scales (10x10m, 20x20m, 50x50m, and 100x100m). I defined communities at a given spatial scale to include species of all tree and shrub stems with DBH ≥ 1cm present together in individual quadrats at that scale. Although I refer to the communities in the FDP as tree communities throughout this paper for the sake of convenience, shrubs with DBH ≥ 1cm were also included in all analyses of community structure.

Phylogenetic data

I constructed a hypothesized phylogenetic tree for the 312 tree species occurring in the FDP using Phylomatic version R20031202 software (Webb and Donoghue 2005), a phylogenetic database and toolkit for the assembly of phylogenetic trees. The tree created by Phylomatic used information from numerous published molecular phylogenies to create a tree containing all of the species in the FDP, based on Phylomatic reference tree R20031202 with the APG II (Angiosperm Phylogeny Group 2003; Stevens 2005) phylogenetic classification of flowering plant families forming the backbone of the tree. In the absence of detailed information on phylogenetic relationships within many of the 55 families and 192 genera found in the FDP, I assumed families and genera were monophyletic and polytomous when placing them on the tree. I assigned branch lengths to the phylogenetic tree using the BLADJ module of the Phylocom version 3.19 software package (Webb et al. 2004), creating a pseudo-chronogram with branch lengths based on clade ages reported by Wikström et al. (2001). Nodes in the phylogenetic tree for which age estimates were available were fixed at their estimated ages (Wikström et al. 2001), and all remaining branch lengths were set by spacing undated nodes in the tree evenly between dated nodes to minimize variance in branch lengths. Age estimates were available for 34 of the 122 internal nodes in the phylogeny. Although 57 of the 122 internal nodes were polytomous, the majority of these polytomies were within families and genera, with the backbone of the tree relatively well resolved and dated. The resulting phylogenetic tree (Figure 4-1) was used for all subsequent analyses of community phylogenetic structure.

Community phylogenetic structure

I calculated several measures of community phylogenetic structure for all quadrats at each spatial scale. All analyses of community phylogenetic structure were conducted using the Phylocom version 3.19 software package (Webb et al. 2004). Three steps were taken to measure community phylogenetic structure in each quadrat. First, I calculated raw phylogenetic distances among species occurring together in each quadrat. Second, I created numerous randomly generated null communities corresponding to each quadrat and estimated raw phylogenetic distances among species occurring together in the null communities. Finally, I calculated measures of standardized effect size (Gotelli and Rohde 2002) of community phylogenetic structure for the raw measures of phylogenetic distance in each quadrat, by comparing observed phylogenetic distances to the distribution of phylogenetic distances for the randomly generated null communities.

I calculated raw phylogenetic distances among species in quadrats in two ways, each of which captures a different aspect of the phylogenetic relatedness of co-occurring species (Webb 2000). The mean pairwise distance (MPD) was calculated as the mean phylogenetic distance among all pairwise combinations of species occurring together in each quadrat, and the mean nearest neighbor distance (MNND) was calculated as the mean phylogenetic distance to the nearest relative for all species occurring together in each quadrat (Webb 2000, Webb et al. 2002).

Null models

To determine whether the phylogenetic structure of local tree communities differed from the phylogenetic community structure expected by chance, I compared observed phylogenetic distances among species in each quadrat to the distribution of phylogenetic distances for randomly generated null communites (Gotelli and Graves 1996). To assess the effect of null model choice on ability to measure community phylogenetic structure, I generated null communities in two ways.

The first method maintained the total species richness of each quadrat, with species in each quadrat chosen equiprobably at random without replacement from the pool of species present in the FDP. I refer to this null model as the 'unconstrained' model since the species richness of each quadrat remained the same in the null communities, but species occurrence frequencies in the null communities were not constrained to be equal to their actual occurrence frequency among quadrats in the FDP data set. This null model assumes that all species present in the FDP are equally able to colonize any quadrat.

The second method maintained both the total species richness of each quadrat as well as the occurrence frequency of each species by randomly swapping species occurrences among all quadrats at a scale subject to the constraint that the species richness of each quadrat remain constant and that the relative frequency of all species occurrences in quadrats remain constant. I refer to this null model as the 'constrained' model since the occurrence frequencies of species in the null community were constrained to be equal to their actual frequency in quadrats at that spatial scale. This null model assumes that a species' ability to colonize a quadrat is proportional to its frequency in the FDP.

The constrained null communities were generated using the independent swap algorithm (Gotelli 2000, Gotelli and Entsminger 2003) by holding the row and column sums of the quadrat/species occurrence matrix constant while swapping species among quadrats using a checkerboard swap. The checkerboard swap searches the quadrat/species matrix for submatrices of the form (0,1)(1,0) or (1,0)(0,1) (where 1 and 0 represent species presence or absence in two quadrats) and swaps species presences between quadrats when these checkerboard submatrices are found. This maintains species frequencies and quadrat species richnesses while randomizing patterns of species co-occurrence. Each null community was created by swapping subsequent matrices many times relative to the number of species presences in the quadrat/species matrix, creating serially independent randomized matrices. The first null community for each spatial scale was created by checkerboard swapping the original quadrat/species matrix for that scale 30 000 times, with each subsequent null community generated by checkerboard swapping the previous matrix 10 000 times.

I recorded the mean and standard deviation of mean pairwise distance (MPD) and mean nearest neighbor distance (MNND) among species in each quadrat for 1000 null communities generated using the constrained and unconstrained null models. I then calculated measures of standardized effect size (Gotelli and Rohde 2002) of the observed phylogenetic distances among species occurring in each quadrat relative to the distribution of distances calculated for null communities in each quadrat. These effect size measures compared the observed phylogenetic distance in each quadrat to the distribution of phylogenetic distances in null communities corresponding to that quadrat, and can be used to test for phylogenetic clustering or overdispersion. I used two metrics of community phylogenetic structure similar to those first proposed by Webb (2000), but based on comparisons with different null models.

The Net Relatedness Index (NRI) of each quadrat (Webb 2000, Webb et al. 2002) was defined as:

$$NRI = -\frac{MPD - MPD_{null}}{SD(MPD_{null})} \qquad (4.1)$$

where MPD is the mean pairwise phylogenetic distance among species in the quadrat, MPD_{null} is the mean MPD for that quadrat in 1000 null communities, and $SD(MPD_{null})$ is the standard deviation of MPD for that quadrat in 1000 null communities. NRI has been proposed as a measure of tree-wide phylogenetic clustering and overdispersion of species (Webb 2000). Positive NRI scores indicate that species occuring together in a quadrat are more closely phylogenetically related than expected by chance, generally due to tree-wide phylogenetic clustering of co-occurring species. Negative NRI scores indicate that co-occurring species are less phylogenetically related than expected by chance, generally due to tree-wide phylogenetic overdispersion of co-occurring species.

The Nearest Taxon Index (NTI) of each quadrat (Webb et al. 2002) was defined as:

$$NTI = -\frac{MNND - MNND_{null}}{SD(MNND_{null})} \qquad (4.2)$$

where MNND is the mean nearest neighbor phylogenetic distance among species in the quadrat, $MNND_{null}$ is the mean MNND for that quadrat in 1000 null communities, and $SD(MNND_{null})$ is the standard deviation of MNND for that quadrat in 1000 null communities. NTI has been proposed as a measure of terminal (branch tip) phylogenetic clustering of species on a phylogeny (Webb 2000). If species tend to occur together with other closely related species (e.g. with congeners or confamilials), NTI scores will generally be positive due to this terminal phylogenetic clustering of species towards the tips of the phylogenetic tree. If species tend not to occur together with other closely related species, NTI scores will be negative due to terminal phylogenetic overdispersion.

Estimating plot-wide phylogenetic structure

To test whether the average phylogenetic structure of local tree communities at a given spatial scale differed from random, I calculated the mean phylogenetic structure of all quadrats at each scale as the mean NRI and NTI of all quadrats at that scale. If the mean NRI or NTI for all quadrats at a given spatial scale differed from zero according to a one-sample *t*-test, I could conclude that the tree communities at that scale were significantly phylogenetically clustered or overdispersed on average, since both NRI and NTI are standardized effect sizes whose expected values are zero for phylogenetically random communities, positive for phylogenetically clustered communities, and negative for phylogenetically overdispersed communities, with approximately 95% of NRI and NTI values expected to fall in the range of -2 to +2 for random communities (Gotelli and Rohde 2002). I estimated phylogenetic structure for each quadrat using the constrained and unconstrained null models.

I also assessed the phylogenetic structure of each quadrat by comparing the observed MPD and MNND values in each quadrat to the distribution of these values in the null communities. Quadrats were considered to be significantly phylogenetically overdispersed or clustered if they occurred in the lowest or highest 2.5% percentiles of the distribution of distances from the null communities, respectively ($\alpha = 0.05$). A one-tailed binomial test was then used to assess whether the numbers of quadrats with significantly overdispersed or clustered phylogenetic distances were greater than expected at each spatial scale.

Differences in sample sizes among spatial scales were accounted for using bootstrap estimation (Manly 1997) of the mean and standard error of NRI and NTI at each spatial scale. At the largest spatial scale examined (100x100m), the sample size was 50 quadrats. At smaller spatial scales (50x50m, 20x20m, 10x10m), I estimated the mean and standard error of phylogenetic structure (NRI and NTI) for 4 999 random draws without replacement of 50 quadrats. The bootstrap estimates of mean and standard errors of NRI and NTI were then used to calculate a bootstrap *t* statistic and *P* value for NRI and NTI at each spatial scale. This method allowed direct comparisons of the mean and standard error of NRI and NTI values among spatial scales, taking into account the original differences in sample size at each spatial scale.

Because phylogenetic similarities among co-occurring species were positively spatially autocorrelated (Figure 4-2), I also tested whether NRI and NTI values at each spatial scale differed from zero using generalized least squares models with simultaneous spatial autoregression (SAR) covariance structures in S+SpatialStats (Kaluzny et al. 1998). These models calculate an estimate of the mean and standard error of the coefficients in the model (NRI or NTI values), taking into account the non-independence of spatially adjacent samples (Cressie 1993). In all cases, adding a first-order spatial neighbor autoregressive term to the model removed autocorrelation from the residuals and improved the fit of the model relative to a non-spatial model, and so I report only the results of the spatial models.

Null model comparisons

To compare null models, I employed a method similar to that used by Gotelli (2000), whereby random communities are created by shuffling 'real' data using null models in order to randomize patterns of species co-occurrence, and then the randomized data are analyzed using the same set of null models. I randomly chose fifty 20x20m quadrats from the FDP to conduct the randomization study. I first randomized the original data using the unconstrained or constrained null models. This created a new set of samples with the same phylogenetic relationships among species and sample species richnesses as the original data, but for which species co-occurrences were completely randomized. I then calculated phylogenetic distances (MPD and MNND) and standardized effect sizes of community phylogenetic structure (NRI and NTI) for the randomized samples using 1000 runs of the unconstrained and constrained null models. This process was repeated 500 times for each combination of randomization and analysis null models.

I estimated the proportion of randomized samples for which each null model found a significantly non-random phylogenetic distance among species (number of times observed distances were in the top or bottom 2.5% percentiles of randomized distances, an estimate of the Type I error rate), as well as calculating the average degree of phylogenetic clustering or overdispersion (mean NRI and NTI) estimated by each null model.

Null models that maintain species frequencies have been criticized for potentially including the effects of any process which acts to determine the occurrence frequency of a species (Colwell and Winkler 1984). If this were occurring, I might expect some phylogenetic signal in the distribution of species occurrence frequencies. To determine whether the frequencies of species in the FDP exhibited a phylogenetic signal, I calculated phylogenetic distances among all pairs of species occurring in the entire FDP at each spatial scale examined. I then calculated the dissimilarity of species frequencies as the square root of the absolute difference in occurrence frequency rank for all pairs of species, and tested the significance of the correlation among the resulting phylogenetic and frequency distance matrices using a Mantel test (Legendre and Legendre 1998).

Habitat phylogenetic structure

Based on Harms et al.'s (2001) classification of 20x20m quadrats within the FDP into seven habitat types (high plateau, low plateau, mixed, slope, stream, swamp, and young), I asked whether the phylogenetic structure of tree communities at this spatial scale differed among habitats within the FDP. I tested for overall differences in phylogenetic structure among habitats using generalized least squares models with simultaneous spatial autoregression (SAR) covariance structures as described previously for the plot-wide tests. I estimated the overall significance of differences in phylogenetic structure among habitats using NRI and NTI scores based on the constrained null model, as well as estimating the mean and standard error of NRI and NTI scores within each habitat type to test for significant phylogenetic clustering or overdispersion. In all cases, adding a first-order spatial neighbor autoregressive term to the model removed autocorrelation from the residuals and improved the fit of the model relative to a non-spatial model, and so I report only the results of the spatial models.

Results

Null model comparisons

When analyzing community data generated by randomizing fifty randomly selected 20x20m quadrats from the FDP with the unconstrained null model, both null models performed well (Table 4-1), with appropriate Type I error rates and mean NRI and NTI values of approximately zero. When analyzing data generated by randomizing these quadrats with the constrained null model, the unconstrained null model concluded that the randomized data were significantly phylogenetically clustered or overdispersed (mean NRI and NTI different from zero), although the Type I error rates of both null models remained correct (Table 4-1).

A comparison of the phylogenetic distances among co-occurring species in all 1250 20x20m quadrats in the FDP with the corresponding mean pairwise phylogenetic distances in null communities (Figure 4-3) showed that phylogenetic distances in the null communities were much less variable than the observed distances, especially the mean pairwise distances. Mean phylogenetic distances among co-occurring species in the unconstrained null communities were higher than the mean distances in the observed and constrained null communities. As a result of the higher mean phylogenetic distances in the unconstrained null communities, NRI and NTI values calculated for each quadrat using the different null models were tightly correlated (Figure 4-4), but NRI and NTI values calculated using the constrained null model tended to be higher than those calculated using the unconstrained null model.

I found no statistically significant relationships between phylogenetic distances among species and the square root of differences in species frequency ranks at any spatial scale (Mantel tests, $P > 0.5$ at all scales), although there was a slight but non-significant trend of the most closely related species pairs having similar frequency ranks (Figure 4-5).

Community phylogenetic structure

The phylogenetic structure of tree communities in the FDP was highly variable among quadrats and dependent on the choice of null model. Using the unconstrained null model, tree communities in the FDP were phylogenetically clustered on average (mean NRI and NTI > 0; Table 4-2, Figure 4-6) at spatial scales from 10x10m to 50x50m. Although the mean NRI and NTI were greater than zero at most scales examined, relatively few quadrats were significantly phylogenetically clustered or overdispersed according to the unconstrained null model (Table 4-3, Figure 4-6).

According to the constrained null model, mean community phylogenetic structure (NRI and NTI) across the entire plot did not differ from zero, except at the smallest spatial scale examined (10x10m), where the mean NRI was greater than zero, indicating a slight overall trend of phylogenetic clustering at this scale. The magnitude of this effect was very small, and after accounting for differences in sample size among spatial scales using bootstrap resampling (Table 4-4), the average phylogenetic structure (NRI and NTI) of tree communities did not differ from zero at all spatial scales examined according to the constrained null model. However, there was substantial variation in phylogenetic structure around the mean at all spatial scales examined (Figure 4-6), and more quadrats than expected contained communities that were significantly phylogenetically overdispersed or clustered (Table 4-3).

In the 20x20m quadrats (Figure 4-2), the phylogenetic structure of a quadrat was positively correlated with the phylogenetic structures of that quadrat's first-order spatial neighbors (NRI: Moran's I = 0.34, $P<0.01$; NTI: Moran's I = 0.14, $P<0.01$), whereas the residuals of the spatial autoregression models for these values were not significantly spatially correlated (NRI residuals: Moran's I = -0.05, $P=0.93$; NTI residuals: Moran's I = -0.02, $P=0.80$). Similar patterns of significant positive spatial autocorrelation of NRI and NTI values and non-significant spatial autocorrelation of residuals from the spatial models were observed at all spatial scales examined.

Habitat influences on community phylogenetic structure

Based on the constrained null model, the phylogenetic structure of the tree communities in the FDP differed among habitats at the spatial scale (20x20m) for which habitat data were available (Figure 4-1, Table 4-5). Species occurring together in the high plateau, low plateau, and young habitats tended to be significantly phylogenetically clustered (NRI > 0 or NTI > 0, $P < 0.05$), while communities in the swamp habitat tended to contain species that were phylogenetically overdispersed (NRI = -1.096, $P < 0.0001$). Communities in the slope habitat exhibited both tree-wide phylogenetic overdispersion (NRI = -0.286, $P = 0.0005$) and marginally significant terminal phylogenetic clustering (NTI =0.130, $P = 0.0589$).

Discussion

Null model choice and community phylogenetic structure

Measures of phylogenetic distances among species in a community must be compared with the phylogenetic distances generated by a null model in order to determine whether a community is more phylogenetically clustered or overdispersed than expected by chance. A potential problem with analyses of the phylogenetic structure of a community is that null models may simultaneously affect not only the co-occurrence patterns of species, but also their occurrence frequencies across samples and the distribution of frequencies on the phylogeny.

Randomization tests showed that the unconstrained null model may indicate non-random community phylogenetic structure (mean NRI and NTI different from zero) when used with data containing non-uniform species frequencies, even when patterns of species co-occurrence in samples are completely random (Table 4-1). A similar pattern was found in the tree communities within the FDP (Figure 4-3). In the unconstrained null communities, mean pairwise phylogenetic distance converged on the average pairwise phylogenetic distance among all species occurring in the FDP, with every species and its associated phylogenetic distance to other species given equal weighting. In the constrained null communities, species and associated phylogenetic branches were effectively weighted by their frequency within the plot, leading to similar mean phylogenetic distances in the observed and constrained null communities.

These results highlight the sensitivity of measures of phylogenetic community structure not only to patterns of species co-occurrence, but also to phylogenetic tree topology, branch lengths, and species frequencies. By giving species such as the tree fern *Cnemidaria petiolaria* (which is both rare and distantly related to all other species in the FDP) an equal chance of occurring in every null community, phylogenetic distances among species in the unconstrained null communities were inflated relative to the observed communities, causing mean NRI and NTI values to be greater than zero on average. Conversely, by maintaining species frequencies in null communities, the constrained null communities tended to have the same mean phylogenetic structure as the observed communities, leading to a distribution of NRI and NTI values whose mean was close to zero.

When using the unconstrained null model with the FDP data, it is difficult to attribute any differences in phylogenetic structure between the observed and null communities to the impact of ecological processes on the phylogenetic structure of the community, since they may simply be due to differences in species frequencies between the observed and null communities. Additionally, given the widespread spatial aggregation of trees (Condit et al. 2000) and strong dispersal limitation (Hubbell et al. 1999) demonstrated to occur in the FDP and other ecological communities, the assumption that all species are equally able to colonize any sample in the null communities is not realistic.

Much of the previous debate surrounding the use of null models in ecology has focused on the relative merits of null models that maintain or do not maintain species frequencies (Gotelli and Graves 1996). The problem of null models which do not maintain species frequencies being overly statistically liberal has been described previously as the "Jack Horner effect" (Wilson 1995). Conversely, null models that maintain species frequencies have been criticized for potentially 'smuggling in' the effects of processes such as competition or environmental filtering on species frequencies and community phylogenetic structure (the Narcissus effect; Colwell and Winkler 1984), and for potentially being too statistically conservative. If this were the case, I might expect some relationship between species' frequencies and their phylogenetic relatedness, but relationships between frequency similarity and phylogenetic distance were not statistically significant at any spatial scale examined according to Mantel tests (Figure 4-2). I might also expect the constrained null model to find non-random community structure in fewer quadrats within the FDP if it was too statistically conservative, but in fact the constrained model found many more quadrats to be phylogenetically non-random compared to the unconstrained null model (Table 4-2), although mean NRI and NTI values from the constrained null model were always close to zero.

Recent attempts to resolve the null model debate have focused on the use of simulation studies to quantify the Type I and II error rates of different null models when confronted with randomized or simulated community co-occurrence data (Gotelli 2000). Although I quantified the Type I error rate and bias of null models in this study, I did not assess the Type II error rate, which will need to be measured using simulation studies with data generated using different models of the interaction between phylogenetic relationships, trait evolution and community structure.

Several additional issues related to the use of null models to measure community phylogenetic structure remain unresolved. The constrained null model used in this study maintains observed species frequencies in null communities, but swaps species presences among samples, and thus can only be used with species presence/absence data matrices. Species abundances within samples contain useful information that is discarded when using this approach, but it is probably not possible to simultaneously constrain species frequencies, abundances and sample species richnesses.

The constrained null model also ignores species in the regional species pool when generating null communities, since only species present in local communities are used to generate the null communities. To determine how community phylogenetic structure varies across a larger range of spatial scales (Webb et al. 2002, Cavender-Bares et al. 2006), it will be necessary to compare the phylogenetic relatedness of species present in communities at one scale to those present in some regional species pool. It is not clear how to separate the effects of variation in species frequency from the effects of ecological and evolutionary processes on community phylogenetic structure for these types of data, but clearly more research on the effects of null model and species pool choice is needed.

Community phylogenetic structure in the FDP

Although the average phylogenetic structure of the tree communities in the FDP was close to random on average across the entire plot at spatial scales from 10x10m to 100x100m according to the constrained null model (Tables 4-2 & 4-4), the phylogenetic relatedness of tree species occurring together in individual quadrats varied greatly from phylogenetic overdispersion to phylogenetic clustering (Table 4-2, Figure 4-6), and more quadrats than expected exhibited significant phylogenetic clustering and overdispersion at most spatial scales (Table 4-1, Figure 4-6). Habitats within the plot differed strongly in their phylogenetic structure (Table 4-5, Figure 4-2). At the spatial scale for which habitat data were available, phylogenetic clustering in some habitats combined with phylogenetic overdispersion in other habitats appeared to result in a pattern of phylogenetic structure indistinguishable from random on average across the entire FDP, obscuring the strong differences in phylogenetic structure among habitats within the FDP.

In the seasonally dry plateau habitats and in the young secondary growth forests within the plot, co-occurring species were phylogenetically clustered (Table 4-5). The tree-wide phylogenetic clustering in the plateau habitats was largely due to the co-occurrence of numerous species from large, highly speciose clades such as the eurosids. Numerous species from these clades tended to occur together in plateau quadrats, leading to a trend of tree-wide phylogenetic clustering of co-occurring species in the plateau. Similarly, in the young habitats species from families and genera concentrated in a few orders commonly occurred together in most quadrats, leading to a pattern of both tree-wide and terminal phylogenetic clustering.

Given the broad phylogenetic niche and trait conservatism documented across plants in general (Prinzing et al. 2001, Cavender-Bares et al. 2006, Silvertown 2006) and tropical trees in particular (Chazdon et al. 2003), the phylogenetic clustering in the plateau and young forests in the FDP suggests that some sort of environmental filter is structuring tree communities in these habitats. Habitats such as the dry plateaus within the FDP would be predicted to contain phylogenetically clustered species, since the relatively stressful dry season soil moisture conditions in these environments would be more likely to act as an environmental filter on broadly conserved ecological traits than in moister habitats (Harms et al. 2001, Webb et al. 2002).

Communities in the relatively moist slope and swamp habitats were phylogenetically overdispersed (Table 4-5). Tree-wide phylogenetic overdispersion in the swamp habitats was caused by the presence of species from families such as the Moraceae and Arecaceae (Harms et al. 2001) that are widely scattered across the angiosperm phylogeny. In the slope habitats, a pattern of tree-wide overdispersion but terminal phylogenetic clustering was found.

Several processes could give rise to these patterns of phylogenetic overdispersion, depending on the interaction between the phylogenetic history of trait evolution and contemporary ecological interactions in these habitats. Even within a single habitat type, the hierarchical nature of trait and niche evolution (Ackerly et al. 2006, Silvertown et al. 2006), interactions among multiple ecological processes (Webb et al. 2002), and the phylogenetic history of species habitat associations (Brooks and McLennan 1991) could lead to complicated patterns of community phylogenetic structure, making it difficult to attribute these patterns to any one process.

More data on the ecological traits of species in the FDP and the evolutionary history of habitat associations in tropical trees will be necessary to determine the relative importance of processes such as environmental filtering or competitive exclusion in different habitats within the FDP. However, it is clear that either the relative importance of non-neutral ecological processes or the evolutionary history of niches, traits or habitat associations must vary along environmental gradients within the FDP to explain the observed variation in phylogenetic structure among habitats.

Literature Cited

Ackerly, D. D., D. W. Schwilk, and C. O. Webb. 2006. Niche evolution and adaptive radiation: testing the order of trait divergence. Ecology **87**:S50-61.

Angiosperm Phylogeny Group. 2003. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. Botanical Journal of the Linnean Society **141**:399-436.

Brooks, D. R., and D. A. McLennan. 1991. Phylogeny, ecology, and behavior: a research program in comparative biology. University of Chicago Press, Chicago, U.S.A.

Cavender-Bares, J., D. D. Ackerly, D.A. Baum, and F.A. Bazzaz. 2004. Phylogenetic overdispersion in Floridian oak communities. American Naturalist **163**:823-843.

Cavender-Bares, J., A. Keen, and B. Miles. 2006. Phylogenetic structure of Floridian plant communities depends on taxonomic and spatial scale. Ecology **87**:S109-122.

Chazdon, R. L., C. Careaga, C. Webb, and O. Vargas. 2003. Community and phylogenetic structure of reproductive traits of woody species in wet tropical forests. Ecological Monographs **73**:331-348.

Clark, D.B., D.A. Clark, and J.M. Read. 1988. Edaphic variation and the mesoscale distribution of tree species in a neotropical rain forest. Journal of Ecology **86**:101-112.

Colwell, R.K., and D.W. Winkler. 1984. A null model for null models in biogeography. Pages 344-359 *in* D. R. Strong, D. Simberloff, L. G. Abele and A. B. Thistle, editors. Ecological communities: Conceptual issues and the evidence. Princeton University Press, Princeton, N.J., U.S.A.

Condit, R. 1998. Tropical Forest Census Plots. Springer-Verlag, Berlin, Germany.

Condit, R., Hubbell, S.P., and Foster, R.B. 1996. Changes in tree species abundance in a Neotropical forest: impact of climate change. Journal of Tropical Ecology **12**: 231-256.

Condit, R., Ashton, P.S., Baker, P., Bunyavejchewin, S., Gunatilleke, S., Gunatilleke, N., Hubbell, S.P., Foster, R.B., Hua Seng, L., Itoh, A., LaFrankie, J.V., Losos, E., Manokaran, N., Sukumar, R., and Yamakura, T. 2000. Spatial patterns in the distribution of tropical tree species. Science **288**:1414-1418.

Cressie, N.A. 1993. Statistics for spatial data. Revised edition. John Wiley and Sons, New York, New York, USA.

Dietrich, W.E., Windsor, D.M., and Dunne, T. 1996. Geology, climate, and hydrology of Barro Colorado Island. Pages 21-46 *in* E.G. Leigh, A.S. Rand, and D.M. Windsor, editors. The ecology of a tropical forest. Smithsonian Institution Press, Washington, DC, USA.

Gotelli, N.J. 2000. Null model analysis of species co-occurrence patterns. Ecology **81**: 2606-2621.

Gotelli, N.J. 2004. Research frontiers in null model analysis. Global Ecology & Biogeography **10**:337-343.

Gotelli, N.J., and Graves, G.R. 1996. Null models in ecology. Smithsonian Institution Press, Washington, DC, USA.

Gotelli, N.J. and K. Rohde. 2002. Co-occurrence of ectoparasites of marine fishes: a null model analysis. Ecology Letters **5**:86-94.

Gotelli, N.J., and Entsminger, G.L. 2003. Swap algorithms in null model analysis. Ecology **84**:532-535.

Harms, K.E., R. Condit, S.P. Hubbell, and R.B. Foster. 2001. Habitat associations of tree and shrubs in a 50-ha neotropical forest plot. Journal of Ecology **89**:947-959.

Hubbell, S.P. 2001. The unified neutral theory of biodiversity and biogeography. Princeton University Press, Princeton, New Jersey, USA.

Hubbell, S.P., and Foster, R.B. 1983. Diversity of canopy trees in a neotropical forest and implications for conservation. Pages 25-41 in S. L. Sutton, T. C. Whitmore, and A. C. Chadwick, eds. Tropical Rain Forest: Ecology and Management. Blackwell Scientific Publications, Oxford, UK.

Hubbell, S.P., Foster, R B., O'Brien, S.T., Harms, K E., Condit, R., Wechsler, B., Wright, S J., and Loo de Lao, S. 1999. Light-gap disturbances, recruitment limitation, and tree diversity in a neotropical forest. Science **283**:554–557.

Kaluzny, S.P., S.C. Vega, T.P. Cardoso, and A.A. Shelly. 1998. S+SpatialStats user's manual for Windows and Unix. Springer-Verlag, New York, New York, U.S.A.

Legendre, P., and L. Legendre. 1998. Numerical ecology. 2nd English edition. Elsevier, New York, U.S.A.

Levin, S.A. 1992. The problem of pattern and scale in ecology. Ecology **73**:1943-1967.

Manly, B.F.J. 1997. Randomization, bootstrap and Monte Carlo methods in biology. 2nd edition. Chapman and Hall, London, UK.

Novotny, V., Y.Basset, S.E. Miller, G.D. Weiblen, B. Bremer, L. Cizek, and P. Drozd. 2002. Low host specificity of herbivorous insects in a tropical forest. Nature **416**:841-844.

Prinzing, A., W. Durka, S. Klotz, and R. Brandl. 2001. The niche of higher plants: evidence for phylogenetic conservatism. Proceedings of the Royal Society of London Series B Biological Sciences **268**:2383-2389.

Silvertown, J., M. Dodd, D. Gowing, C. Lawson, and K. McConway. 2006. Phylogeny and the hierarchical organization of plant diversity. Ecology **87**:S39-S49.

Stevens, P.F. 2005. Angiosperm Phylogeny Website. Version 6. http://www.mobot.org/MOBOT/research/APweb/.

Uriarte, M., R. Condit, C.D. Canham, and S.P. Hubbell. 2004. A spatially explicit model of sapling growth in a tropical forest: does the identity of neighbours matter? Journal of Ecology **92**:348-360.

Webb, C. O. 2000. Exploring the phylogenetic structure of ecological communities: an example for rain forest trees. American Naturalist **156**:145-155.

Webb, C. O., and D. R. Peart. 2000. Habitat associations of trees and seedlings in a Bornean rain forest. Journal of Ecology **88**:464-478.

Webb, C.O., and M.J. Donoghue. 2005. Phylomatic: tree assembly for applied phylogenetics. Molecular Ecology Notes **5**:181-183.

Webb, C. O., D. D. Ackerly, M. A. McPeek, and M. J. Donoghue. 2002. Phylogenies and community ecology. Annual Review of Ecology and Systematics **33**: 475-505.

Webb, C.O., D.D. Ackerly, and S.W. Kembel. 2004. Phylocom: software for the analysis of community phylogenetic structure and trait evolution. Version 3.19. http://www.phylodiversity.net/phylocom/.

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

Weiher, E., and P.A. Keddy, editors. 1999. Ecological assembly rules: perspectives, advances, retreats. Cambridge University Press, Cambridge, UK.

Wikström, N., V. Savolainen, and M.W. Chase. 2001. Evolution of the angiosperms: calibrating the family tree. Proceedings of the Royal Society of London Series B **268**:2211-2219.

Wilson, J.B. 1995. Null models for assembly rules: the Jack Horner effect is more insidious than the Narcissus effect. Oikos **72**:139–144.

Wright, S. J. 2002. Plant diversity in tropical forests: a review of mechanisms of species coexistence. Oecologia **130**:1-14.

Table 4-1. Results of a randomization study to assess the ability of different null models to measure community phylogenetic structure for 50 randomly selected 20x20m quadrats in the 50-hectare Forest Dynamics Plot on Barro Colorado Island, Panama. Species co-occurrences in these quadrats were first randomized using either the constrained or unconstrained null model (see methods section for description). Phylogenetic distances among co-occurring species (mean pairwise phylogenetic distance (MPD) and mean nearest neighbor phylogenetic distance (MNND)) were first calculated for the randomized data. The distributions of phylogenetic distances in null communities were then calculated for 999 subsequent randomizations of the data using the analysis null model, and used to calculate measure of community phylogenetic structure for each randomization (Net Relatedness Index (NRI) and Nearest Taxon Index (NTI), see methods section for description). This process was repeated 500 times for each combination of randomization and analysis null models. The average community phylogenetic structure (mean NRI and NTI) and Type I error rate (proportion of randomizations indicating a significant phylogenetic structure (absolute value of NRI or NTI > 2, $P<0.05$)) were calculated for each combination of randomization and analysis null models. Bold type indicates mean NRI or NTI values that were significantly different from zero according to a one-sample *t*-test (N=50 quadrats X 500 runs, $P<0.05$).

		MPD (Ma) (Randomization)	MPD (Ma) (Analyses)		NRI	
Randomization method	Analysis null model	Mean	Mean	Standard Deviation	Mean	Type I error rate
Constrained	Constrained	214.3	214.4	5.9	0.00	0.06
	Unconstrained	214.3	217.9	7.3	**0.49**	0.04
Unconstrained	Constrained	217.9	217.9	7.2	0.00	0.06
	Unconstrained	217.9	217.9	7.2	0.00	0.06

		MNND (Ma) (Randomization)	MNND (Ma) (Analyses)		NTI	
Randomization method	Analysis null model	Mean	Mean	Standard Deviation	Mean	Type I error rate
Constrained	Constrained	78.0	78.1	6.6	0.02	0.05
	Unconstrained	78.0	79.2	7.2	**0.18**	0.05
Unconstrained	Constrained	79.2	79.2	7.1	0.00	0.06
	Unconstrained	79.2	79.2	7.2	0.00	0.05

Table 4-2. Tree community phylogenetic structure in quadrats at four spatial scales within the 50-hectare Forest Dynamics Plot on Barro Colorado Island, Panama. Net Relatedness Index (NRI) and Nearest Taxon Index (NTI) are measures of community phylogenetic structure based on constrained and unconstrained null models (see methods section for description). Positive NRI and NTI values indicate phylogenetic clustering and negative values indicate phylogenetic overdispersion of species occurring together in a quadrat. Parameter estimates at each scale are based on a spatial generalized least squares model with a first-order spatial neighbor simultaneous spatial autoregression term (SAR). Significant *P* values indicate that the phylogenetic structure at a given spatial scale differed from zero according to a two-tailed *t*-test.

			Net Relatedness Index (NRI)			Nearest Taxon Index (NTI)		
Null model	Spatial scale	N	Estimated Mean	Standard Error	*P* value	Estimated Mean	Standard Error	*P* value
Unconstrained	10x10m	5000	0.260	0.008	0.0001	0.142	0.012	0.0001
	20x20m	1250	0.389	0.016	0.0001	0.154	0.021	0.0001
	50x50m	200	0.771	0.047	0.0001	0.453	0.045	0.0001
	100x100m	50	0.074	0.099	0.4605	-0.019	0.099	0.8459
Constrained	10x10m	5000	0.061	0.016	0.0001	0.070	0.014	0.0001
	20x20m	1250	0.056	0.033	0.0952	0.020	0.028	0.4854
	50x50m	200	0.051	0.093	0.5825	-0.046	0.076	0.5427
	100x100m	50	0.043	0.180	0.8110	0.003	0.161	0.9858

Table 4-3. Number of quadrats with statistically significant phylogenetic clustering or overdispersion at four spatial scales in the 50-hectare Forest Dynamics Plot on Barro Colorado Island, Panama. Phylogenetic distances among co-occurring species in each quadrat (mean pairwise phylogenetic distance (MPD) and mean nearest neighbor phylogenetic distance (MNND)) were compared with phylogenetic distances in 1000 null communities generated using the constrained and unconstrained null models (see methods section for description). Quadrats were considered to be significantly phylogenetically overdispersed or clustered if they occurred in the lowest or highest 2.5% percentiles of the distribution of distances from the null communities, respectively ($\alpha = 0.05$). A one-tailed binomial test was then used to assess whether the numbers of quadrats with significantly overdispersed or clustered phylogenetic distances were greater than expected at each spatial scale.

			MPD (Ma)				MNND (Ma)			
		Total quadrats	Overdispersed quadrats		Clustered quadrats		Overdispersed quadrats		Clustered quadrats	
Null model	Spatial scale	N	N	Binomial *P* value	N	Binomial *P* value	N	Binomial *P* value	N	Binomial *P* value
Unconstrained	10x10m	5000	69	1.0000	4	1.0000	92	0.9992	17	1.0000
	20x20m	1250	36	0.2171	1	1.0000	10	1.0000	0	1.0000
	50x50m	200	17	<0.001	1	0.9937	1	0.9937	0	1.0000
	100x100m	50	0	1.0000	3	0.1294	0	1.0000	1	0.7180
Constrained	10x10m	5000	188	<0.001	204	<0.001	150	0.0151	98	0.9950
	20x20m	1250	76	<0.001	63	<0.001	30	0.6144	40	0.0716
	50x50m	200	18	<0.001	6	0.3840	4	0.7385	6	0.3840
	100x100m	50	3	0.1294	1	0.7180	4	0.0362	2	0.3565

Table 4-4. Tree community phylogenetic structure in quadrats at four spatial scales within the 50-hectare Forest Dynamics Plot on Barro Colorado Island, Panama. Net Relatedness Index (NRI) and Nearest Taxon Index (NTI) are measures of community phylogenetic structure based on constrained and unconstrained null models (see methods section for description). Positive NRI and NTI values indicate phylogenetic clustering and negative values indicate phylogenetic overdispersion of species occurring together in a quadrat. Parameter estimates at each scale are based on 4999 bootstrap resamples of 50 quadrats from that spatial scale, except at the 100x100m scale where parameters represent the actual parameter estimates for the 50 quadrats at that scale. Significant *P* values indicate that the phylogenetic structure at a given spatial scale differed from zero according to a two-tailed *t*-test.

			Net Relatedness Index (NRI)			Nearest Taxon Index (NTI)		
Null model	Spatial scale	N	Estimated Mean	Standard Error	*P* value	Estimated Mean	Standard Error	*P* value
Unconstrained	10x10m	50	0.261	0.077	0.0013	0.143	0.118	0.2327
	20x20m	50	0.388	0.079	<0.0001	0.156	0.103	0.1347
	50x50m	50	0.771	0.094	<0.0001	0.452	0.090	<0.0001
	100x100m	50	0.074	0.099	0.4583	-0.019	0.099	0.8455
Constrained	10x10m	50	0.058	0.156	0.7128	0.070	0.140	0.6201
	20x20m	50	0.053	0.157	0.7389	0.021	0.139	0.8786
	50x50m	50	0.047	0.186	0.8028	-0.047	0.151	0.7546
	100x100m	50	0.043	0.180	0.8122	0.003	0.161	0.9852

Table 4-5. Tree community phylogenetic structure in 20x20m quadrats in different habitats within the 50-hectare Forest Dynamics Plot on Barro Colorado Island, Panama. Net Relatedness Index (NRI) and Nearest Taxon Index (NTI) are measures of community phylogenetic structure based on a constrained null model which shuffled species co-occurrence patterns in null communities while maintaining observed species occurrence frequencies and quadrat species richnesses (see methods section for description). Positive NRI and NTI values indicate phylogenetic clustering and negative values indicate phylogenetic overdispersion of species occurring together in a quadrat. Parameter estimates in each habitat are based on a spatial generalized least squares model with a first-order spatial neighbor simultaneous spatial autoregression term (SAR). Overall differences among habitats in NRI and NTI were statistically significant according to the spatial GLS tests ($P<0.0001$). Significant P values in the Table 4-indicate that the phylogenetic structure in a habitat differed from zero (significant phylogenetic clustering or overdispersion) according to a two-tailed *t*-test.

		Net Relatedness Index (NRI)			Nearest Taxon Index (NTI)		
Habitat	N	Mean	Standard Error	*P* value	Mean	Standard Error	*P* value
High Plateau	170	0.338	0.117	0.0039	0.021	0.094	0.8216
Low Plateau	620	0.117	0.060	0.0533	-0.054	0.049	0.2619
Mixed	66	0.140	0.146	0.3393	0.141	0.129	0.2723
Slope	284	-0.286	0.827	0.0005	0.130	0.069	0.0589
Stream	32	0.198	0.219	0.3666	-0.315	0.191	0.0990
Swamp	30	-1.096	0.244	0.0001	-0.116	0.206	0.5720
Young	48	0.570	0.206	0.0057	0.440	0.169	0.0095

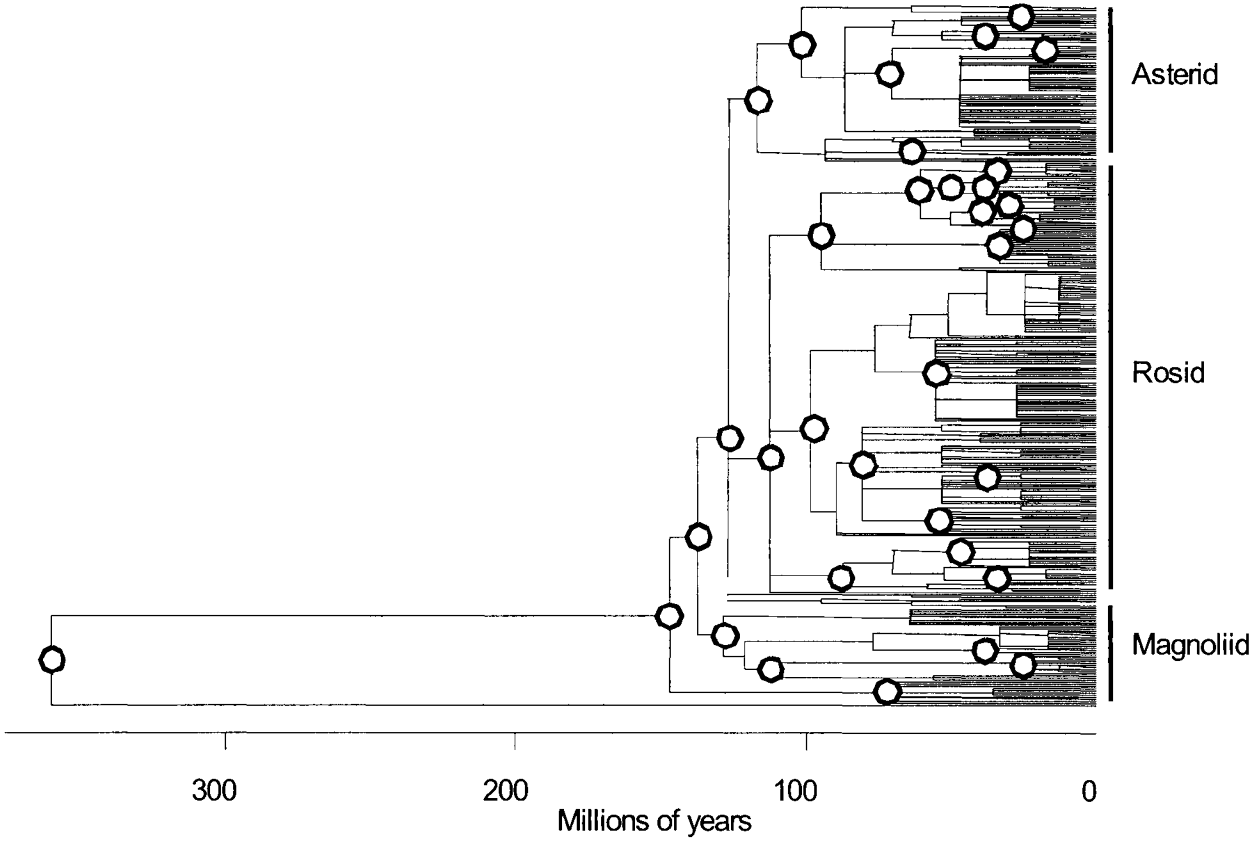

Figure 4-1. Hypothesized phylogenetic relationships among woody plant species occurring in the 50-hectare Forest Dynamics Plot on Barro Colorado Island, Panama. Circles indicate nodes dated based on divergence dates reported by Wikström et al (2001).

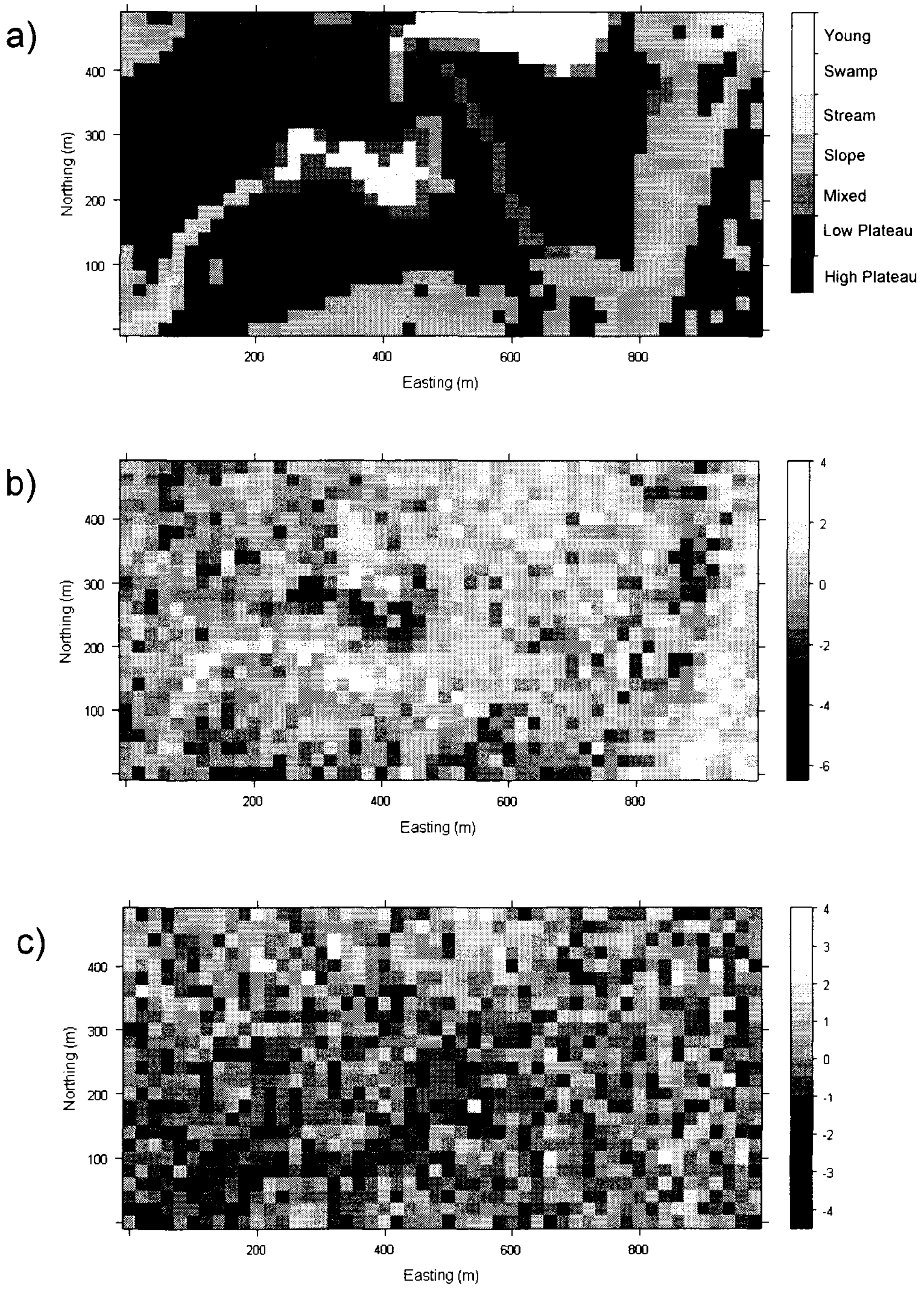

Figure 4-2. Spatial patterns of a) habitats (from Harms et al. 2001), b) Net Relatedness Index (NRI), and c) Nearest Taxon Index (NTI) in 20x20m quadrats within the 50-hectare Forest Dynamics Plot on Barro Colorado Island, Panama. Net Relatedness Index (NRI) and Nearest Taxon Index (NTI) are measures of community phylogenetic structure based on a constrained null model (see methods section for description). Positive NRI and NTI values indicate phylogenetic clustering and negative values indicate phylogenetic overdispersion of species occurring together in a quadrat.

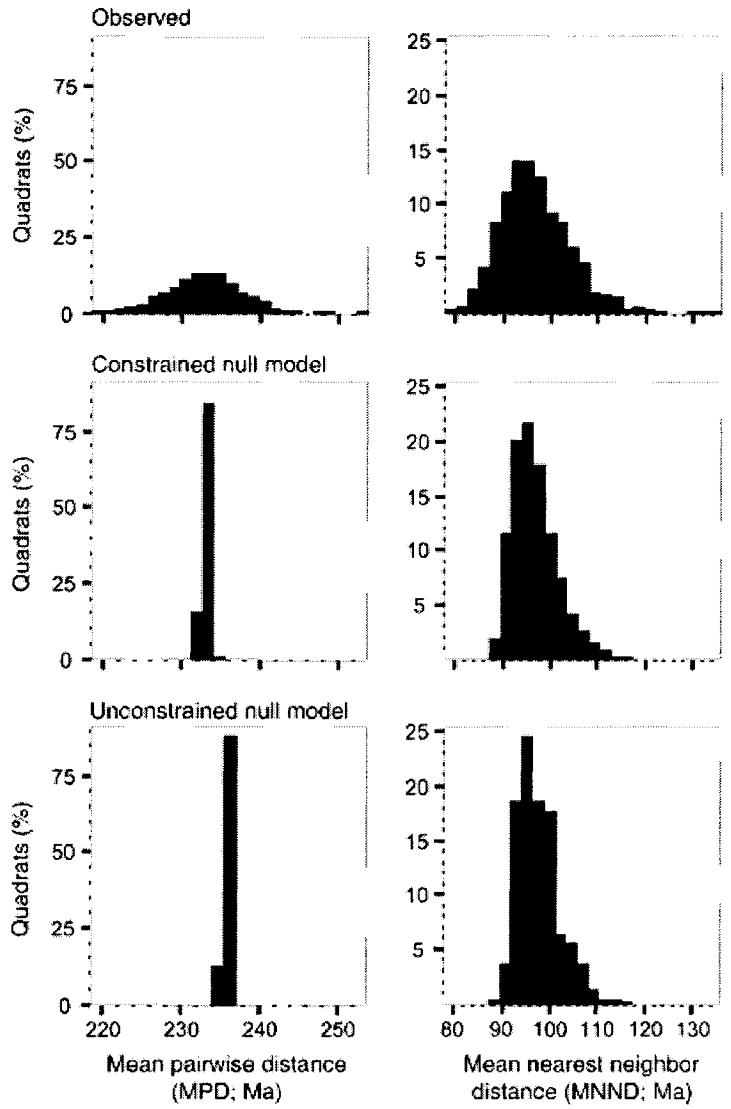

Figure 4-3. Tree community phylogenetic structure in 20x20m quadrats within the 50-hectare Forest Dynamics Plot on Barro Colorado Island, Panama. Observed phylogenetic distances among co-occurring species are presented for 1250 quadrats, along with phylogenetic distances among species in corresponding null communities generated using a constrained and an unconstrained null model (see methods section for description).

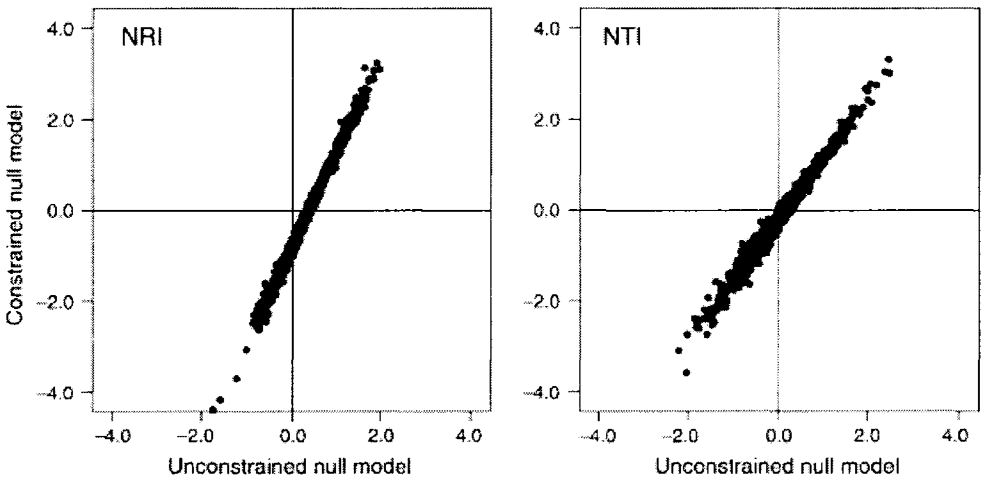

Figure 4-4. Relationship between measures of phylogenetic community structure calculated using two null models in 1250 20x20m quadrats within the 50-hectare Forest Dynamics Plot (FDP) on Barro Colorado Island, Panama. Net Relatedness Index (NRI) and Nearest Taxon Index (NTI) are measures of community phylogenetic structure based on constrained and unconstrained null models (see methods section for description).

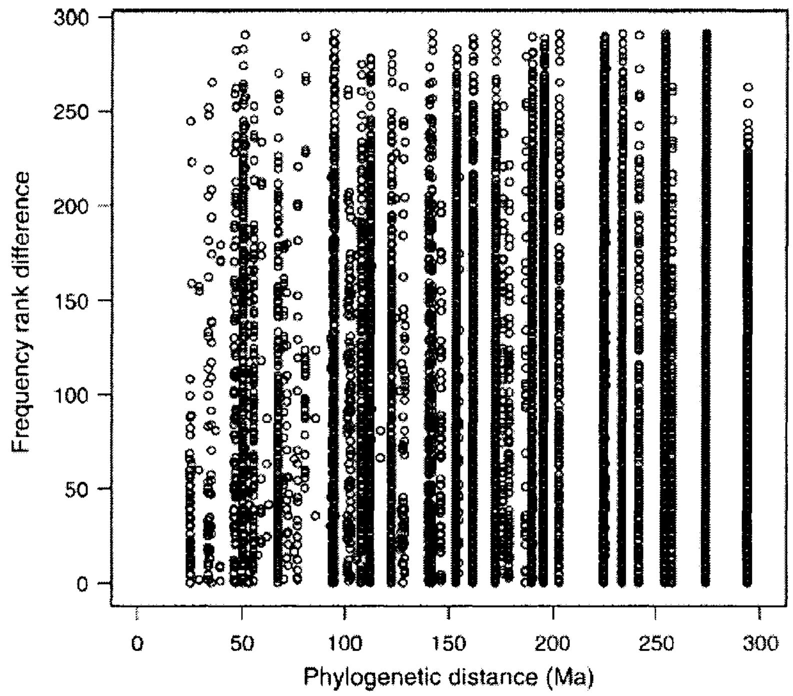

Figure 4-5. Relationship between phylogenetic distances among species and similarity of species occurrence frequency ranks in 20x20m quadrats within the 50-hectare Forest Dynamics Plot (FDP) on Barro Colorado Island, Panama. Data points represent all pairwise combinations of species present in the FDP. Frequency rank differences were calculated as the absolute difference in the frequency occurrence ranks of each species pair. Phylogenetic distances were calculated as the branch length (Ma, millions of years) connecting each species pair. Mantel tests indicated that relationships between square-root transformed frequency rank difference and phylogenetic distance were not statistically significant at any spatial scale examined.

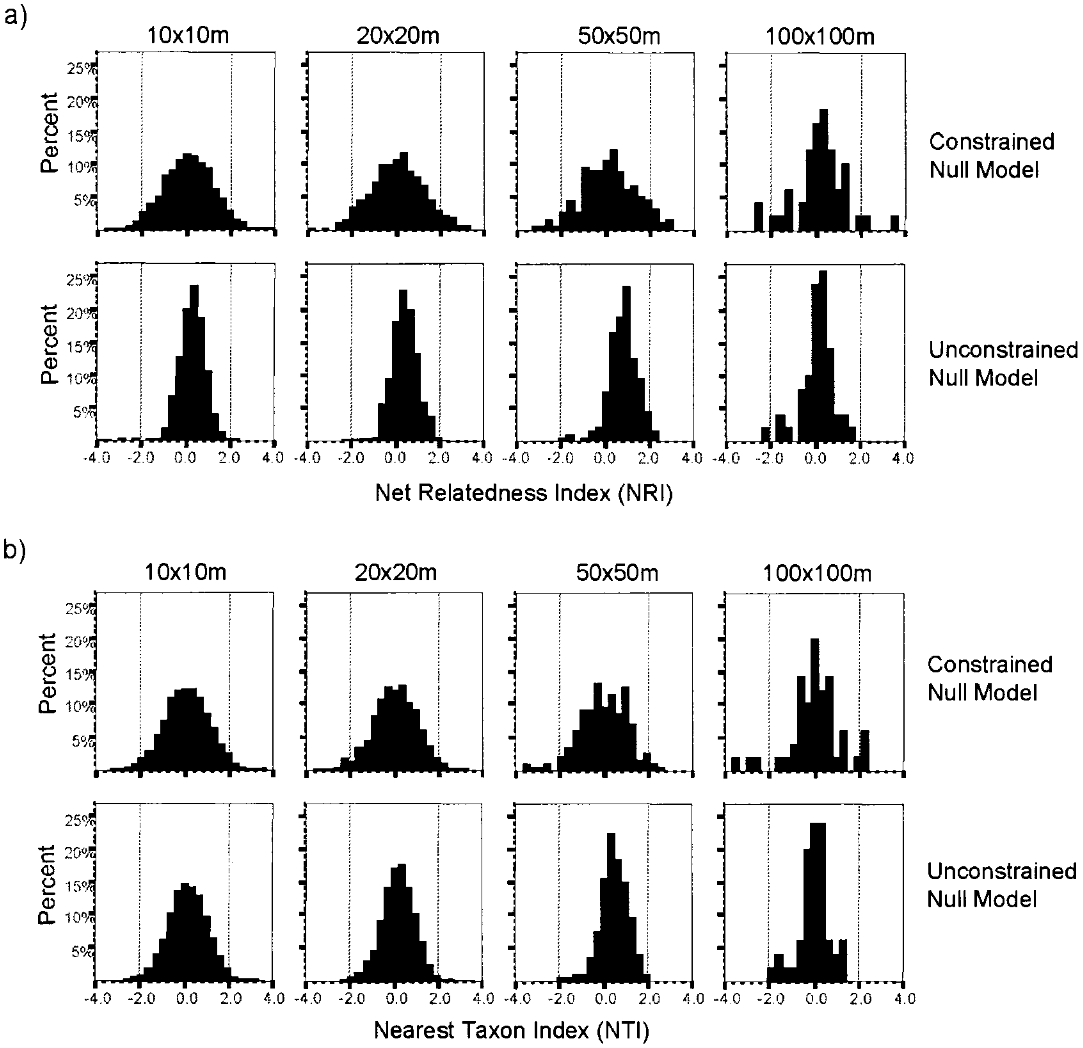

Figure 4-6. Tree community phylogenetic structure in quadrats at four spatial scales within the 50-hectare Forest Dynamics Plot on Barro Colorado Island, Panama. Net Relatedness Index (NRI) and Nearest Taxon Index (NTI) are measures of community phylogenetic structure based on constrained and unconstrained null models (see methods section for description). Positive NRI and NTI values indicate phylogenetic clustering and negative values indicate phylogenetic overdispersion of species occurring together in a quadrat. Dashed lines indicate expected 95% confidence intervals.

Chapter 5 Phenotypic plasticity and integration of leaf and root traits of *Arabidopsis thaliana* in response to nutrient availability

Introduction

Plants are sessile organisms that must cope with environmental heterogeneity at a range of spatial and temporal scales in order to survive and reproduce. Plant adaptations to environmental heterogeneity include variation in a range of morphological, physiological and life-history traits, as well as variation in the phenotypic plasticity of those traits (Schlichting and Pigliucci 1998). In addition to widespread evidence for natural selection on plant morphological and physiological traits in different environments (Geber and Griffen 2003), there is also evidence that selection may act directly on the phenotypic plasticity of some traits (Dorn et al. 2000), or on the phenotypic integration of suites of traits (Pigliucci 2003a), selecting for or against the ability to change the phenotype in response to different environmental signals (Tienderen 1991, Alpert and Simms 2002).

Despite the importance of roots for nutrient uptake, relatively little is understood about the phenotypic plasticity and integration of root traits or their relationships with fitness in response to nutrient availability. In this study, I grew genotypes of the annual plant *Arabidopsis thaliana* in high and low nutrient availability environments in order to address several questions. First, I tested for phenotypic plasticity in response to nutrient availability for a variety of above- and belowground phenotypic traits. Second, I described patterns of phenotypic integration of above- and below-ground traits, and asked whether patterns of phenotypic integration are affected by nutrient availability. Third, I tested for evidence of selection on these traits and costs of phenotypic plasticity, and asked whether selection or plasticity costs varied with nutrient availability.

Trait variation and plasticity in Arabidopsis thaliana

Mouse-ear cress (*Arabidopsis thaliana* (L.) Heynh, Brassicaceae) is an annual-biennial plant species that is common in disturbed or open habitats in temperate zones throughout the world (Mitchell-Olds 2001). Genotypes of *A. thaliana* tend to fall along a continuum from early-flowering to late-flowering (Pigliucci 2003b). The time of flowering and a concurrent shift from a strictly annual life-history strategy towards a biennial or winter annual strategy is correlated with latitudinal gradients in climate conditions (Stinchcombe et al. 2004), and a suite of other vegetative traits including leaf and root morphology and plant relative growth rate (Li et al. 1998). Genotypes of *A thaliana* vary in their root system size and root branching architecture (Chevalier et al. 2003, Fitz Gerald et al. 2006), with fast-growing genotypes tending to have higher specific root length (SRL; root length per unit mass), and higher nutrient uptake rates per unit length of root (Krannitz et al. 1991b, Li et al. 1998).

The phenotypic plasticity of *Arabidopsis thaliana* in response to a variety of environmental factors has been widely studied (Mitchell-Olds 2001). Some of the earliest studies of plasticity in *A. thaliana* focused on responses to nutrient availability (Pigliucci and Schlichting 1995). Plastic responses to nutrient availability in *A. thaliana* include changes in seed production and infloresence structure, as well as changes to vegetative traits including rosette diameter and number of leaves (Zhang and Lechowicz 1994, Pigliucci and Schlichting 1995, Pigliucci and Hayden 2001), but due to the logistical difficulties of working with roots, relatively little is known about the belowground plasticity of *A. thaliana* in response to nutrient availability.

Phenotypic integration above- and belowground

Phenotypic integration is the study of correlations among phenotypic traits and how these correlations change in response to the environment (Pigliucci 2003a). Suites of correlated phenotypic traits may evolve due to selection, or due to fundamental constraints such as genetic correlations among traits (Grime 2001, Pigliucci 2001, Westoby et al. 2002). Several studies have examined phenotypic integration of functional traits in *Arabdiopsis thaliana*, generally finding that vegetative and reproductive traits are highly integrated, but with little integration between these two types of traits, with correlations among some traits shifting depending on environmental conditions due to plasticity (Pigliucci and Hayden 2001, Pigliucci and Kolodynska 2002). Due to a general lack of knowledge about the ecology of roots, and due to the logistical difficulty of working with roots, most studies of phenotypic integration have ignored the belowground portion of the plant phenotype. It is unclear how root traits are related to aboveground phenotypic traits, or what effect inclusion of information on root traits will have on our understanding of whole-plant phenotypic integration and character correlations in *A. thaliana*. On the one hand, root traits may be highly correlated with aboveground traits as part of an integrated vegetative phenotype. However, if selection or constraint act differently on above- and belowground structures, including information on root traits may change conclusions about the nature of whole-plant trait integration in *A. thaliana.*

Phenotypic traits, phenotypic plasticity and fitness

Many plant morphological, physiological and life-history traits have been shown to be under selection due to their relationship with relative fitness, although the measured strength of selection is typically quite weak (Kingsolver et al. 2001). Phenotypic plasticity is a change in the phenotype of an organism in response to environmental variation (Bradshaw 1965, Scheiner 1993). Many aspects of the phenotype change during development and in response to the environment, and phenotypic plasticity may be caused by passive ontogenetic or allometric responses of traits to resource availability (Weiner 2004), as well as by selection for or against plasticity due to direct or maintenance costs of expressing a plastic phenotype (DeWitt et al. 1998). Patterns of selection on phenotypic plasticity may vary among environments, depending on the spatial and temporal scale of signals and resources in the environment (Alpert and Simms 2002). Plasticity may also have effects on fitness independent of the adaptive value of phenotypes produced in different environments if there are costs of maintaining a more plastic phenotype (Scheiner and Berrigan 1998).

Studies of root morphology and plasticity in *Arabidopsis thaliana* have shown that plasticity of root attributes including root system size, architecture, and root hair production may have important fitness consequences (Fitter et al. 2002), but the fitness consequences of root traits and plasticities may vary depending on nutrient availability or the identity of limiting nutrients (Bates and Lynch 2001, Linkohr et al. 2002). For example, root hair production by *A. thaliana* affects plant fitness only when phosphorous is limiting (Fitter et al. 2002). Few studies have explicitly tested for relationships between root plasticity and fitness in different environments, and the overall relationships between root traits, plasticity and fitness are not well understood for this species.

Objectives

While the plasticity, integration and fitness of *Arabidopsis thaliana* plants in response to nutrient availability have been widely studied, relatively little is known about the belowground portion of the *A. thaliana* phenotype. In this study, I address a series of questions related to this lack of information about roots, using data from an experiment where above- and belowground phenotypic traits and fitness were measured on *Arabidopsis thaliana* plants grown at two level of nutrient availability:

1) Do vegetative (leaf and root) and reproductive traits of *Arabidopsis thaliana* exhibit phenotypic plasticity in response to nutrient availability?
2) Is there evidence for integration of phenotypic traits in *Arabidopsis thaliana*, and if so, how do patterns of above- and belowground phenotypic integration change between environments?
3) Is there evidence for selection on phenotypic traits and their plasticities in response to nutrient availability? If so, how does selection vary between low and high nutrient availability environments? Is phenotypic plasticity adaptive, and are there maintenance costs of plasticity?

Methods

Study species

Seeds from 16 genotypes of *Arabidopsis thaliana* were obtained from the *Arabidopsis* Information Resource Centre (http://www.*Arabidopsis*.org). Genotypes included 8 ecotypes collected from a range of source populations, as well as 8 mutant genotypes derived from the *col-0* Columbia isoline background (Table 5-1). Genotypes were chosen for inclusion in this study based on their use in previous studies of phenotypic plasticity or ecophysiology in *A. thaliana*, and to obtain a range of phenotypic variation by selecting ecotypes and mutants with major phenotypic effects on life-history or leaf and root traits (Cahill et al. 2005). Seeds of all genotypes were grown for one generation in constant growth chamber conditions (16h:8h light:dark, 24°C temperature, Metro-Mix growth medium) and harvested in order to provide enough seed for use in this study. Due to a near total failure of germination and survival of seeds of the *eto1-1* and *rhd-1* mutant genotypes, and the extreme dwarf phenotype of the surviving plants, these genotypes were excluded from all further analyses in this study.

Growth conditions and experimental design

Seeds were stratified in Petri plates with moist filter paper at 4°C for one week prior to planting to ensure synchronous germination. The experiment consisted of 12 replicate blocks, each a plastic tray containing 32 - 6 x 6 x 6 cm cells. Each cell was filled with a 3:1 sand:soil mixture in order to allow extraction of roots from the soil. Within each tray, each genotype was planted into four cells, with two cells randomly assigned to each of a high or low nutrient addition treatment. Plants were thinned to one germinated seed per cell within 3 days of planting.

Nutrient addition treatments were applied to individual cells, with 2mL of either full-strength Hoagland's solution (Epstein 1972; 'high nutrient' treatment) or 10% concentration Hoagland's solution ('low nutrient' treatment) applied to each cell twice per week for the duration of the experiment. Plants were watered as necessary to prevent drought stress. To prevent dilution of the nutrient treatments, I avoided watering for at least 24 hours after nutrient addition. Trays were arranged randomly on a bench and grown in a controlled environment growth chamber for the remainder of the experiment (16h:8h light:dark, 24°C temperature).

Trait and fitness measures

Each of the two cells of each genotype by nutrient addition treatment combination per tray were assigned to one of two harvest treatments. 'Bolting harvest' plants were harvested at the time of bolting (flowering stem initiation and elongation), while 'seed harvest' plants were harvested at the time of final seed set (total senescence of aboveground vegetative and flowering structures). All plants were monitored on a daily basis and each plant's time of bolting was recorded as the number of days from germination to flowering stem initiation and elongation. The number of leaves and rosette diameter of each plant were measured at bolting time. Bolting harvest plants were harvested at the time of bolting, washed and separated into leaf and root tissue. Leaves were scanned and total leaf area was determined using WinFOLIA software (Regent Instruments Inc.). Roots were extracted from the soil and scanned and total root length and average root diameter were determined using WinRHIZO software (Regent Instruments Inc.). Leaves and roots were then dried at 70°C for three days and weighed to determine leaf and root biomass. I calculated specific leaf area (SLA; leaf area per unit leaf biomass) and specific root length (SRL; root length per unit root biomass) for each bolting harvest plant. For seed harvest plants, the height of the infloresence, number of fruits, and number of basal and lateral flowering branches were measured at the time of final seed set and aboveground senescence. Average individual seed masses were measured for five seed harvest plants from each genotype and nutrient addition treatment combination. Survival of each genotype was calculated as the proportion of plants surviving to bolting time in each nutrient treatment.

Phenotypic plasticity

I estimated the magnitude of variation in all traits among genotypes and environments based on measurements on individual plants. Seed mass and survival were only calculated as average values for genotypes and thus not included in this analysis. Analyses were conducted using linear mixed effects models with restricted maximum likelihood parameter estimation (Pinheiro and Bates 2000) due to the unbalanced nature of the data created by differential mortality of individual plants among genotypes and treatments. Genotype and environment were considered fixed factors since genotypes were not randomly selected from the population of available *A. thaliana* genotypes for inclusion in this study. Tray was included as a random blocking factor. Analyses were performed on individual plants with all variables transformed as necessary to meet model assumptions of normality (Table 5-2). For each trait, I report marginal F statistics and associated *P*-values for both fixed factors and their interaction ($\alpha = 0.05$), along with estimates of trait means in low and high nutrient environments.

Phenotypic integration and trait correlations

To visualize changes in patterns of phenotypic integration of traits in the two environments, I calculated a pairwise correlation matrix among genotype trait means in each environment. I then calculated a change-in-correlation matrix by calculating the absolute change in each trait's correlation with other traits between low and high nutrient environments. A cluster analysis of the change-in-correlation matrix (UPGMA clustering algorithm based on absolute differences among rows and columns of the matrix; Legendre and Legendre 1998) was used to measure the similarity of each trait's change in correlations with other traits. These analyses allowed me to identify clusters of traits whose correlations with other traits changed in similar ways between environments.

Selection on phenotypic traits

I employed univariate and multivariate selection gradient analyses (Lande and Arnold 1983) to determine the nature of selection on all measured traits. Most vegetative traits could only be measured through destructive sampling of bolting harvest plants, while fitness and reproductive traits could only be measured on seed harvest plants. I therefore used genotypic trait averages in low and high nutrient environments in place of individual plant data when calculating selection gradients. This approach precluded the use of regression approaches to simultaneously measure direct and indirect selection on all traits (the selection differential approach), since there were more explanatory variables (traits) than samples (genotypes), and because of the high collinearity of many of the measured traits (Mitchell-Olds and Shaw 1987). I instead estimated the strength of direct selection on each trait individually, in addition to a multivariate approach that summarized relationships among all measured traits using a principal components analysis (PCA) before estimating selection gradients between PCA axes and relative fitness.

I measured the absolute fitness of genotypes in each environment in two ways. The first fitness measure was the average number of siliques per plant (fertility) for each genotype. The second was the average number of siliques per plant multiplied by the proportion of plants that survived to seed set for each genotype, equivalent to the estimated proportional total fruit production by a genotype relative to other genotypes (cumulative fitness). The relative fitness of each genotype was defined as the absolute fitness of the genotype in an environment, divided by the mean absolute fitness across genotypes in that environment.

Exploratory analyses indicated that relationships between traits and fitness were linear when present and so I did not include quadratic terms in further analyses. Transformed trait means in each environment were standardized to zero mean and unit variance for selection gradient analyses. I report estimates of standardized selection gradients, which are the slopes (β) of the relationship between standardized genotypic trait values and relative fitness (Lande and Arnold 1983) for each trait in each environment.

To assess how selection on the multivariate phenotype differed among environments, I calculated multivariate selection gradients as the relationship between relative fitness and standardized genotype scores on the first two axes of a principal components analysis (PCA) based on correlation matrices of scaled, transformed traits in each environment (Mitchell-Olds and Shaw 1987).

Costs of plasticity

To test for costs of phenotypic plasticity, I fit a linear regression model $w = X + pl.X$ for each trait in each environment, where w = genotype relative fitnesses, X = standardized trait means, and $pl.X$ = standardized trait plasticities (Scheiner and Berrigan 1998). I defined the phenotypic plasticity of a trait as the absolute difference in each genotype's mean trait value between environments. After accounting for the relationship between relative fitness and trait means, a negative relationship between relative fitness and plasticity indicates a maintenance cost of plasticity, meaning that it is costly for a genotype to be plastic across environments, independent of the mean trait values in those environments (DeWitt et al. 1998).

Evidence for selection and costs of plasticity

Due to the small sample sizes used in the selection and plasticity cost analyses (N = 14 genotypes per environment), and a desire to detect potentially weak trends of selection on traits and plasticities (Kingsolver et al. 2001), I considered selection gradients and plasticity cost estimates whose 90% confidence intervals did not include zero to provide evidence for selection or a cost of plasticity, equivalent to using a liberal P-value cutoff ($\alpha = 0.10$) in a test of statistical significance.

Results

Phenotypic plasticity

Nearly all phenotypic traits differed among genotypes (Table 5-3). All reproductive traits were phenotypically plastic in response to nutrient availability, with plants of most genotypes producing taller inflorescences with more basal and lateral branches and more siliques in the high nutrient environment. (Figure 5-1). The only vegetative traits that exhibited consistent plasticity across genotypes were rosette diameter, leaf area and SLA, all of which increased in response to high nutrient availability. The only clear example of genotypic variation in plasticity (significant GxE interaction) was for the number of lateral branches ($P = 0.04$), which increased in high nutrient conditions for 11 of 14 genotypes but decreased for the other genotypes (Figure 5-1).

Phenotypic integration and trait correlations

In both environments, several clusters of correlated traits explained much of the phenotypic variation among genotypes. The first two axes from a principal components analysis of genotype trait averages (Figure 5-2) explained 54% and 17%, respectively, of the variation in traits among genotypes in the high nutrient environment, and 57% and 18% in the low nutrient environment. The main trend of phenotypic variation among genotypes in both environments was a gradient in flowering time and associated traits. A genotype's position along the first PCA axis in both environments indicated where the genotype falls in the continuum from early-flowering to late-flowering phenotypes. Early-flowering genotypes with high scores along the first PCA axis tended to possess high SLA leaves, more basal flowering branches, and a relatively small vegetative investment at the time of bolting (low vegetative biomass, leaf area, root length, and number of leaves). This main trend was similar between environments (Figure 5-2), but several individual traits differed in their correlations with other traits (Figure 5-3). In the low nutrient environment, seed mass and average root diameter also increased along the first axis while SRL decreased, and the number of lateral branches, inflorescence height and rosette diameter increased along the second axis. In the high nutrient environment, SRL, inflorescence height and rosette diameter increased and seed mass and root average diameter decreased along the second axis.

The phenotypic traits with the largest changes in their correlation with other traits were SRL, average root diameter, seed mass, and number of lateral branches (Figure 5-3). SRL, average root diameter and seed mass changed from being closely correlated with other traits varying along the flowering-time gradient in the low nutrient environment, to being uncorrelated with these same traits in the high nutrient environment (Figure 5-2).

Selection on phenotypic traits

Late-flowering genotypes had consistently lower relative fitness in both environments, and as a result the suite of traits associated with later flowering also tended to be negatively related to relative fitness (Table 5-4). There was evidence for positive selection on most reproductive traits for both measures of fitness at high and low nutrient availability, although number of lateral branches was only under positive selection in the low nutrient environment. Selection on vegetative traits was more variable between environments and fitness measures. At high nutrient availability, vegetative traits related to total leaf and root system size were under negative selection when fertility was used as a measure of relative fitness. Seed mass was positively related to relative fitness in the low nutrient environment, and SLA was positively related to cumulative fitness in both environments. Genotypes with thicker roots had higher relative fitness in the low nutrient environment.

Multivariate selection analyses revealed patterns of selection similar to those found in the univariate analyses (Table 5-5). Relative fitness increased with increasing genotype scores along both the first and second PCA axes in the low nutrient environment, but in the high nutrient environment only the first axis was related to relative fitness. Early-flowering genotypes had higher relative fitness in both environments, but the strength of selection was stronger when fitness was measured as fertility, especially in the high nutrient environment.

Costs of plasticity

Maintenance costs of phenotypic plasticity were detected for a few traits. After accounting for selection on mean values of these traits in each environment, plasticity of leaf area and number of lateral branches were costly in the low nutrient environment, and plasticity of bolting time and seed mass were costly in the high nutrient environment (Table 5-6).

Discussion

Phenotypic plasticity of reproductive, leaf and root traits

Based on trait measurements on individual plants, I detected phenotypic plasticity in response to nutrient availability for all measured reproductive traits, but only three of ten measured vegetative traits (Table 5-3). This result is consistent with previous studies that have found that reproductive traits tend to be plastic in response to nutrient availability, while vegetative traits are much less plastic (Pigliucci and Schlichting 1995). It is not clear whether this lack of detectable plasticity in most vegetative traits is due to high intergenotypic variability for these traits, or due to canalizing selection, a lack of genetic variation, or inability to sense and respond to nutrient availability (Pigliucci and Byrd 1998). While the number of lateral branches was the only trait to show statistically significant variation in plasticity among genotypes, several other traits such as average root diameter varied among environments for a subset of genotypes, and these traits generally showed marginally statistically significant GxE interactions (Table 5-3). This may be a result of plasticity for these traits only occurring in late-flowering genotypes, or due to high intra-genotypic variability for these traits.

Phenotypic integration above- and belowground

Phenotypic integration, measured as patterns of correlations among traits, was mostly consistent between the high and low nutrient environments (Figure 5-2). Average values of SRL and root diameter did not differ between environments, but the correlation of these traits with other traits did change (Figure 5-3), with early-flowering genotypes producing low SRL roots with a larger average diameter only in the low nutrient environment. Decreases in SRL and thicker roots have been found to maintain plant growth and fitness in low nutrient environments by avoiding wasteful production of tissues with a high instantaneous rate of nutrient uptake in these environments (Ryser and Lambers 1995). Early-flowering genotypes appear to be capable of producing thick, low SRL roots in response to nutrient stress while maintaining high SLA leaves and high relative fitness across both environments. Previous studies have found that SLA and SRL were positively correlated among *A. thaliana* genotypes (Li et al. 1998), but these studies grew plants in conditions of high nutrient availability, which may explain the different results if these traits are plastic in response to nutrient availability.

Selection on root traits and plasticities

Belowground traits related to overall root system size and length were negatively correlated with fitness only in the high nutrient environment, while root morphological traits such as SRL and average root diameter were under positive selection only in the low nutrient environment. The overall strength of selection on the multivariate phenotype was weaker in the high nutrient environment (Table 5-5), especially when fitness was measured as cumulative fitness, indicating that differential survival of genotypes in the low nutrient treatment had important effects on patterns of selection. The negative selection on many vegetative traits in both environments is likely a result of the higher overall fitness of early flowering genotypes, which flowered at a consistently smaller vegetative size, and did not increase their vegetative allocation in response to increased nutrient availability. Plant survival in stressful environments will be determined by more than just total root system length or biomass, and root traits such as SRL and root diameter might have important implications for survival at a range of nutrient availabilities, potentially through their effects on ecophysiology and plant anchorage (Fitter 2002).

For the phenotypic plasticity of a trait to be considered adaptive or maladaptive, a trait must be plastic, and the direction of plastic changes in the trait must be the same as the direction of selection on the trait in those environments, or opposite the direction of selection in the case of maladaptive plasticity (Dorn et al. 2000). Using these criteria, the phenotypic plasticity of most reproductive traits was adaptive, since values of reproductive traits increased in the high nutrient environment, and there was selection for higher reproductive trait values in both environments (Tables 5-3 and 5-4). Only a few vegetative traits were plastic according to GxE analyses (Table 5-3), and the plasticity of these traits ranged from adaptive (SLA), to passive (rosette diameter), to maladaptive (leaf area). Only a few weak costs of plasticity to nutrient availability were detected (Table 5-6), indicating that selection on plasticity acted primarily through changes in fitness in different environments rather than maintainance costs of producing a plastic phenotype. By measuring vegetative traits at a common developmental stage (time of bolting) rather than at a fixed time, I was able to rule out nutrient effects on growth rate or ontogenetic stage at measurement time as causes of observed phenotypic plasticity (Coleman et al. 1994).

Genotypes producing larger seeds had higher relative fitness in the low nutrient environment (Table 5-4), but seed mass plasticity was costly in the high nutrient environment (Table 5-6). Seed mass differs from the other traits measured in this study in that relationships between seed mass and fitness are not truly measures of selection, since seed mass effects on fitness would not be expected to take place until the following generation through maternal effects (Donohue 2002). Relationships between seed mass, plasticity and fitness may simply reflect changes in overall plant vigor and fertility in some environments, with actual selection on seed mass potentially occurring in the subsequent generations when it may affect the relative fitness of offspring. Large seeds allow *A. thaliana* plants to grow and to survive longer in low nutrient environments (Krannitz et al. 1991a). While I would expect larger seeds to provide offspring with a greater fitness advantage when nutrients are limiting, tradeoffs between seed size and fecundity (Clauss and Aarssen 1994) could also explain the negative relationship between parental fertility and plasticity of seed mass, which might only be expected in high nutrient environments where overall seed production is limited by the fecundity-seed mass tradeoff instead of nutrient availability.

The relevance of estimates of selection and costs of plasticity will ultimately depend on whether measurements in a growth chamber can be extrapolated to relative fitness in natural communities. Many generations of growth in artificial conditions has probably resulted in selection for *A. thaliana* genotypes which are able to quickly produce large amounts of seed with low investment in the vegetative traits that might be more important for plant fitness in a natural setting. Interactions with numerous environmental factors other than nutrient availability could have important effects on the relative fitness of *A. thaliana* genotypes in natural communities. While annual plants such as *A. thaliana* are one of the few organisms for which estimates of seed production are generally good surrogate measures of relative fitness in field conditions (Crone 2001), the small sample size used in this study and the artificial nature of the growth environment suggest that these results should be interpreted with caution.

Conclusions

I found consistent evidence for adaptive plasticity of reproductive traits of *Arabidopsis thaliana* in response to nutrient availability, but vegetative traits were less consistently plastic and relationships between vegetative plasticity and fitness were highly variable. Patterns of phenotypic integration in different environments were mostly similar above and belowground, with genotypes falling along a gradient of flowering time and associated traits in both environments. Selection gradient and plasticity cost analyses suggested an overall pattern of selection for low vegetative investment, rapid flowering and high reproductive output in both environments, and selection for reproductive plasticity but a canalized vegetative genotype with a small vegetative size regardless of nutrient availability. Several root traits (SRL and root diameter) were correlated with plant fitness only in the low nutrient environment, and the correlation of these traits with other phenotypic traits changed depending on nutrient availability.

Literature Cited

Alpert, P., and E. L. Simms. 2002. The relative advantages of plasticity and fixity in different environments: when is it good for a plant to adjust? Evolutionary Ecology **16**:285-297.

Bates, T. R., and J. P. Lynch. 2001. Root hairs confer a competitive advantage under low phosphorus availability. Plant and Soil **236**:243-250.

Bradshaw, A. D. 1965. Evolutionary significance of phenotypic plasticity in plants. Advances in Genetics **13**:115–155.

Cahill, J. F., S. W. Kembel, and D. J. Gustafson. 2005. Differential genetic influences on competitive effect and response in *Arabidopsis thaliana*. Journal of Ecology **93**:958-967.

Chevalier, F., M. Pata, P. Nacry, P. Doumas, and M. Rossignol. 2003. Effects of phosphate availability on the root system architecture: large-scale analysis of the natural variation between *Arabidopsis* accessions. Plant Cell and Environment **26**:1839-1850.

Clauss, M. J., and L. W. Aarssen. 1994. Phenotypic plasticity of size-fecundity relationships in *Arabidopsis thaliana*. Journal of Ecology **82**:447-455.

Coleman, J. S., K. D. M. Mcconnaughay, and D. D. Ackerly. 1994. Interpreting phenotypic variation in plants. Trends in Ecology and Evolution **9**:187-191.

Crone, E. E. 2001. Is survivorship a better fitness surrogate than fecundity? Evolution **55**:2611-2614.

DeWitt, T. J., A. Sih, and D. S. Wilson. 1998. Costs and limits of phenotypic plasticity. Trends in Ecology & Evolution **13**:77-81.

Donohue, K. 2002. Germination timing influences natural selection on life-history characters in *Arabidopsis thaliana*. Ecology **83**:1006-1016.

Dorn, L. A., E. H. Pyle, and J. Schmitt. 2000. Plasticity to light cues and resources in *Arabidopsis thaliana*: Testing for adaptive value and costs. Evolution **54**:1982-1994.

Epstein, E. 1972. Mineral nutrition of plants : principles and perspectives. Wiley, New York.

Fitter, A. 2002. Characteristics and functions of root systems. Pages 15-32 *in* Y. Waishel, A. Eshel, and U. Kafkafi, editors. Plant roots: the hidden half. Marcel Dekker, New York, NY.

Fitter, A., L. Williamson, B. Linkohr, and O. Leyser. 2002. Root system architecture determines fitness in an *Arabidopsis* mutant in competition for immobile phosphate ions but not for nitrate ions. Proceedings of the Royal Society of London, Series B: Biological Sciences **269**:2017-2022.

Fitz Gerald, J. N., M. D. Lehti-Shiu, P. A. Ingram, K. I. Deak, T. Biesiada, and J. E. Malamy. 2006. Identification of quantitative trait loci that regulate *Arabidopsis* root system size and plasticity. Genetics **172**:485-498.

Geber, M. A., and L. R. Griffen. 2003. Inheritance and natural selection on functional traits. International Journal of Plant Sciences **164**:S21-S42.

Grime, J. P. 2001. Plant strategies, vegetation processes and ecosystem properties. Wiley, Chichester, U.K.

Kingsolver, J. G., H. E. Hoekstra, J. M. Hoekstra, D. Berrigan, S. N. Vignieri, C. E. Hill, A. Hoang, P. Gibert, and P. Beerli. 2001. The strength of phenotypic selection in natural populations. American Naturalist **157**:245-261.

Krannitz, P. G., L. W. Aarssen, and J. M. Dow. 1991a. The effect of genetically based differences in seed size on seedling survival in *Arabidopsis thaliana* (Brassicaceae). American Journal of Botany **78**:446-450.

Krannitz, P. G., L. W. Aarssen, and D. D. Lefebvre. 1991b. Relationships between physiological and morphological attributes related to phosphate uptake in 25 genotypes of *Arabidopsis thaliana*. Plant and Soil **133**:169-175.

Lande, R., and S. J. Arnold. 1983. The measurement of selection on correlated characters. Evolution **37**:1210-1226.

Legendre, P., and L. Legendre. 1998. Numerical ecology, 2nd English edition. Elsevier, New York.

Li, B., J. I. Suzuki, and T. Hara. 1998. Latitudinal variation in plant size and relative growth rate in *Arabidopsis thaliana*. Oecologia **115**:293-301.

Linkohr, B. I., L. C. Williamson, A. H. Fitter, and H. M. O. Leyser. 2002. Nitrate and phosphate availability and distribution have different effects on root system architecture of *Arabidopsis*. Plant Journal **29**:751-760.

Mitchell-Olds, T. 2001. *Arabidopsis thaliana* and its wild relatives: a model system for ecology and evolution. Trends in Ecology and Evolution **16**:693-700.

Mitchell-Olds, T., and R. G. Shaw. 1987. Regression Analysis of Natural Selection: Statistical Inference and Biological Interpretation. Evolution **41**:1149-1161.

Pigliucci, M. 2001. Phenotypic plasticity: beyond nature and nurture. Johns Hopkins Univ Press, Baltimore, MD.

Pigliucci, M. 2003a. Phenotypic integration: studying the ecology and evolution of complex phenotypes. Ecology Letters **6**:265-272.

Pigliucci, M. 2003b. Selection in a model system: Ecological genetics of flowering time in *Arabidopsis thaliana*. Ecology **84**:1700-1712.

Pigliucci, M., and N. Byrd. 1998. Genetics and evolution of phenotypic plasticity to nutrient stress in *Arabidopsis*: drift, constraints or selection? Biological Journal of the Linnean Society **64**:17-40.

Pigliucci, M., and K. Hayden. 2001. Phenotypic plasticity is the major determinant of changes in phenotypic integration in *Arabidopsis*. New Phytologist **152**:419-430.

Pigliucci, M., and A. Kolodynska. 2002. Phenotypic plasticity and integration in response to flooded conditions in natural accessions of *Arabidopsis thaliana* (L.) Heynh (Brassicaceae). Annals of Botany **90**:199-207.

Pigliucci, M., and C. D. Schlichting. 1995. Reaction norms of *Arabidopsis* (Brassicaceae). III. Response to nutrients in 26 populations from a worldwide collection. American Journal of Botany **82**:1117-1125.

Pinheiro, J. C., and D. M. Bates. 2000. Mixed-effects models in S and S-PLUS. Springer, New York.

Ryser, P., and L. Eek. 2000. Consequences of phenotypic plasticity vs. interspecific differences in leaf and root traits for acquisition of aboveground and belowground resources. American Journal of Botany **87**:402-411.

Ryser, P., and H. Lambers. 1995. Root and leaf attributes accounting for the performance of fast-growing and slow-growing grasses at different nutrient supply. Plant and Soil **170**:251-265.

Scheiner, S. M. 1993. Genetics and evolution of phenotypic Pplasticity. Annual Review of Ecology and Systematics **24**:35-68.

Scheiner, S. M., and D. Berrigan. 1998. The genetics of phenotypic plasticity. VIII. The cost of plasticity in *Daphnia pulex*. Evolution **52**:368-378.

Schlichting, C., and M. Pigliucci. 1998. Phenotypic evolution : a reaction norm perspective. Sinauer Press, Sunderland, MA.

Stinchcombe, J. R., C. Weinig, M. Ungerer, K. M. Olsen, C. Mays, S. S. Halldorsdottir, M. D. Purugganan, and J. Schmitt. 2004. A latitudinal cline in flowering time in *Arabidopsis thaliana* modulated by the flowering time gene FRIGIDA. Proceedings of the National Academy of Sciences of the United States of America **101**:4712-4717.

Tienderen, P. H. V. 1991. Evolution of generalists and specialist in spatially heterogeneous environments. Evolution **45**:1317-1331.

Tjoelker, M. G., J. M. Craine, D. Wedin, P. B. Reich, and D. Tilman. 2005. Linking leaf and root trait syndromes among 39 grassland and savannah species. New Phytologist **167**:493-508.

Weiner, J. 2004. Allocation, plasticity and allometry in plants. Perspectives in Plant Ecology Evolution and Systematics **6**:207-215.

Westoby, M., D. S. Falster, A. T. Moles, P. A. Vesk, and I. J. Wright. 2002. Plant ecological strategies: Some leading dimensions of variation between species. Annual Review of Ecology and Systematics **33**:125-159.

Wolfe, L. M., and S. J. Mazer. 2005. Patterns of phenotypic plasticity and their fitness consequences in wild radish (*Raphanus sativus*: Brassicaceae). International Journal of Plant Sciences **166**:631-640.

Zhang, J. H., and M. J. Lechowicz. 1994. Correlation between time of flowering and phenotypic plasticity in *Arabidopsis thaliana* (Brassicaceae). American Journal of Botany **81**:1336-1342.

Table 5-1. List of *Arabdiopsis thaliana* genotypes used in this study.

Name	TAIR Stock #	Description	Phenotype
Aa-0	CS934	Aua/Rhon, Germany ecotype	Late flowering
An-1	CS944	Antwerpen, Belgium ecotype	Early flowering
Col-0	Obtained from Lehle Seeds	Columbia ecotype/isoline	Late flowering
En-2	CS1138	Enkheim, Germany ecotype	Late flowering
Jl-3	CS1252	Vranov u Brna, Czech Republic ecotype	Early flowering
No-0	CS1394	Nossen, Germany ecotype	Early flowering
Rld-2	CS1641	Rschew, Russia ecotype	Early flowering
Sei-0	CS1504	Seis am Schlern, Italy ecotype	Late flowering
aux1-7	CS3074	Auxin mutant	Increased root elongation
axr4-2	CS8019	Auxin mutant	Reduced lateral root formation
Eto1-1	CS3072	Ethylene mutant	Small rosette, increased root hair formation
gl1-2	CS3126	Glabrous mutant	Young leaves lack trichomes
pho2-1	CS8508	Phosphate mutant	Accumulates phosphate in leaf tissues
phyB-9	CS6217	Phytochrome mutant	Small rosette, long stem and root hairs
rhd1-1	CS2257	Root hair deficient mutant	Root hairs lacking or deformed
xv-1	CS3133	Xanthaviridis mutant	Dwarf plants

Table 5-2. List of traits measured on 14 genotypes of *Arabidopsis thaliana* grown in low and high nutrient availability environments.

Trait type	**Trait**	**Units**	**Transformation**	**Abbreviation**	**Time of measurement**
Fitness	Siliques	#	Square root	siliques	Seed set
	Survival	%	None	survival	Bolting
Reproductive	Inflorescence height	cm	$\log_{10}$	height	Seed set
	Basal branches	#	Square root	basal	Seed set
	Lateral branches	#	Square root	lateral	Seed set
	Seed mass	g	$\log_{10}$	seedmass	Seed set
Vegetative	Rosette diameter	mm	$\log_{10}$	rosdiam	Bolting
	Number of leaves	#	Square root	numleaves	Bolting
	Days to bolt	days	$\log_{10}$	daystobolt	Bolting
	Leaf mass	g	$\log_{10}$	leafmass	Bolting
	Root mass	g	$\log_{10}$	rootmass	Bolting
	Leaf area	cm^2	$\log_{10}$	leafarea	Bolting
	Root length	mm	$\log_{10}$	rootlen	Bolting
	Root diameter	mm	$\log_{10}$	rootdiam	Bolting
	Specific leaf area	cm^2/g	$\log_{10}$	SLA	Bolting
	Specific root length	m/g	$\log_{10}$	SRL	Bolting

Table 5-3. Results of a linear mixed model analysis of variation in functional traits among *Arabidopsis thaliana* plants of 14 genotypes grown in low and high nutrient environments. N indicates the number of plants each trait was measured on. Bold values indicate *P*<0.05.

							Mean by environment	
Variable	**N**	**Statistic**	**Intercept**	**Genotype**	**Environment**	**G x E**	**Low**	**High**
Inflorescence height	259	F	2915.4	8.9	24.5	1.0	24.8	27.4
		P	**<0.0001**	**<0.0001**	**<0.0001**	0.4288		
Basal branches	259	F	2094.7	4.1	13.8	1.6	1.6	2.0
		P	**<0.0001**	**<0.0001**	**0.0003**	0.1012		
Lateral branches	259	F	480.4	1.7	33.5	1.8	2.3	3.1
		P	**<0.0001**	0.0540	**<0.0001**	**0.0385**		
Siliques	256	F	1374.7	2.1	77.8	0.5	53.9	80.9
		P	**<0.0001**	**0.0153**	**<0.0001**	0.9296		
Rosette diameter	536	F	5840.3	15.9	21.5	1.0	31.6	34.4
		P	**<0.0001**	**<0.0001**	**<0.0001**	0.4147		
Number of leaves	536	F	43362.8	72.1	2.1	1.7	10.6	10.9
		P	**<0.0001**	**<0.0001**	0.1497	0.0652		
Days to bolt	537	F	30079.2	41.7	0.0	0.7	24.0	24.0
		P	**<0.0001**	**<0.0001**	0.8331	0.7891		
Leaf mass	266	F	1076.4	15.9	3.5	0.8	0.017	0.021
		P	**<0.0001**	**<0.0001**	0.0622	0.6270		
Root mass	269	F	2105.8	17.7	0.0	1.7	0.004	0.004
		P	**<0.0001**	**<0.0001**	0.9973	0.0678		
Leaf area	273	F	2204.1	13.5	16.8	1.6	5.1	6.3
		P	**<0.0001**	**<0.0001**	**0.0001**	0.0869		
Root length	268	F	2511.7	22.2	0.0	1.2	463.9	445.9
		P	**<0.0001**	**<0.0001**	0.9903	0.2561		
Root diameter	268	F	57053.3	1.4	0.0	1.1	0.156	0.156
		P	**<0.0001**	0.1579	0.9076	0.4017		
SLA	266	F	1952.1	14.5	5.8	0.7	375.8	401.1
		P	**<0.0001**	**<0.0001**	**0.0173**	0.8016		
SRL	264	F	49448.7	2.4	1.5	1.6	112.2	120.2
		P	**<0.0001**	**0.0060**	0.2173	0.0964		

Table 5-4. Standardized selection gradients for traits measured on 14 *Arabidopsis thaliana* genotypes grown in low and high nutrient. Selection gradients (β) represent the slope (± standard error) of the relationship between standardized trait values and relative fitness measured as fertility or cumulative fitness. Bold values indicate $P<0.05$; bold and italic values indicate $P<0.10$.

		Relative fitness: Fertility			Relative fitness: Cumulative fitness		
Nutrients	Trait	β	s.e.	P	β	s.e.	P
High	Height	0.07	0.04	***0.0645***	0.06	0.08	0.4670
	Basal branches	0.07	0.03	**0.0361**	0.12	0.06	***0.0776***
	Lateral branches	0.00	0.04	0.9520	0.01	0.08	0.8910
	Days to bolt	-0.07	0.03	**0.0317**	-0.13	0.06	***0.0672***
	Number of leaves	-0.07	0.03	**0.0230**	-0.12	0.06	***0.0703***
	Rosette diameter	-0.02	0.04	0.6630	-0.02	0.08	0.7690
	Root diameter	-0.04	0.04	0.2620	0.04	0.07	0.6370
	Leaf mass	-0.07	0.03	**0.0376**	-0.10	0.06	0.1420
	Root mass	-0.05	0.03	***0.0633***	-0.09	0.05	0.1250
	Leaf area	-0.07	0.03	**0.0210**	-0.09	0.06	0.1560
	Root length	-0.06	0.03	0.0740	-0.10	0.06	0.1180
	SLA	0.05	0.04	0.1780	0.14	0.07	***0.0581***
	SRL	-0.02	0.03	0.6350	-0.04	0.06	0.5810
	Seed mass	-0.03	0.03	0.3920	0.08	0.06	0.1880
	Survival	0.00	0.03	0.9290			
Low	Height	0.10	0.05	***0.0612***	0.15	0.08	***0.0793***
	Basal branches	0.13	0.05	**0.0164**	0.18	0.09	***0.0545***
	Lateral branches	0.14	0.07	***0.0763***	0.25	0.11	***0.0501***
	Days to bolt	-0.12	0.04	**0.0124**	-0.20	0.06	**0.0057**
	Number of leaves	-0.09	0.05	***0.0605***	-0.17	0.07	**0.0328**
	Rosette diameter	0.01	0.05	0.8220	0.00	0.09	0.9640
	Root diameter	0.09	0.04	***0.0572***	0.17	0.07	**0.0285**
	Leaf mass	-0.06	0.05	0.2760	-0.12	0.08	0.1730
	Root mass	-0.08	0.06	0.2310	-0.12	0.11	0.2880
	Leaf area	-0.05	0.06	0.4630	-0.07	0.11	0.5040
	Root length	-0.08	0.05	0.1180	-0.12	0.08	0.1490
	SLA	0.07	0.05	0.1460	0.14	0.07	***0.0662***
	SRL	-0.08	0.06	0.2470	-0.17	0.10	0.1030
	Seed mass	0.15	0.04	**0.0053**	0.29	0.06	**0.0003**
	Survival	0.12	0.05	**0.0391**			

Table 5-5. Estimates of selection on the multivariate phenotype of 14 *Arabidopsis thaliana* genotypes grown in high and low nutrient availability environments, based on selection gradient analysis of relationships between relative fitness measured as fertility or cumulative fitness versus the first two axes of a principal components analysis (PCA) of genotypes and traits. Selection gradients (β) represent the slope (± standard error) of the relationship between standardized trait values and relative fitness measured as fertility or cumulative fitness. Values in parentheses indiciate the percent variance explained by each PCA axis. Axis loadings indicate the relationship between variables and the PCA axes.

	Low		High	
Selection gradients	PCA Axis 1 (57%)	PCA Axis 2 (18%)	PCA Axis 1 (54%)	PCA Axis 2 (17%)
Fertility				
β	0.10	0.09	0.07	0.02
s.e.	0.04	0.04	0.03	0.03
P-value	0.04	0.06	0.03	0.44
Cumulative fitness				
Beta	0.18	0.14	0.11	-0.06
s.e.	0.07	0.07	0.06	0.06
P-value	0.02	0.05	0.11	0.36
Axis loadings				
Height	0.05	0.58	0.15	0.39
Basal branches	0.25	-0.12	0.29	-0.08
Lateral branches	0.03	0.56	-0.03	0.12
Rosette diameter	-0.19	0.47	-0.24	0.28
Number of leaves	-0.34	-0.10	-0.36	-0.02
Days to bolt	-0.32	-0.17	-0.32	0.00
Leaf mass	-0.33	-0.02	-0.35	0.03
Root mass	-0.31	-0.01	-0.34	-0.07
Leaf area	-0.30	0.06	-0.36	-0.05
Root length	-0.33	0.01	-0.35	0.02
Root diameter	0.17	-0.08	-0.10	-0.50
SLA	0.32	0.06	0.32	-0.23
SRL	-0.26	0.19	-0.01	0.47
Seed mass	0.26	0.15	-0.06	-0.45

Table 5-6. Estimates of costs of phenotypic plasticity for traits measured on 14 genotypes of *Arabidopsis thaliana* grown in high and low nutrient availability environments. Plasticity costs were measured as the slope of a linear regression of the relationship between standardized plasticity (absolute change in a trait between environments) and relative fitness measured as fertility or cumulative fitness, controlling for selection on standardized trait means in each environment. See methods section for further details. Bold values indicate $P<0.05$; bold and italic values indicate $P<0.10$.

		Relative fitness: Fertility			Relative fitness: Cumulative fitness		
Nutrients	Trait	Slope	s.e.	*P*	Slope	s.e.	*P*
High	Height	0.02	0.03	0.4433	-0.07	0.07	0.3050
	Basal branches	-0.01	0.04	0.7064	-0.02	0.08	0.8410
	Lateral branches	-0.08	0.05	0.1680	-0.23	0.09	**0.0330**
	Days to bolt	0.02	0.03	0.4835	-0.12	0.05	**0.0387**
	Number of leaves	-0.01	0.03	0.6197	0.04	0.06	0.5279
	Rosette diameter	-0.02	0.04	0.5380	-0.05	0.07	0.5220
	Root diameter	0.01	0.04	0.7540	-0.13	0.08	0.1360
	Leaf mass	-0.03	0.03	0.4110	0.05	0.07	0.4770
	Root mass	0.04	0.05	0.4404	-0.04	0.10	0.7150
	Leaf area	-0.03	0.04	0.5170	-0.04	0.09	0.6390
	Root length	0.01	0.03	0.7520	-0.03	0.07	0.6380
	SLA	0.03	0.03	0.3930	-0.06	0.06	0.3770
	SRL	-0.02	0.06	0.7290	0.12	0.10	0.2880
	Seed mass	-0.10	0.04	**0.0267**	-0.27	0.05	**0.0003**
	Survival	-0.07	0.04	0.1340	N/A		
Low	Height	0.00	0.06	0.9600	0.00	0.09	0.9980
	Basal branches	0.04	0.05	0.4281	0.11	0.08	0.1974
	Lateral branches	-0.07	0.06	0.2560	-0.10	0.09	0.2990
	Days to bolt	0.02	0.05	0.6374	0.04	0.07	0.5637
	Number of leaves	-0.08	0.05	***0.0949***	-0.10	0.07	0.2082
	Rosette diameter	-0.09	0.06	0.1630	-0.16	0.09	0.1130
	Root diameter	-0.05	0.07	0.5100	-0.05	0.11	0.6390
	Leaf mass	-0.06	0.05	0.2800	-0.06	0.08	0.4580
	Root mass	-0.03	0.06	0.6060	-0.10	0.09	0.2900
	Leaf area	-0.10	0.05	**0.0477**	-0.17	0.08	**0.0415**
	Root length	0.02	0.05	0.7170	-0.05	0.09	0.5830
	SLA	-0.05	0.06	0.4270	-0.05	0.10	0.6400
	SRL	0.00	0.05	0.9840	-0.02	0.09	0.8510
	Seed mass	0.02	0.05	0.7515	0.06	0.06	0.2943
	Survival	-0.02	0.05	0.6242	N/A		

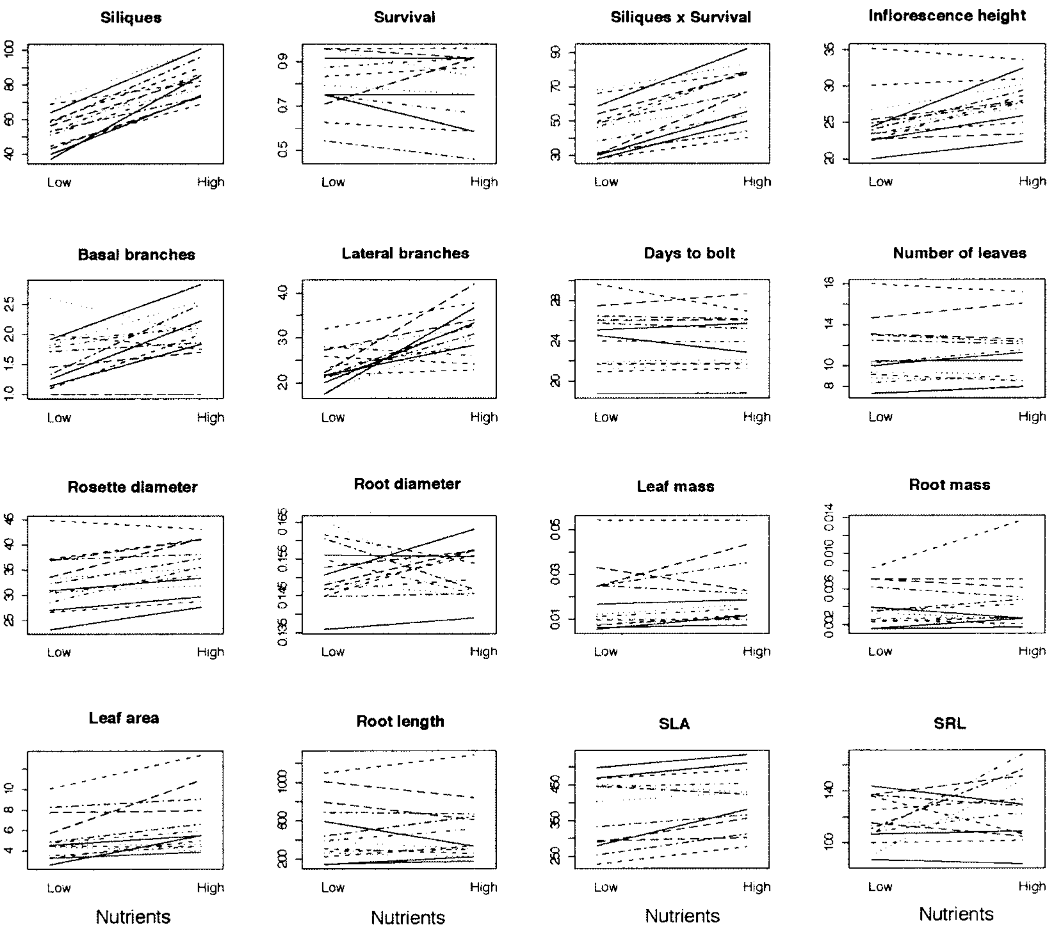

Figure 5-1. Reaction norms for untransformed mean values of 16 traits measured on 14 *Arabidopsis thaliana* genotypes grown in low and high nutrient environments.

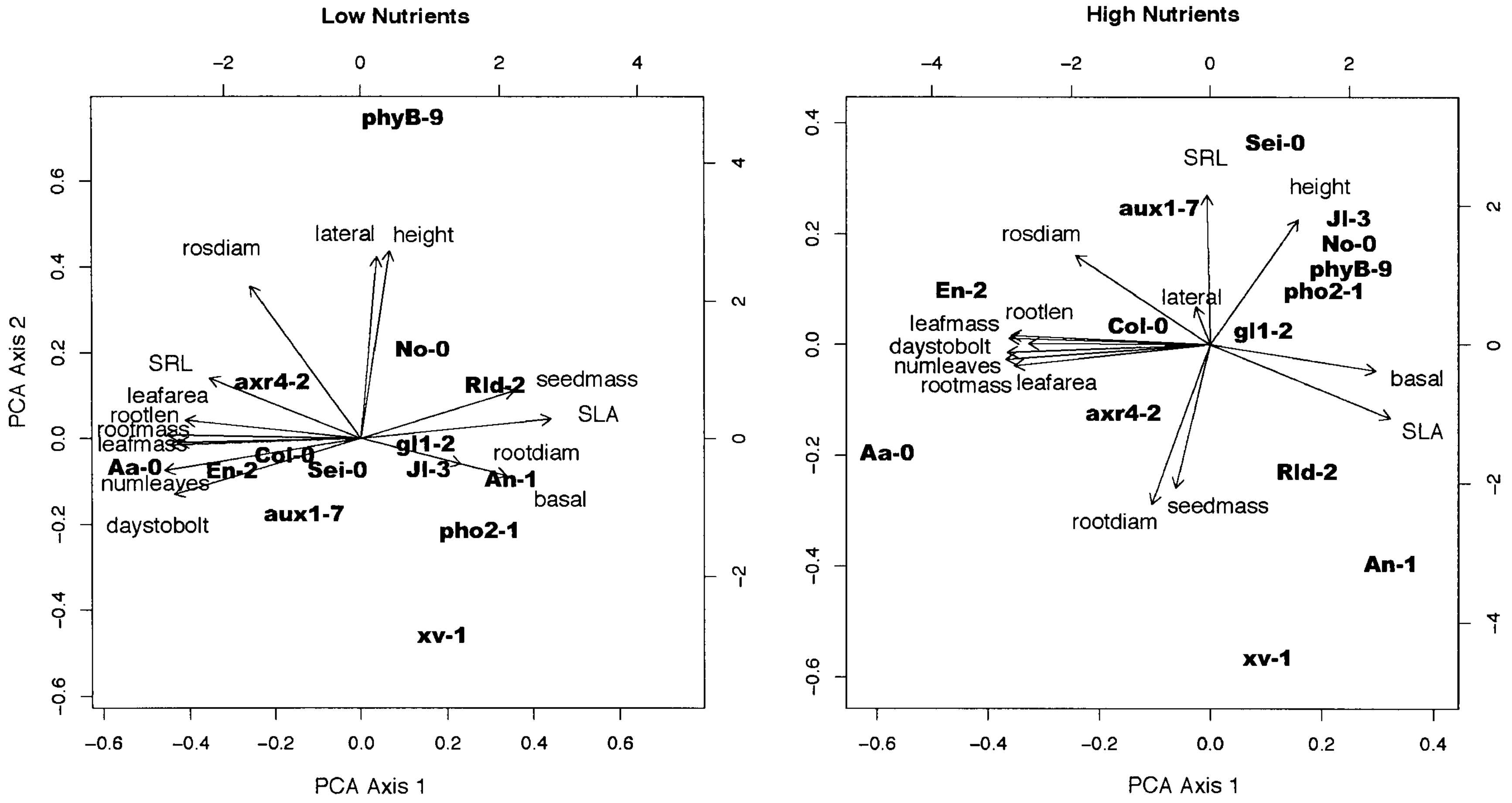

Figure 5-2. Genotype and trait biplot scores on the first two axes of principal components analyses (PCA) of scaled, transformed genotype means in low and high nutrient environments. Genotype and trait abbreviations are explained in Tables 5-1 and 5-2.

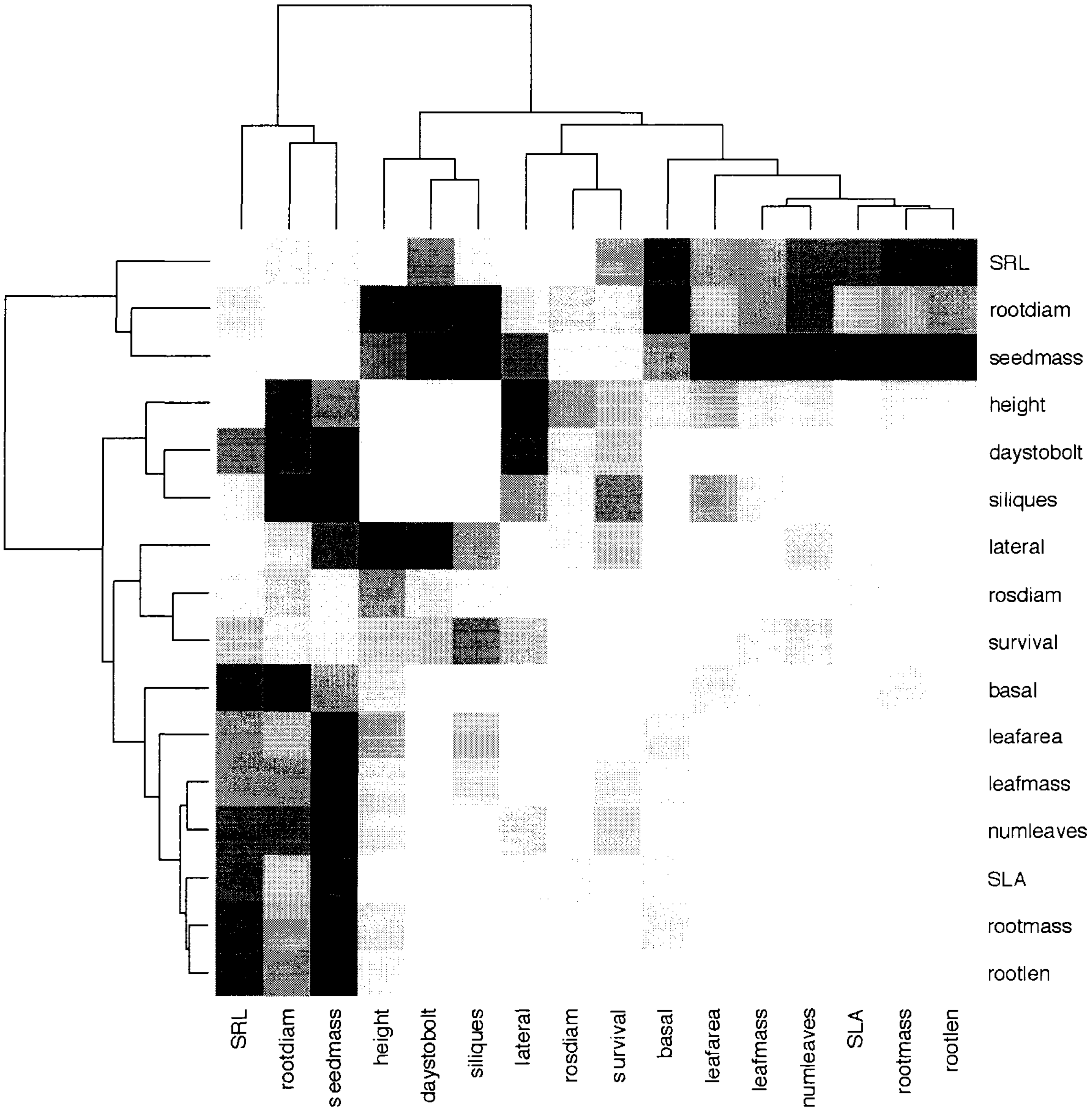

Figure 5-3. Change-in-correlation matrix for 16 traits measured on 14 *Arabidopsis thaliana* genotypes grown in low and high nutrient environments. Cell shading indicates the absolute magnitude of changes in the correlations between traits in high versus low nutrient environments, with darker shading indicating greater change in correlation with another trait between environments. Cluster diagrams at the matrix margins indicate the similarity of each trait's overall pattern of change in correlation to other traits based on absolute distance among rows/columns and an UPGMA clustering algorithm.

Chapter 6 Discussion and Conclusions

Discussion

The main objectives of this thesis were to understand relationships among plant leaf and root functional traits, their evolutionary histories, and their influence on the ecology of populations and communities. My original rationale for asking these questions was a lack of knowledge in the literature regarding root functional trait variation among and within species, and a lack of studies that integrated knowledge on functional traits, community phylogenetic structure and community trait similarity in plants.

How do leaf and root traits vary among and within communities?

In Chapters 2 and 3 I studied patterns of community structure and leaf and root trait variation in mixedgrass and fescue grasslands. Mean trait values varied little among different communities, but individual species characteristic of different communities had distinct sets of leaf and root traits. The drier environmental conditions in mixedgrass communities were associated with stronger clustering of leaf and root traits such as tissue density and thickness, which are thought to determine the stress tolerance of species (Fonseca et al. 2000). It is interesting to note that even in these two distinct grassland communities that contain species with different biogeographic affinities, I found few differences in mean trait values between community types, although there was a greater range of trait values among species in fescue grasslands, and species in fescue grasslands tended to have larger, thinner leaves with high specific leaf area relative to those in mixedgrass grasslands.

How do leaf and root traits vary among and within species?

Related species from lineages that diverged millions of years ago appear to have filled similar niches in both community types, resulting in little difference in the mean trait values in the different grasslands, but a great deal of trait variation within communities. Within-community trait variation was largely due to interspecific trait differences rather than phenotypic plasticity or local adaptation, since all measured traits except leaf area varied at the same rate among and within species arranged along gradients of community mean trait values. The explanation of within-community trait variation remains an open question that needs to be addressed by future studies (Reich et al. 2003).

Trait values differed among plants from the same species growing in the field and a common garden. It is interesting to note that the range of specific leaf area (SLA) and specific root length (SRL) values within *Arabidopsis thaliana* (Chapter 5) was similar to the range of values for these traits among species in Alberta grasslands. Many studies have measured traits on greenhouse-grown seedlings in order to understand functional effects and responses of species growing in the field (Grime et al. 1997). My results indicate that plasticity and local adaptation may make it difficult to measure traits on plants in a common garden and apply them to plants growing in the field, and that more research is needed on the relative contribution of intraspecific trait variation to interactions among species in natural ecosystems (Callaway et al. 2003).

The vast majority of studies of trait variation among species and communities have interpreted trait correlations among species as evidence for the adaptive evolution of trait syndromes in different habitats (reviewed by Reich et al. 2003). This thesis provides a cautionary example of how comparative methods can dramatically change conclusions about the adaptive significance of trait correlations (Harvey and Pagel 1991). Contrary to theory (Chapin et al. 1993) and empirical evidence (Craine et al. 2001) suggesting that leaf and root traits are correlated due to evolution of integrated whole-plant resource uptake strategies, suites of leaf and root traits appear to have evolved independently in Alberta grassland plants. The correlations among corresponding leaf and root traits such as tissue density and thickness found in this study and in other temperate grasslands were due to the phylogenetic conservatism of traits among a few dominant families, rather than correlated evolution of suites of leaf and root traits. These lineages diverged millions of years ago, and an open question is why and how variation in these traits among families arose and have been maintained for so long, and whether leaf and root trait variation in these clades arose before or after they colonized the mixedgrass and fescue prairies (Ackerly 2003).

In Chapter 5, I focused on patterns of trait variation within a single species. I observed patterns that were similar in many ways to the patterns of among-species trait variation, in that corresponding leaf and root traits of *Arabidopsis thaliana* such as SLA and SRL were negatively correlated or unrelated, depending on the growth environment. However, the overall vegetative phenotype above- and belowground was integrated, with a tradeoff between high reproductive output versus high investment in vegetative biomass above- and belowground. Root morphological traits including root diameter and SRL contributed more to fitness in low nutrient availability environments.

What can trait and phylogenetic similarity tell us about community assembly?

In both Chapters 3 and 4, I found that stressful habitats tended to show more evidence of trait clustering and of phylogenetic clustering relative to other habitat types. This suggests that conditions in these habitats act as a stronger environmental filter, although this remains a conjecture based on a qualitative assessment of environmental conditions in these habitats since I did not measure stress directly. Comparisons of the relative phylogenetic and trait similarity along environmental gradients promises to be a useful method for understanding community assembly rules and patterns of species diversity (Warwick and Clarke 1998, Weiher et al. 1998).

Trait clustering within grassland communities was associated with weak patterns of phylogenetic overdispersion or clustering, depending on the evolutionary history of traits in different communities and lineages. This result differs from previous studies, which have generally found consistent patterns of phylogenetic clustering at broad scales and overdispersion at smaller spatial and phylogenetic scales (Cavender-Bares et al. 2004, Cavender-Bares et al. 2006, Slingsby and Verboom 2006). While many studies have attributed patterns of phylogenetic overdispersion to the effects of competition (Webb et al. 2002), if diffuse competition selects for a set of traits that confer the ability to compete for resources in a habitat, competition could actually lead to trait and phylogenetic clustering within communities (Grime 2006). Traits and niches evolve in a hierarchical fashion (Silvertown et al. 2006), leading to a potential disconnect between the scales of trait evolution, phylogenetic relatedness and ecological processes. Future studies should move beyond description of patterns to explicitly test the links between traits, phylogenetic relatedness and plant fitness and population dynamics in different environments and at different scales (Callaway et al. 2003).

In Chapter 3, I measured relationships between co-occurrence and phylogenetic distances among individual species, while in Chapter 4 I measured the average similarity of species occurring together in samples. Each of these methods has been used by a number of recent studies (Cavender-Bares et al. 2006, Horner-Devine and Bohannan 2006, Kembel and Hubbell 2006, Lovette and Hochachka 2006, Slingsby and Verboom 2006), but it should be acknowledged that these methods may actually be measuring different aspects of community structure. The sample-based methods used in Chapter 4 are analogous to traditional measures of species diversity, and similar methods have been used to describe the phylogenetic diversity of communities in different environments (e.g. Warwick and Clarke 1998). A worthwhile avenue for future research would be to compare the performance of the methods used in Chapters 3 and 4 using simulation studies, since each may be sensitive to different aspects of community structure.

The phylogenetic similarity of co-occurring species will ultimately depend on the degree to which phylogenetic branch lengths separating species and the traits measured by a study reflect the actual niche similarity of species. Methods for choosing and assessing branch lengths have been developed in studies of trait evolution (Mooers et al. 1999), but these methods have not yet been applied in studies of community phylogenetic structure. Studies of phylogenetic and functional similarity of co-occurring species may ultimately have difficulty detecting the effects of limiting similarity on community structure, since they can only compare the species and traits present in a post-competition species pool (the Narcissus effect; Colwell and Winkler 1984). This problem could potentially be addressed by comparing local communities to null communities assembled from some species pool, but this raises the issue of how to define the species pool for a given community (Zobel et al. 1998, Loreau 2000). In general, more research on the effects of branch length choice, null model choice and species pool definition in studies of phylogenetic and trait similarity is needed.

Conclusions

In conclusion, the main findings of this thesis can be summarized as:

1) Leaf and root morphological traits linked to resource uptake strategies vary predictably among and within grassland communities, but the traits of individual

species vary more along environmental gradients than do mean trait values across entire communities. Trait variation among communities was much smaller than the magnitude of trait variation within communities. Plants growing in mixedgrass communities, and at drier plots within each site, tended to have a suite of traits linked to a stress-tolerant strategy, including smaller, thicker leaves with lower SLA and dense roots with lower SRL.

2) Communities in more stressful environments tend to contain species that are more similar in terms of their traits and phylogenetic relatedness, indicating the importance of environmental filtering of traits during community assembly.
3) Within *Arabidopsis thaliana*, root traits such as root diameter and SRL are more important for maintaining plant fitness when nutrients are limiting, but otherwise above- and belowground vegetative traits are highly integrated across environments, and early-flowering genotypes had higher fitness in both environments.
4) Many leaf and root traits vary a great deal within and among species. The lack of correlation between traits measured in the field and in a common garden suggests that more research on causes and patterns of trait variation are needed before applying traits measured on seedlings or in a common garden to plants growing in the field.
5) Measures of community trait and phylogenetic similarity are sensitive to a number of methodological issues related to scale, branch length and null models, and more caution is needed when trying to infer process from pattern in these types of studies.

The questions addressed in this thesis continue to be areas of active research, and it is my hope that this thesis has contributed to the integration of research on the evolution and ecology of plant form and function above- and belowground, and suggested numerous questions that deserve further study.

Literature Cited

Ackerly, D. D. 2003. Community assembly, niche conservatism, and adaptive evolution in changing environments. International Journal of Plant Sciences **164**:S165-S184.

Callaway, R. M., S. C. Pennings, and C. L. Richards. 2003. Phenotypic plasticity and interactions among plants. Ecology **84**:1115-1128.

Cavender-Bares, J., D. D. Ackerly, D. A. Baum, and F. A. Bazzaz. 2004. Phylogenetic overdispersion in Floridian oak communities. American Naturalist **163**:823-843.

Cavender-Bares, J., A. Keen, and B. Miles. 2006. Phylogenetic structure of Floridian plant communities depends on taxonomic and spatial scale. Ecology **87**:S109-122.

Chapin, F. S., K. Autumn, and F. Pugnaire. 1993. Evolution of suites of traits in response to environmental stress. American Naturalist **142**:S78-S92.

Colwell, R. K., and D. W. Winkler. 1984. A null model for null models in biogeography. Pages 344-359 *in* D. R. Strong, D. Simberloff, L. G. Abele, and A. B. Thistle, editors. Ecological communities: Conceptual issues and the evidence. Princeton University Press, Princeton, NJ.

Craine, J. M., J. Froehle, D. G. Tilman, D. A. Wedin, and F. S. Chapin. 2001. The relationships among root and leaf traits of 76 grassland species and relative abundance along fertility and disturbance gradients. Oikos **93**:274-285.

Fonseca, C. R., J. M. Overton, B. Collins, and M. Westoby. 2000. Shifts in trait-combinations along rainfall and phosphorus gradients. Journal of Ecology **88**:964-977.

Grime, J. P. 2006. Trait convergence and trait divergence in herbaceous plant communities: Mechanisms and consequences. Journal of Vegetation Science **17**:255-260.

Grime, J. P., K. Thompson, R. Hunt, J. G. Hodgson, J. H. C. Cornelissen, I. H. Rorison, G. A. F. Hendry, T. W. Ashenden, A. P. Askew, S. R. Band, R. E. Booth, C. C. Bossard, B. D. Campbell, J. E. L. Cooper, A. W. Davison, P. L. Gupta, W. Hall, D. W. Hand, M. A. Hannah, S. H. Hillier, D. J. Hodkinson, A. Jalili, Z. Liu, J. M. L. Mackey, N. Matthews, M. A. Mowforth, A. M. Neal, R. J. Reader, K. Reiling, W. RossFraser, R. E. Spencer, F. Sutton, D. E. Tasker, P. C. Thorpe, and J.

Whitehouse. 1997. Integrated screening validates primary axes of specialisation in plants. Oikos **79**:259-281.

Harvey, P. H., and M. D. Pagel. 1991. The comparative method in evolutionary biology. Oxford University Press, Oxford.

Horner-Devine, M. C., and B. J. Bohannan. 2006. Phylogenetic clustering and overdispersion in bacterial communities. Ecology **87**:S100-108.

Kembel, S. W., and S. P. Hubbell. 2006. The phylogenetic structure of a Neotropical forest tree community. Ecology **87**:S86-S99.

Loreau, M. 2000. Are communities saturated? On the relationship between alpha, beta and gamma diversity. Ecology Letters **3**:73-76.

Lovette, I. J., and W. M. Hochachka. 2006. Simultaneous effects of phylogenetic niche conservatism and competition on avian community structure. Ecology **87**:S14-28.

Mooers, A. O., S. M. Vamosi, and D. Schluter. 1999. Using phylogenies to test macroevolutionary hypotheses of trait evolution in Cranes (Gruinae). American Naturalist **154**:249-259.

Reich, P. B., I. J. Wright, J. Cavender-Bares, J. M. Craine, J. Oleksyn, M. Westoby, and M. B. Walters. 2003. The evolution of plant functional variation: Traits, spectra, and strategies. International Journal of Plant Sciences **164**:S143-S164.

Silvertown, J., M. Dodd, D. Gowing, C. Lawson, and K. McConway. 2006. Phylogeny and the hierarchical organization of plant diversity. Ecology **87**:S39-S49.

Slingsby, J. A., and G. A. Verboom. 2006. Phylogenetic relatedness limits co-occurrence at fine spatial scales: Evidence from the schoenoid sedges (Cyperaceae: Schoeneae) of the Cape Floristic Region, South Africa. American Naturalist **168**:14-27.

Warwick, R. M., and K. R. Clarke. 1998. Taxonomic distinctness and environmental assessment. Journal of Applied Ecology **35**:532-543.

Webb, C. O., D. D. Ackerly, M. A. McPeek, and M. J. Donoghue. 2002. Phylogenies and community ecology. Annual Review of Ecology and Systematics **33**:475-505.

Weiher, E., G. D. P. Clarke, and P. A. Keddy. 1998. Community assembly rules, morphological dispersion, and the coexistence of plant species. Oikos **81**:309-322.

Zobel, M., E. van der Maarel, and C. Dupre. 1998. Species pool: the concept, its determination and significance for community restoration. Applied Vegetation Science **1**:55-66.