Testing a trait-based model of fern community assembly

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Preface

This is not a manuscript-based thesis. Instead I have written a thesis in the traditional format – a narrative explaining the work that I have done throughout my MSc., as well as the conclusions I have drawn from my analyses.

Acknowledgments

I would first and foremost like to thank my supervisor, Martin Lechowicz, for his generous guidance and continued support throughout my M.Sc. He provided me with the tools to overcome challenges and then with challenges to overcome. I feel privileged that I had the opportunity to benefit from his great wisdom and shall endeavour to live up to the high standard of scientific inquiry that he has set for me in any future research that I may pursue.

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In the scope of an M.Sc. thesis I could not have attempted a test of Shipley's MaxEnt approach if I had to gather data on community abundance as well as on the functional traits of the species in a community. I therefore am grateful to Ben Gilbert for allowing me to use the community data from his 2003 M.Sc. thesis as well as the unpublished data taken when he and Professor Lechowicz resampled those communities in 2004.

The analytical aspect of my thesis could not have been done without Bill Shipley who helped me overcome various obstacles in my test of his MaxEnt approach. Additionally, I could not have completed my analyses without the generosity of Eric Pedersen, Michael Pedruski, and Allen Larocque, who all helped me with the steep learning curve of writing R code.

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Abstract

The MaxEnt (maximum entropy) approach is a recently introduced analytic framework that utilizes plant traits to predict community assembly along environmental gradients. I aimed to provide a rigorous test of this approach using forest understory fern communities. I executed the MaxEnt algorithm by inputting observed community-aggregated traits (CATs), and accurately determined species abundance distributions (SADs). These results support the notion that trait-based environmental filtering at least in part drives fern community assembly, but a more general application of the MaxEnt approach depends on its ability to predict SADs using CATs independently estimated from environmental data. In an initial exploration of this predictive ability I was unable to accurately predict SADs for fern communities despite screening many measures of environmental conditions. Very recent studies, however, provide new tools that can be used to further analyze my data and may yet establish the utility of the MaxEnt approach in predicting SADs.

Résumé

Le modèle MaxEnt (entropie maximale) est un nouveau cadre analytique qui utilise des caractéristiques de plantes afin de prédire l'assemblage de communautés suivant un gradient environnemental. J'ai voulu tester cette approche de façon rigoureuse en utilisant des communautés de fougères de sous-bois. En exécutant l'algorithme du modèle MaxEnt avec des traits biologiques agrégés au niveau des communautés, j'ai pu déterminer la distribution et l'abondance des espèces. Ces résultats appuient la notion que l'assemblage des communautés de fougères est au moins en partie déterminé par un filtre environnemental reposant sur les traits biologiques. Toutefois, une application plus générale de l'approche MaxEnt dépend de son habileté à prédire la distribution et l'abondance des espèces en utilisant les traits agrégés estimés indépendamment des données environnementales. Dans une première évaluation des capacités prédictives du modèle, il fut impossible de prédire les distributions et abondances des espèces pour des communautés de fougères malgré l'essaie de multiples mesures de conditions environnementales. Néanmoins, plusieurs études récentes fournissent de nouveaux outils qui peuvent être utilisés dans des analyses plus poussées de mes données et pourraient établir l'utilité du modèle MaxEnt pour prédire la distribution et l'abondance des espèces.

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Introduction

In November 2006, Shipley, Vile and Garnier (Shipley *et al*. 2006) published a novel method for predicting the composition of plant communities – a theory for species distribution and community assembly along environmental gradients. This method was immediately criticized (e.g. Marks and Muller-Landau 2007; Roxburgh and Mokany 2007; Haegeman and Loreau 2008; Haegeman and Loreau 2009), and Shipley responded to these criticisms in turn (Shipley 2007; Shipley 2009a; Shipley 2009b). Shipley subsequently published a book (Shipley 2009c) elaborating and defending his theory, and the debate about the validity and utility of the approach continues presently. For example, a recent publication of *Oikos* (April 2010) features four commissioned articles that consider different aspects of the MaxEnt approach (He 2010; McGill and Nekola 2010; Roxburgh and Mokany 2010; Shipley 2010). Additionally, the original article by Shipley *et al*. (2006) has been cited 73 times in the 3.75 years since its publication. Despite this lively discourse, the theory remains little tested (Shipley *et al*. 2010; Sonnier *et al*. 2010). If the MaxEnt approach is proven to work then it would not only further our understanding of community assembly, but also be used in conservation efforts such as determining the impact of a changing environment on plant communities, as well as predict the success of an invasive species in a given area. My thesis concerns the test of Shipley's MaxEnt approach, but to put my own work in context I first need to briefly review the long history of research on community assembly and allied topics.

Early ecology and concepts of succession

Community assembly theory has been discussed and debated for at least the last century, with a particular focus on plant communities. Until the mid-19th century the discipline of ecology had not really taken form, although the roots of the discipline in the natural history of plant and animal species were well established (McIntosh 1975). By the mid-19th century the foundations for contemporary views of community assembly began to emerge with the publication of Darwin's (1859) *Origin of Species* and Haeckel's (1866) introduction of the term "ecology" in his publication on the *General Morphology of Organisms*. Allee (1949) translated Haeckel's definition of ecology as "the body of knowledge concerning the economy of nature – the total relations of the animal to both its inorganic and organic environment; including above all, its friendly and inimical relations with those animals and plants with which it comes directly or indirectly into contact – in a word ecology is the study of all those complex interrelations referred to by Darwin as the conditions of the struggle for existence."

Even with a formal definition, there was no real paradigm organizing ecological studies until the early 20th century when Frederic Clements proposed a holistic concept of ecology in which communities were viewed as "superorganisms" that could be classified as an association type (McIntosh 1975; Simberloff 1980). Clements' proposed that succession was unidirectional and predictable, always leading to a homogeneous climax community in a state of stable equilibrium (McIntosh 1975; Simberloff 1980). This view was widely accepted because the concept of a deterministic path is appealing, both in that it is easy to comprehend and in that it is aesthetically pleasing (Simberloff 1980).

Although Clements' ideas dominated community ecology research in the early 20th century, he had critics from the very beginning – most notably Henry Allen Gleason (McIntosh 1975). Gleason propounded an individualistic concept of ecology in which communities were not analogous to organisms, but rather they were assemblages of coexisting individuals (Gleason 1939; McIntosh 1975). Gleason's individualistic concept stated that community structure is dependent on a number of variables, including: a) the characteristics of individuals, b) the nature of the surrounding populations from which species are dispersed, c) the probabilities of species dispersing to an area, and d) environmental conditions that fluctuate in time and space selecting for individuals that are adapted to the local conditions (Gleason 1939; McIntosh 1975). It follows that communities are dynamic and temporary – to some degree constantly in a state of disequilibrium (McIntosh 1975; Simberloff 1980; Nicolson 1990). Moreover, no two areas are identical in terms of the species that occur in them, the abundance of each species, or the way that the areas are spatially arranged (McIntosh 1975). In essence, every community, as well as every part of a community, is the product of countless variables that are unique to the specific area in which the community is found. Each community is therefore independent of every other community, except in regard to the chance dispersal of species from one community to another (Gleason 1939). Gleason affirmed the existence of communities composed of groups of plants with similar requirements to survive, but denied Clements' holistic concept and its invocation of plant association types to categorize communities in a strict manner (Gleason 1939). It eventually became evident that Clements' holistic concept of succession did not fit with vegetation patterns around the globe, but that Gleason's individualistic concept did (Nicolson 1990). In time Gleason gained support from other eminent ecologists, and a paradigm shift occurred (McIntosh 1967a; Simberloff 1980; Nicolson 1990; Austin 1999). Contemporary plant community ecology is still underpinned by Gleason's individualistic paradigm.

Niche theory

As illustrated by the paradigm shift from Clements' holistic view of ecology to Gleason's individualistic perspective, there is constant vacillation between theories in community ecology; yet the discipline is threaded together by a common, albeit ambiguous goal: predicting the composition of communities (Keddy and Weiher 1999, p. 2). Niche theory is a framework of ideas that has been embraced by ecologists in their attempts to reach this goal. Niche theory is a logical extension of Gleason's individualistic concept and has driven the majority of ecological research since its beginnings until the present day, including the new theory proposed by Shipley *et al*. (2006). A niche is a multidimensional measure of the position of an individual or population along an environmental gradient in a locality (Vandermeer 1972). The dimensions represent the different abiotic and biotic variables that are suitable for the survival of the species occupying that niche (Vandermeer 1972). Chase and Leibold (2003) explain that there are two major aspects of a niche: 1) species requirements for survival in a locality, as was focused on by Grinnell (1917) and Hutchinson (1957), and 2) the impact of a species on its environment, which was the focus of Elton (1927) and MacArthur and Levins (1967). Grinnellian niches are comprised of variables that are essentially non-interactive environmental conditions that do not invoke the concept of competition and help us understand the broad ecological and geographic properties of a species (Soberón 2007). Alternatively, Eltonian niches are comprised of variables that are resources over which consumers compete and interact locally (Soberón 2007). Chase and Leibold (2003) have proposed a "synthetic" niche concept, which draws from both the Grinnellian and Eltonian niche, and they develop a framework to understand niches as the complex culmination of a number of ecological processes at different scales. Niche theory is therefore an ecological framework that in general is used to understand nature at all scales in space and time by focusing on the abilities of individuals to cope with varying abiotic and biotic conditions to survive in an area (Vandermeer 1972; Chase and Leibold 2003; Gilbert and Lechowicz 2004; Herault 2007).

Niche theory-based research has dominated the discipline of community assembly since the principle of competitive exclusion was introduced by Gause (1934). This principle essentially states that complete competitors cannot coexist, i.e. that no two species can occupy the same niche (Hardin 1960; Vandermeer 1972). For two or more species to coexist they must use the available and necessary environmental resources differentially, and hence occupy different niches. Since Gause's principle of competitive exclusion, many other ecological principles based in niche theory have been introduced. For example, Diamond (1975) famously created the notion of "assembly rules" to tackle the underlying problem of how communities assemble from a species pool. These rules are ecological restrictions to species presence or abundance in communities (Wilson and Whittaker 1995; Keddy and Weiher 1999, p. 131). While the concept of assembly rules has been improved and applied to sundry systems over time (e.g. Keddy 1992, Weiher and Keddy 1995; Wilson and Watkins 1994; Wilson and Whittaker 1995; Belyea and Lancaster 1999), it originated from Diamond's research into the distribution of bird species on islands in the New Guinea archipelago. Diamond (1975) was able to construct niches for the species he investigated by observing the interspecific differences in resource utilization on the islands and predicting which species could coexist.

The continuum concept

In addition to niche theory encompassing interactions among species, as was described in Gause's principle of competitive exclusion and Diamond's original case study, niche theory also includes interactions between species and the environment. These interactions between the biotic and abiotic elements of a community are examined in the vegetation continuum concept. The vegetation continuum concept is based in niche theory and was developed by many ecologists (e.g. Curtis and McIntosh 1951; Whittaker 1956) as an extension of Gleason's individualistic hypothesis (McIntosh 1967a). A continuum, in terms of plant community ecology, refers to a gradient along which species distributions gradually change both spatially and temporally, according to the relationship between species and the environment (McIntosh 1967a). The continuum concept supports Gleason's individualist hypothesis because it asserts that species do not occur in clearly defined associations. Instead community composition varies according to how individual species interact with the environment differentially along an environmental gradient.

Whittaker (1960) realized that environmental gradients can occur within local communities as well as span multiple communities. He therefore defined three hierarchical levels of biodiversity – alpha, beta, and gamma – to assess vegetation patterns and community assembly along environmental gradients at different spatial scales. Alpha diversity refers to species richness at a local scale, or within a habitat. The species included in this level co-occur and interact with one another. Beta diversity refers to species turnover taking place between habitats. Finally, gamma diversity measures the species richness of a whole landscape, or region (Whittaker 1960; McIntosh 1967b; Whittaker *et al*. 2001; Silvertown *et al*. 2006). Each biodiversity level contains heterogeneity within itself due to “short” environmental gradients; however there is greater heterogeneity among each scale (Whittaker 1960). There is ambiguity surrounding these diversity levels as a habitat can be defined in many different ways, according to the purpose of a study. Nonetheless, it is important to note that scale greatly impacts vegetation patterns and is integral to the study of community assembly along environmental gradients.

Utilizing ordination methods for gradient analysis

Qualitative evidence for the gradual change of species along a gradient is plentiful and has been utilized to understand different aspects of species’ niches within and across communities (see McIntosh 1967a for a historical review of the supporting research). However, more objective sampling techniques and methods of quantitative analyses in the form of ordination have been developed to provide greater understanding of how communities assemble along environmental gradients and to aid in constructing species’ niches at different spatial scales (McIntosh 1967a; ter Braak 1983).

Ordination arranges species, communities, or environments in relation to ecological gradients for the purpose of revealing information about the relationships among them, and reducing complex data to a few dimensions (McIntosh 1967a; Austin 1985). In other words, ordination clusters data so that quadrats or species that are similar to one another are placed closer to each other and dissimilar quadrats or species are farther away from each other. Ordinations can be interpreted to infer under which environmental conditions a species typically occurs (Gauch *et al*. 1977; Whittaker 1978).

Whittaker’s (1956, 1967) gradient analyses inspired the development and use of different methods of both indirect and direct ordination in community ecology. Indirect gradient analysis, sometimes referred to as vegetation ordination or indirect ordination, is a type of ordination in which the environmental causes of vegetation structure are examined (Austin 1985). Plots are first placed along an axis according to their floristic similarity. This ordination is then interpreted by correlating the axes with environmental factors

(Whittaker 1967; ter Braak 1986). There are many forms of indirect ordination, ranging from linear models such as polar ordination, principle component analysis, principle coordinate analysis, canonical variates analysis, and canonical correlation analysis, to multivariate techniques like detrended correspondence analysis and metric or non-metric multidimensional scaling (Austin 1985).

Similarly to indirect gradient analysis, direct gradient analysis (DGA) is a type of ordination that illustrates patterns of species distribution in relation to important environmental factors. However, DGA uses ordination axes that incorporate environmental variables so to directly relate community variation to environmental variation. In its simplest form, DGA is a form of regression analysis involving plotting species abundance against one or two environmental variables; this was the DGA method Whittaker (1967) employed (ter Braak 1986). Direct ordination can be made more complex by comparing sets of variables (canonical correlation analysis). However, canonical correlation analysis assumes linear relationships between sets of variables, which are not likely in community ecology (Minchin 1987). Ter Braak (1986) revolutionized ordination methods when he developed canonical correspondence analysis (CCA). This method combines regression techniques with correspondence analysis and assumes a Gaussian relationship between sets of variables rather than a linear one.

Both indirect and direct ordination methods have their advantages and disadvantages. Some indirect ordination studies have displayed discontinuities in ordination space. These have often been explained to be the result drastic changes in environmental conditions, which are bound to occur at large spatial scales; however indirect ordinations invoke assumptions regarding the nature of the distribution of the variables and their relationship to one another, and therefore about how species respond to gradients (Minchin 1987; Austin 2005, p. 63). Indirect ordination methods are useful for data exploration, but they cannot be used to test hypotheses and thus cannot really support theories like the continuum concept (Austin 1985; Minchin 1987). Alternatively, hypothesis testing is possible for the CCA to determine if species composition is significantly related to the environment (ter Braak 1986); however, while Gaussian relationships are more common than linear ones, they are certainly not present for all species (Minchin 1987). Redundancy analysis is another direct gradient analysis technique that is commonly used. Although it is a linear method it can be used to determine how much of the variance in one set of variables can be explained by the other (ter Braak 1986).

Ordination techniques have been used in a great deal of research to increase understanding of how niches are formed by the relationship between environmental gradients and various species, and hence of community assembly processes (Silvertown and Law 1987; Austin and Gaywood 1994). However, this information is descriptive and phenomenological, and does not allow for predictions of species abundance along environmental gradients. Indeed, niche-based research entails studying the influence of all biotic and abiotic idiosyncrasies on community assembly. This is such an overwhelming task that it has impeded the creation of a general predictive theory of community ecology and has led some to question the utility of niche theory and to come up with alternative ecological theories (Keddy and Weiher 1999, p.2; Roughgarden 2009).

Neutral theory

Recently, Hubbell (2001) reacted to the inability of niche theory to explain general ecological patterns by proposing a “unified neutral theory of biodiversity,” which was formalized in a book and has been heralded as a working model of species distribution and abundance. Neutral theory has been broached a few times in the last several decades in different forms, with varying success in attracting the attention of ecologists (e.g. MacArthur and Wilson (1963, 1967); Caswell 1976; Hubbell 1979) (Bell *et al*. 2006; Leigh 2007). According to Hubbell’s (2001) theory, all individuals in a trophic level are equivalent in the sense that they have identical birth and death rates, as well as equal dispersal and speciation abilities. As such, abiotic and biotic variables are assumed to be inconsequential in the establishment and persistence of any species. Rather, a species will colonize an area simply if that area has been made available (for any number of reasons) and that species is able to disperse and grow there. Because dispersal drops off rapidly with distance, neighbouring species are more likely to colonize a newly available area than species that disperse from great distances. Dispersal limitation is thus hypothesized to strongly influence community composition, as floristic similarity between sites will decrease with increasing distance (Gilbert and Lechowicz 2004; Hubbell 2005; Jones *et al*. 2006). The neutral framework does not claim that all species are identical in their biological traits, but rather that these traits are not responsible for patterns in community ecology. Alternatively, chance events involving species demography and dispersal are the foundation of these patterns (Hubbell 2001; Hubbell 2005; Jones *et al*. 2006; Herault 2007).

The inability to quantify general patterns using niche theory gives all the more power to neutral theory which has achieved success in capturing the patterns of species-area, species-turnover, and rank-abundance (Hubbell 2005; Herault 2007). The implication that niche theory research and differences among species are irrelevant to questions of community assembly has understandably spurred great debate and research in community ecology (e.g. Condit *et al*. 2002; Karst *et al*. 2005; Jones *et al.* 2006; Tuomisto *et al*. 2003), which has led to the realization that neither approach is complete in its ability to predict the relative abundance of species at a site. Many researchers have therefore directed their efforts towards general concepts that incorporate aspects of both niche and neutral theory, e.g. the application of the trait-based approach that Shipley *et al*. (2006) developed.

Potential for trait-based approaches to community assembly

Species can be characterized and grouped by the various morphological, physiological, and/or phenological features affecting their survival and reproduction, i.e. functional traits, which typically vary more among than within species (McGill *et al*. 2006; Violle *et al*. 2007). Plant functional types are groups of species that are respond to environmental factors in a similar way, and/or share similar roles in an ecosystem, because they express certain traits in a similar manner (Lavorel 1997; Lavorel and Garnier 2002; Cornelissen *et al*. 2003; Herault 2007). Many ecologists use the term "functional trait" to signify plant traits that define plant functional types and are particularly relevant to vegetation and ecosystem dynamics (Cornelissen *et al*. 2003). Violle *et al*. (2007) demonstrate that the term is ambiguous and often carries different meanings in the current literature. They propose that functional traits be defined as traits that impact fitness indirectly via their effects on growth, reproduction and survival.

Trait-based community ecology has built upon research into the relationship between species and environmental gradients (i.e. the continuum concept) to broaden the scope of the aforementioned assembly rules (e.g. Diaz and Cabido 1997; Diaz *et al*. 1998; McIntyre *et al*. 1999; Wang and Ni 2005; Silvertown *et al*. 2006; Ackerly and Cornwell 2007; Cornwell and Ackerly 2009). This vein of research theorizes that the environment serves as a filter that only permits species that have certain functional trait combinations to survive in a given environment. Instead of the environment filtering out unsuitable genotypes, as is the case in evolution, unsuitable traits are being selected against (Keddy 1992; Weiher and Keddy 1995; Belyea and Lancaster 1999; Booth and Swanton 2002).

The concept of environmental filtering is influenced by the spatial scale that is being observed. Although some traits appear important to vegetation patterns at multiple spatial scales, others appear to be more relevant at a particular spatial scale. For example, traits that are associated with climatic factors may be termed “gamma-traits” because they affect the range of a species on a continental scale. Alternatively, traits that are associated with factors involved in the ability of a plant to garner resources in a specific environment may be termed “alpha-traits” because they affect the survival of a species in a given locality (Morin and Lechowicz 2008). Silvertown *et al*. (2006) as well as Ackerly and Cornwell (2007) propose that there are alpha, beta and gamma niches that can be defined by alpha, beta and gamma traits, respectively, as well as environmental factors that influence vegetation dynamics at these scales. Furthermore, it should be noted that some traits may affect whether or not a species occurs in an area at all, i.e. a species’ distribution, while others are relevant to the abundance at which a species occurs if it is in fact present in a given area (Morin and Lechowicz 2008). All of this suggests that understanding the scale of a study and choosing traits appropriately is very important in trait-based research. Studying alpha traits will help elucidate community assembly processes within habitats, and beta traits will facilitate understanding of community assembly along vast environmental gradients that span many different habitats (Ackerly and Cornwell 2007).

The differentiation between alpha and beta traits may also provide insight into the trait correlations that have been discovered (Ackerly and Cornwell 2007). A large amount of research into interspecific trait correlations has provided evidence that a relatively small number of plant functional traits greatly influence many aspects of plant life history, and explain plant responses to biotic and abiotic factors at different scales (Lavorel and Garnier 2002; Wright *et al*. 2004). For example, a trait correlation study was carried out by Wright *et al*. (2004) that used the "GLOPNET" data set, which contains 2,548 species at 175 sites. This meta-analysis focused on leaf functional traits from a wide array of species and showed that these traits are highly correlated across the globe. This "worldwide leaf economics spectrum" demonstrates that trait expression is constrained by trade-offs involving physiology and competitive interactions (Wright *et al*. 2004).

Additionally, through an analysis of 640 species and 12 traits, Diaz *et al*. 2004) demonstrated that certain patterns of trait specialization are common across floras from Argentina, England, Iran and Spain. A principle component analysis (PCA) illustrated that traits associated with obtaining and using resources (specific leaf area, leaf thickness, leaf size, and leaf tensile strength) were highly correlated, accounting for 24% of the variance, while those associated with plant size (leaf area, seed mass, woodiness, and height) accounted for 17% of the variance. It is evident that necessary tradeoffs between form and function must be fulfilled by plants to survive. These PCA results suggest that such tradeoffs, and thereby the expression of various traits, are very similar across taxa because there is a common need among plants to reduce water, light and nutrient stress and to use these critical resources efficiently.

Functional traits reflect species' "strategies" to survive in different environmental conditions, but environmental filters ultimately act upon whole individuals who possess a myriad of traits (Grime 2002; Westoby *et al*. 2002). Therefore interspecific correlations between plant traits reflect a process of natural selection that facilitates certain combinations of traits over others at each scale (Westoby *et al*. 2002). Even if species express these traits differently, according to what the particular environment they occur in necessitates, similar tradeoffs will cause trait correlation among species across the globe. Trait-based research has thereby increased understanding of plant functional types and how ecological communities function at different scales.

In summary to this point, I have reviewed evidence supporting the idea that plant functional traits contain a lot of information relevant to community assembly, and that the scale of a study influences which traits are most significant to the distribution and abundance of species. In addition to providing insight into the environmental filtering hypothesis, and the fundamental tradeoffs governing plant functional types, trait-based research has the potential to develop general principles in community ecology. When attempting to understand community assembly along environmental gradients and predict community composition, species-based studies are limited to specific areas and thus cannot be extrapolated to understand community dynamics in different habitats. Alternatively, as the analyses by Diaz *et al*. (2004) and Wright *et al*. (2004) indicate, trait-based research allows for hypotheses to be generalized to multiple systems. Additionally, moving past the species concept eliminates the inevitable complexity of examining pairwise species interactions (Weiher and Keddy 1995; McGill *et al*. 2006). Moreover, trait-based research functionally groups species together, further reducing the perceived complexity of ecological communities and directing focus toward more fundamental processes in community assembly (Weiher and Keddy 1995; Lavorel 1997; Mcgill *et al*. 2006; Ackerly and Cornwell 2007; Herault 2007). Nonetheless, the problem of predicting community assembly has yet to be completely solved (Keddy 1992; Roughgarden 2009). Shipley, Vile and Garnier (2006) have come up with a possible solution that draws upon previous research, but also introduces some new ideas rooted in a trait-based approach to the problem of community assembly.

An explanation of Shipley, Vile and Garnier's (2006) MaxEnt Approach

Shipley, Vile and Garnier (2006) developed a trait-based model that in principle could reconcile the niche and neutral theory approaches to understanding community assembly. This model utilizes plant traits as constraints driving the distribution and abundance of species along environmental gradients. This aspect of the MaxEnt approach falls within traditional approaches to predicting community assembly as it is assumed that the environment filters out the least suited plant traits so that only select species form a community in any given habitat.

The novel aspect of Shipley, Vile and Garnier's (2006) model (hereafter called the MaxEnt approach) is that it employs Bayesian statistics and entropy maximization to ensure that species abundance distribution (SAD) predictions only incorporate observed trait assembly rules and no other information (Shipley 2009c). This approach is plausible because SAD predictions should be solely based on trait data that encodes important information about species' evolutionary histories and the process of community assembly, and not irrelevant information or other made-up assumptions. This Bayesian approach integrates niche and neutral theory because species are considered ecologically equivalent and randomly assembled (neutral theory), except with respect to their functional traits governing the environmental filtering process (niche theory) (Shipley *et al*. 2006).

Community-aggregated traits

The plant trait data incorporated into the MaxEnt approach are actually "community-aggregated traits," which are average trait values weighted by species abundance in a series of sample plots. To obtain these community-aggregated traits (CATs), selected plant traits must first be measured for all the species in a species pool. This is done by sampling a number of individuals in populations across the entire area that the species pool is derived from. Once these measurements are made, the average value of each trait is calculated for each species. After this, relative abundance must be determined for each species in the communities being studied. The average trait values for each species are then weighted by the relative abundance of each species and summed to produce a single aggregate value for each trait in each community; these weighted average values are the CATs. Weighting the trait data in this manner allows for trait comparison among communities rather than just among coexisting taxa.

Bayesian statistics and entropy maximization

In physics, entropy maximization has proven to be extremely useful in predicting many variables of a system using only a few constraints (Mcgill 2006; Haegeman and Loreau 2008; He 2010). For example, maximum entropy has been used to develop complex weather system models (McGill 2006). In general, Bayesian techniques such as this transform a probability distribution of some system component that is based on *a priori* information into a more accurate probability distribution once additional information regarding the system is gained in the form of constraints (Shipley 2009c). Since it is impossible to consider all the factors involved in community assembly, applying the ecologically relevant information that is available in species traits in conjunction with the technique of entropy maximization, as is done in the MaxEnt approach, appears to be a promising solution to the problem of predicting the distribution and abundance of plants along environmental gradients.

The entropy maximization procedure works by assigning certain probabilities to the possible states of a system; in the case of the MaxEnt approach, states are species (Shipley 2009c). To predict the probability distributions of species in a community, one first derives an *a priori*, or “prior” distribution that is not yet informed by any trait information. At the simplest or “maximally uninformative” level, this prior distribution can be based on only the information that a species occurs in the species pool and might contribute to a community (Shipley 2009c). In this case each species is mutually exclusive, species are not ordered in any way, and the probabilities of each species occurring add to unity. Consequently, the species in the species pool are assumed to yield a community in which every species has an equal abundance, or a uniform prior distribution. Alternatively, if other SAD information is known, it can be used to create a somewhat better informed prior distribution. For example, the relative frequency of each species counted in a vegetation survey of the region encompassing the species pool could provide a prior distribution based on the assumption that the relative abundance of a species in the community is determined by its relative abundance in the regional species pool – an expectation in accord with neutral theory. To reiterate, the prior distribution does not have to be maximally uninformative, but it must not contain any information pertaining to the community-aggregated trait constraints that will be used in the MaxEnt approach to predict the actual species distributions.

Once this prior distribution is established, the community-aggregated trait information is added to the system to act as a constraint and create potential *a posteriori* probability distributions that are hopefully similar to the actual relative abundances of all the species in the communities being examined. There are potentially many possible probability distributions that allow for the community-aggregated trait constraints but the MaxEnt approach selects the one with maximum entropy (Shipley 2009c). To do otherwise would be to predict an SAD that is based on information outside of the prior distribution and the predetermined constraints, which is a logical contradiction as these data should represent all the information available (Shipley 2009c). This also has the outcome of maximizing species diversity given the species richness of a community.

Criticisms of MaxEnt and Shipley's (2009) response

Despite the plausibility of using the MaxEnt approach to predict SADs across communities from a species pool, the approach has gotten harsh criticism (e.g. Marks and Muller-Landau 2007; Roxburgh and Mokany 2007; Haegeman and Loreau 2008; Haegeman and Loreau 2009). Here I will convey the criticisms Shipley's (2006) approach has received and Shipley's (2009a) response to these criticisms.

The ostensible difficulty with imposing such community-aggregated trait constraints on ecological systems first manifests itself in the apparent inability of the MaxEnt approach to predict abundances of rare species (Marks and Muller-Landau 2007; Haegeman and Loreau 2008). In Shipley *et al.*'s (2006) test of their own model, most of the abundance data points are clustered together, as there are many species in their experiment with low abundance and relatively few with high abundance. Even though this is typical for SADs in most plant communities, it makes the use of linear regression a poor choice for measuring the success of the prediction. When the concentrated data representing less abundant species are separated from the outlier data and transformed (e.g. logarithmically or by the n^{th} root), it seems as though the predictive power of the MaxEnt approach is not as strong as first suggested (Marks and Muller-Landau 2007; Haegeman and Loreau 2008). In fact, when Haegeman and Loreau (2008) reanalyze the original data by Shipley *et al.* (2006) it becomes apparent that the estimation of species abundance is too high for rare species, and too low for the most common species.

Haegeman and Loreau (2008) also claim that only the more prevalent species are well predicted because the constraints are weighted averages necessarily determined predominantly by these dominant species. Therefore plant communities that contain a few dominant species will be characterized by a community-aggregated trait value that is heavily weighted in favour of characteristics of these species; this subsequently allows for the relatively accurate prediction of only the dominant species' distributions. The community-aggregated trait constraints are therefore considered to be poor at predicting the abundance of rare species; in other words, the CATs are "overly restrictive" as they are responsible for the strong prediction of dominant species rather than operating on all species in the maximum entropy analysis (Haegeman and Loreau 2008). As such, for the MaxEnt approach to work, Haegeman and Loreau (2008) propose that the constraints chosen must contain information that represents all the characteristics of the entire community (i.e. not only the most dominant species), but simultaneously not be so restrictive that predicted species abundances are entirely the result of this added information, and not due to the maximization of entropy.

Shipley responds to these criticisms of his method by explaining that there has been a misinterpretation of what the MaxEnt approach is really doing. In particular, he claims that Haegeman and Loreau (2008) have viewed the MaxEnt approach as a "maximum combinatorial" model, rather than a maximum entropy model derived from Bayesian logic and information theory (Shipley 2009a). While it is true that leaving out constraints carrying information that affect the actual SADs would be the downfall of a model based on traditional combinatorial statistics, this is not true for the Bayesian MaxEnt model, which assumes outright that such conditions have not been obtained (Shipley 2009a; Shipley 2009c).

Furthermore, Shipley (2009a) agrees that all the predictive ability of the MaxEnt approach lies within the constraints, not in entropy maximization, and insists that the value of the latter is that it ensures that only the information available in the constraints is incorporated in the prediction of a probability distribution. Hence MaxEnt is based on *relative* entropy -- new information may be gained and used to modify a prior distribution in the process of developing accurate distribution predictions (Shipley 2009a). Relative entropy specifically refers to the amount of new information gained in the predicted *a posteriori* probability by using the new CAT information in addition to the information encoded in the *a priori* distribution, relative to only using the latter. In this sense, MaxEnt may be better termed "relative maximum entropy" or "MaxREnt," as is done by Dewar and Porté (2008), to elucidate the notion that the predicted SADs were influenced by constraint information that was missing in the prior distributions.

I believe that the lack of ability to predict the SAD of rare species warrants more attention; Shipley (2009a) hardly acknowledges it in his assertion that predictions are based solely on the constraints, which represent all the information available (Shipley 2009c). I can infer from this that Shipley (2009a) believes that if SADs of rare species are not predicted with the information available, then there is little or nothing that can be done. This response does not satisfactorily address the problem in my opinion. Nevertheless, my test of the MaxEnt approach will shed more light on this issue, and perhaps it is sufficient to simply settle for the prediction of common and intermediately abundant species' SADs.

Even if the MaxEnt approach does not produce perfect SAD predictions, the knowledge gained from it may still have great implications in the understanding of plant community assembly, as well as the conservation of terrestrial ecosystems. For example, if rare species are not well predicted then it can be inferred that processes other than environmental filtering (e.g. stochastic dispersal, establishment or disturbance events) have a role in the assembly of plant communities, especially in the establishment of rare species. Moreover, if the MaxEnt approach is able to predict the abundance of some species then there is support for the notion that traits do exhibit meaningful trends on a community level; i.e. that the process of environmental filtering does at least in part drive plant community assembly and that community-aggregated traits contain information about this process.

Relating community-aggregated traits to environmental gradients

Another criticism of the Shipley, Vile and Garnier's (2006) test of their MaxEnt model is that there was an erroneous circularity linking the prior distribution and predicted distribution. Shipley *et al*. (2006) were limited in terms of their dataset and used the same relative abundances of species to both calibrate and test their model. That is, they first computed the community-aggregated trait values from the observed species abundances in the communities in their study sites, and then used these values to form predictions of species abundance in the same sites. Even though they eventually attempted to predict CATs by using a cubic-spline regression to smooth the observed CAT data over a successional age gradient, their method intrinsically tied the predicted and observed SADs together, thus undercutting any assertion that the MaxEnt approach worked in predicting species distributions through an analysis of independent knowledge of plant traits (Marks and Muller-Landau 2007; Roxburgh and Mokany 2007; Haegeman and Loreau 2008).

Shipley's (2009a) response to this dilemma is that it, "simply does not exist." First he states that this supposed circularity is actually a common practice in classical statistical mechanics and Bayesian statistics alike. The example he provides is the utilization of temperature as a constraint on macroscopic average kinetic energy. Shipley (2009a) explicates the process of adjusting the degrees of freedom in a statistical model when using the same data to estimate a distribution and test the accuracy of the prediction. This adjustment allows the prediction and the test to be done with different information, and thus circularity is avoided. In essence this rejoinder explains that this circular MaxEnt approach can be used to determine if a MaxEnt model fits species relative abundance and community-aggregated trait data; i.e. it can show whether or not community-aggregated traits do drive species abundance distributions, at least in part. Others have since agreed with this point of view, and many recent studies have used the circular MaxEnt approach to determine if community-aggregated traits are important to community assembly (e.g. He 2010; Mokany and Roxburgh 2010; Roxburgh and Mokany 2010; Sonnier *et al*. 2010). Shipley (2009a) goes on to explain that measured values are not necessary to ascribe constraints, and in fact the goal of the MaxEnt approach is to eventually base the constraints on environmental gradients.

I will attempt to use the MaxEnt approach in this more predictive manner by developing environmental gradients with which to estimate CATs and predict SADs. This proposed method is a commonly accepted way to circumvent the questionable circularity altogether. I simply will use separate communities sampled along the same environmental gradient in a locality, i.e. at the alpha scale, to calibrate and test the MaxEnt approach. An underlying environmental gradient then can be established for both the calibration and test plots, and a generalized additive model (GAM) can be used to define the relationship between the gradient and the CATs calculated in the calibration plots. A GAM fits a function to data using either parametric or non-parametric means, thereby potentially providing a better fit than other methods (Wood 2010). Since the independent test plots are also arrayed along the same environmental gradient, the

GAM can then be used to estimate what the CATs are expected to be in the test plots. Once these estimated CATs are computed, the MaxEnt approach can be employed to predict SADs in the test plots.

Not only does this method avoid any type of circularity and test the true predictive ability of the MaxEnt approach, but it more firmly draws in the concept of the environmental filtering process by means of arranging communities along an environmental gradient to interpret community assembly processes. Rather than simply assuming that the CATs reflect changes in the environment, the relationship between these CATs and the changing environment can be quantified by a GAM and thus provide insight into the environmental filtering process that was previously described (Keddy 1992; Weiher and Keddy 1995; Belyea and Lancaster 1999; Booth and Swanton 2002).

The MaxEnt approach seems to have the potential to move community ecology a significant step forward. If MaxEnt successfully predicts SADs across environmental gradients, it can be applied to predict how plant communities will change in response to environmental changes (e.g. climate change and land use change), and how successful species may be when introduced purposely or inadvertently in a new region. Furthermore, MaxEnt has the potential to connect the fields of biogeography, functional ecology, and community ecology (Shipley *et al*. 2006). Finally, if the MaxEnt approach works, the question of how communities assemble from a species pool, which links together all research in community ecology, will have a more complete answer. Even if Shipley, Vile and Garnier's MaxEnt approach does not work, its potential makes it deserving of thorough empirical testing.

Testing the MaxEnt approach

Although the MaxEnt approach deserves empirical testing, very few such tests in fact have been performed (e.g. Mokany and Roxburgh 2010; Roxburgh and Mokany 2010; Sonnier *et al*. 2010; Shipley *et al*. *in press*). Here I provide a brief outline of the steps I have taken to provide a meaningful test of the MaxEnt approach, and I then will explain each step in detail in the subsequent text:

1. Decide what sort of plant community to sample.
2. Select a study site and determine the species pool.
3. Select and measure a set of functional traits.
4. Measure species abundance in plant communities located in varying environmental conditions and calculate community-aggregated traits for each study plot.
5. Decide on a prior distribution to incorporate in the MaxEnt approach.
6. Perform a circular test of the MaxEnt approach following established procedures.
    i. Predict SADs by inputting the *observed* CATs and the prior distribution for all the plots to the R code written for the MaxEnt approach in the ‘FD’ package in R (Laliberté and Shipley 2010).
    ii. Compare the predicted SADs with the observed ones using the inferential permutation test developed by Shipley (in press), which is available through the ‘FD’ package in R (Laliberté and Shipley 2010).
    iii. Work to improve the predictions by altering the different components of the MaxEnt approach.
7. Perform a non-circular test of the MaxEnt approach using independent calibration and test plots.
    i. Create an environmental gradient from measured environmental variables, along which the communities can be placed and from which prediction constraints (CATs) can be estimated in test plots.
        a. Examine the environmental conditions in all of the plots and eliminate outliers if necessary.
        b. Divide the remaining plots into two subsets of “calibration” and “test” plots, along an environmental gradient.
        c. Define mathematical relationships linking different environmental gradients to the CATs calculated from observed data in the calibration plots.

        d. Use the mathematical relationships to estimate CATs in the test plots.

    ii. Predict SADs by inputting the *estimated* CATs and the prior distribution for the test plots to the R code written for the MaxEnt approach in the ‘FD’ package in R (Laliberté and Shipley 2010).
    iii. Compare the predicted SADs with the observed ones using the inferential permutation test developed by Shipley (in press).
    iv. Work to improve the predictions by altering the different components of the MaxEnt approach.

In this section I will elaborate on the methods I used to realize steps 1-3. Steps 4-7 will be covered in the section regarding my approach to data analysis.

1. DECIDE WHICH COMMUNITIES TO SAMPLE

Defining a community

To test the MaxEnt approach I first had to determine which communities I was going to sample, which was difficult because it involved questioning the concept of a community in general. Despite over a century of scientific study (McIntosh 1975), the definition of a community is still in debate and changes with taxonomic and spatial scale, as well as the purpose of a study (Booth and Swanton 2002; Ricklefs 2008; Vamosi *et al*. 2009). In its simplest form, a community can be defined as two coexisting species (Fauth *et al*. 1996); however communities are usually much more complex, containing many species from every kingdom of life, no matter the spatial scale. Most researchers do not try to study communities defined by all of the species in a particular area because such communities are intractable. With each species that is considered, new relationships with other biota, as well as the environment are brought into the picture. Studying all of these relationships and the overall ecological patterns that they form is an extremely difficult task to carry out that is prevented by limitations in time and resource availability (Vellend *et al*. 2008).

Many studies therefore focus on more restricted communities composed of relatively few species, but excluding some species may lead to misinterpretations of community assembly rules. Community patterns can be missed because they are caused by excluded species, or because they are manifested in excluded species (Booth and Swanton 2002). While these false impressions are obviously not desirable, it is nonetheless common practice in ecology to direct research towards interactions within restricted populations at a local scale (Ricklefs 2008).

Often researchers elect to study species within a plant functional group, which is determined by species' traits, to elucidate mechanisms of community assembly. This is because species within a functional group are more likely to interact competitively if they coexist than species among different functional groups (Vamosi *et al*. 2009). This dynamic is significant to the central questions of community assembly. Webb *et al*. (2002) conclude that sometimes species with similar traits coexist (phenotypic attraction), and sometimes they do not (phenotypic repulsion). Furthermore, they reviewed the literature relevant to both phylogenetics and community ecology and came up with four possible explanations for these outcomes. Phenotypic attraction occurs when species with similar traits coexist because they can survive in similar environmental conditions. These species can either be 1) closely related and share traits that were conserved throughout evolution, or 2) distantly related and share convergent traits. In both of these cases environmental filtering is the proposed mechanism that brings these species together. Alternatively, phenotypic repulsion occurs when 3) highly unrelated species coexist because competitive exclusion causes closely related taxa to be locally excluded or 4) species with convergent traits interact competitively so that some species are excluded. The first option, i.e. phenotypic attraction due to the clustering of phylogenetically related species, appears more often than the other three options in empirical studies, supporting the idea that environmental filtering is the mechanism shaping the majority of communities (reviewed by Webb *et al*. 2002; Vamosi *et al*. 2009). Despite this, what makes environmental filtering or competitive exclusion the dominant force in each case is yet unknown.

I think this question may be answered by focusing on why phylogenetically related species with similar traits do or do not coexist in a habitat. Such communities would provide a rigorous test of the MaxEnt approach because they are likely assembled by environmental filtering and the MaxEnt approach is based on the environmental filtering hypothesis. If a test using such communities fails to support the MaxEnt approach, then doubt will be cast on the theoretical underpinnings of the MaxEnt approach. As such, I decided to limit the communities I sampled in my test of the MaxEnt approach to include only a subset of phylogenetically related species that display trait conservatism, rather than all of the species in a given area.

Using the fern functional group to test the MaxEnt approach

The fern functional group fulfills the aforementioned criteria for a study group as they are phylogenetically distinct on the whole, and they share traits that other vascular plant groups do not. The classification of "fern" is a broad one that encompasses thousands of species across the globe (Moran 2008). Ferns are considered an ancient group of plants, as molecular and fossil evidence leads us to believe that they originated over 350 mya (Pryer *et al*. 2004; Schuetpelz and Pryer 2008). There is a great amount of physiological, morphological and phenological differences among all these species, stemming from the various evolutionary and phylogenetic pathways that developed over time. Although several monophyletic families have been distinguished, and some are more closely related than others, all ferns are vascular plants that disperse lightweight spores, generally only 30-70 µm long, rather than seeds to reproduce (Whittier and Wagner 1971; Pryer *et al.* 2004).

This lightweight reproductive structure is conducive to anemochory, which reduces the role of dispersal limitation in community assembly and hence lends support to the assumptions inherent in both niche theory and the MaxEnt approach. As Gleason propounded in his individualistic concept of ecology, the structure of a community is greatly influenced by the nature of the surrounding communities and the movement of individuals to and fro. Dispersal limitation is thought to be a particularly important constraint on community structure if neutral processes are more important than niche-related ones (Hubbell 2001); however as a result of fern spores being widely dispersed by wind, dispersal limitation is not likely to be an important factor for fern community assembly. Atmospheric samples have found fern spores at high altitudes, even in jet streams (Moran 2008). Biogeographic and molecular evidence confirms that fern spores can traverse great distances as well, much more so than heavier propagules such as fruits and seeds (Dassler and Farrar 2001; Page 2002; Moran 2008). That said, spores from ferns that grow on the forest floor (as do the majority of fern species I am studying) are not usually exposed to large amounts of wind. Spore rain is therefore much more substantial within a few meters of the parent plant than it is even 50 m away (Peck *et al*. 1990; Penrod and McCormick 1996). Nonetheless, since ferns can disperse virtually anywhere, their distribution and abundance is seemingly less determined by dispersal limitation than in seed-bearing plants. The small mass of fern spores is therefore a trait that reinforces the idea that niche-related processes are the dominant forces in fern community assembly, which supports the logic of the MaxEnt approach.

Ferns also possess other traits that particularly support the niche-based concept of environmental filtering, which in turn makes the fern functional group a good candidate for testing the MaxEnt approach. Ferns are generally less susceptible to herbivory and disease than many other plants are (Page 2002). This bolsters the idea that abiotic factors may be greater drivers of fern community assembly than biotic factors, i.e. that the environmental filtering hypothesis is likely to be the dominant mechanism in community assembly rather than competitive exclusion (Page 2002; Karst *et al*. 2005). In fact it has been proposed that climatic properties influence fern distribution on a regional scale, while edaphic variables do so on a local scale (Karst *et al*. 2005).

Thus, in addition to simply being a phylogenetically related group of species displaying trait conservatism, ferns possess unique traits that support the theory behind the MaxEnt approach and are therefore an excellent study group for rigorously testing it. If my test of the MaxEnt approach using fern community-aggregated traits provides positive results, then I will have confirmed that environmental filtering is indeed the dominant process in fern community assembly along environmental gradients. If the MaxEnt approach proves unable to predict the assembly of fern communities, its capacity to predict that of other plant groups with traits that are less conducive to the notion of environmental filtering will most certainly be called into question.

2. SELECT A STUDY SITE AND DETERMINE THE SPECIES POOL

Study site

After establishing that I wanted to sample fern communities, I had to determine which species of ferns I would include in my test of the MaxEnt approach. This decision involved first choosing a study site that contained many populations of a wide variety of fern species. These characteristics were necessary to provide a large enough sample size for my research and to fulfill the statistical requirements regarding degrees of freedom; the degrees of freedom available in a MaxEnt analysis equal the number of species sampled minus the number of traits measured (Shipley 2009c). As previously discussed, choosing a study site with many coexisting species of ferns will also help me interpret the results of my test of the MaxEnt approach in regards to determining if environmental filtering really occurs on the alpha scale, or if competitive exclusion is the dominant driver of community assembly. Additionally, because the purpose of the MaxEnt approach is to study the assembly of plant species across an environmental gradient within a habitat, the study site ideally should be identified as one type of habitat and contain areas with some variation in environmental conditions from which an environmental gradient can be identified.

With these criteria in mind, I decided to sample fern communities in the Gault Nature Reserve, which is a 10 km^2 tract of old growth forest on Mont Saint Hilaire in southern Quebec (45º32’N, 73º08’W) with high plant diversity. Of the approximately 40 fern species present in southern Quebec (Fleurbec 1993; Marie-Victorin 1995), 38 are found in the Gault Nature Reserve (Bell *et al*. 2001). Although Mont Saint Hilaire can be identified as predominantly a hardwood forest habitat, there exists a great amount of heterogeneity within the forest that in part accounts for the high fern diversity (Maycock 1961; Arii and Lechowicz 2002; Karst *et al*. 2005). This site therefore serves the purposes of my study on the assembly of temperate forest fern communities along environmental gradients extremely well.

Furthermore, this reserve has been the venue for numerous ecological investigations, which provided me with some of the information needed to test the MaxEnt approach. In fact, sixty-nine 50 m^2 permanent plots were established across the reserve by Ben Gilbert (Gilbert and Lechowicz 2004) for which fern abundance and environmental factors have been measured in both 2002 and 2004 (Gilbert and Lechowicz 2004, unpublished data; Figure 1). This is rather convenient since these data are necessary to fulfill steps 4 and 7 in my test of the MaxEnt approach. I therefore tested the MaxEnt approach by trying to predict fern distribution and abundance in these plots – the fern species in each 50 m^2 plot representing a fern community. For reasons I will explain shortly, I focused on only 47 of the 69 plots.

Species pool

As discussed throughout my thesis thus far, the theory behind the MaxEnt approach is that the environment filters species based on their functional traits, so that only a subset of species from a regional species pool is able to survive in a given area. Therefore it is integral to my test of the MaxEnt approach to determine which species are included in the regional species pool, which is a task involving some subjective judgement. The species pool should theoretically be comprised of all the species that could potentially occur in any of the 47 permanent plots I am considering. The high vagility of fern spores means that it is possible that species not observed in the plots that occur elsewhere on the reserve or even off the reserve could be part of the species pool. The potential magnitude of the species pool forced me to impose my own limits on which species to include in my research. I therefore deemed the species pool to include all of the species that were observed in any of the 47 plots I've chosen to study in 2002 and/or 2004. The regional pool is therefore comprised of twenty-one fern species (See Table 1 for species names and relative frequencies).

This somewhat arbitrary definition of the species pool does not affect the test of the MaxEnt approach because as discussed previously, the MaxEnt approach is one of maximum *relative* entropy. The information contained in the species pool is part of the information contained in the prior distribution that I must set, as well as the community-aggregated traits that I must calculate and predict from the environmental gradient. As such, even if the species pool that I set does not completely capture all of the information relevant to the assembly of the 47 local communities I am studying, this partial information should still improve my predictions. If I were to include even more of such relevant information by better ascertaining which species should be in the species pool, then my predictions would be even more accurate. Therefore I am satisfied with limiting the species pool to the twenty-one species that have been observed in the 47 plots. I measured functional traits for each of these species in order to predict species abundances in these plots.

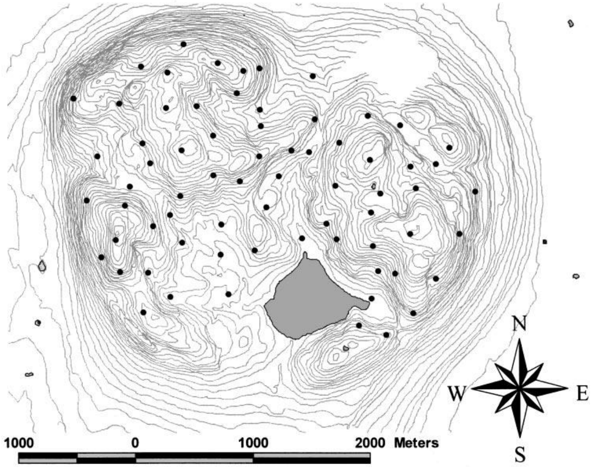

Figure 1. Map of the sixty-nine $50m^2$ permanent plots established on the Gault Nature Reserve in Mont-Saint-Hilaire, Quebec, by Ben Gilbert that were surveyed in 2002 and 2004. Taken from: Gilbert, B. and M.J. Lechowicz. 2004. Neutrality, niches, and dispersal in a temperate forest understory. *PNAS* 101: 7651-7656.

Table 1. List of the 21 fern species and their absolute and relative frequencies in the forty-seven 50m^2 permanent plots established by Ben Gilbert (Gilbert and Lechowicz 2004) that comprise the species pool that I am including in my test of the MaxEnt approach by Shipley *et al*. (2006).

Species	**Family**	**Absolute Frequency**	**Relative Frequency**
Dryopteris intermedia Muhlenberg ex Willdenow	Dryopteridaceae	36	24.5%
Dryopteris marginalis Linnaeus	Dryopteridaceae	25	17.0%
Polystichum acrostichoides Michaux	Dryopteridaceae	14	9.5%
Athyrium filix-femina Linnaeus	Dryopteridaceae	12	8.2%
Deparia acrostichoides Swartz	Dryopteridaceae	10	6.8%
Adiantum pedatum Linnaeus	Pteridaceae	9	6.1%
Cystopteris fragilis Linnaeus	Dryopteridaceae	8	5.4%
Botrychium virginianum Linneaus	Ophioglossaceae	5	3.4%
Dryopteris carthusiana Villars	Dryopteridaceae	5	3.4%
Polypodium virginianum Linnaeus	Polypodiaceae	5	3.4%
Onoclea sensibilis Linnaeus	Dryopteridaceae	3	2.0%
Botrychium matricariifolium Döll	Ophioglossaceae	2	1.4%
Dennstaedtia punctilobula Michaux	Dennstaedtiaceae	2	1.4%
Phegopteris connectilis Michaux	Thelypteridaceae	2	1.4%
Phegopteris hexagonoptera Michaux	Thelypteridaceae	2	1.4%
Pteridium aquilinum Linnaeus	Dennstaedtiaceae	2	1.4%
Dryopteris clintoniana Eaton	Dryopteridaceae	1	0.7%
Gymnocarpium dryopteris Linnaeus	Dryopteridaceae	1	0.7%
Matteuccia struthiopteris Linnaeus	Dryopteridaceae	1	0.7%
Osmunda cinnamomea Linnaeus	Osmundaceae	1	0.7%
Woodsia ilvensis Linnaeus	Dryopteridaceae	1	0.7%

3. SELECT AND MEASURE A SET OF FUNCTIONAL TRAITS

Plant functional traits are characteristics of individuals that impact fitness indirectly via their effects on growth, reproduction and survival (Cornelissen *et al*. 2003; Violle *et al*. 2007). Of key importance to my test of the MaxEnt approach is selecting functional traits to measure that effectively reflect species' adaptations to the environment, since the environmental filtering hypothesis suggests that only species well-suited to a particular habitat will be able to survive there. Since I am focusing on communities that occur in one type of habitat, "alpha-traits" should be focused on. Furthermore, because of sampling limitations and feasibility, as well as the aforementioned statistical considerations regarding degrees of freedom, it was wise to choose the fewest number of traits necessary to encompass the most important ecological constraints on species abundance and distribution. It was very difficult to know *a priori* which traits are the most important to the performance of plants along environmental gradients within a habitat and therefore plant community dynamics; however, a review of plant literature (e.g. Lavorel and Garnier 2002; Cornelissen *et al*. 2003; Violle *et al*. 2007) has led to the following trait categories of interest: growth form, spatial colonization patterns, productivity, phenology, and reproductive capacity.

To represent these categories I measured the following functional traits for each of the 21 species in the species pool: plant height, population density, photosynthetic area, leaf dry matter content, leaf mass per area, chlorophyll content, foliar nitrogen content, foliar carbon to nitrogen ratio, and maximum photosynthetic capacity. I also obtained information regarding whether or not each species has overwintering fronds, as well as during which months they typically sporulate. Finally, I attempted to measure gametophytic moisture tolerance, but was not successful. I will now outline how I selected fronds to sample as well as the specific ecological significance of each trait and how I measured it.

Selecting fronds to sample

The MaxEnt approach using weighted *average* trait values, meaning that I had to sample multiple individuals of each species. I sampled ferns from many different areas of the Gault Nature Reserve to ensure that my samples provided a robust estimate of the mean, and to ensure that phenotypic plasticity was represented in the mean value of each trait. To carry out this broad-range sampling method, I divided the Gault Nature Reserve into ten sectors of roughly the same area to sample from. The sector boundaries were delineated so that they followed the topography of the reserve, as well as the hiking trails that have been established throughout the reserve, in a way that was conducive to sampling the sectors in a sensible and timely manner. I aimed to collect samples of each species from the different sectors, but this was not always possible as some species only grow in certain areas of the reserve or are too rare to find in many places. I managed to obtain between five and ten samples for each species

The only criteria that a fern sporophyte had to satisfy to be eligible for data collection were that it be full-grown (i.e. have fully unfurled fronds) and that it not be visibly damaged or diseased. I sampled both fertile and infertile fronds because often I could only find one or the other in a particular population. Once I encountered a population of a species, I used a variation of the “Ignorant Man” technique (Ward 1974) to determine which fronds I collected trait data for. This is a random sampling method that involved two people: my research assistant and I. My research assistant blindly selected a number from a random numbers table, and without knowing that number I pointed to individual fronds that were eligible for sampling while counting them out loud. When I called out the number that she had selected she told me to stop and that was the frond that I collected data from.

In some cases it is extremely difficult to ascertain whether or not a fern sporophyte is truly an individual or if it is a ramet of an individual that possesses an unseen underground portion connecting many apparently individual fronds. Depending on the species, individual ramets can either grow as multiple fronds arranged in a tight circle that resembles a crown, or as solitary fronds that may or may not form a loose patch or colony. If I encountered a species that formed crowns then I would first randomly select a crown to sample, and then randomly sample a frond within that crown. If the species did not form a crown and there was a large population in a small area, I counted individual full-grown fronds and measured the randomly selected frond. If a particular population was dispersed into small patches in close-proximity to each other, I would first randomly select one patch and then follow the aforementioned selection protocol. Once I selected a focal frond, I proceeded to measure its expression of the aforementioned functional traits. This sampling method was developed to ensure that individual traits and community patterns were impartially observed.

Sampling traits

Plant height: I measured plant height as the vertical distance from the ground to the upper boundary of the fern frond in its naturally curved state (i.e. the frond was not straightened during measurement). This trait is associated with competitive capacity through shading effects and is typically correlated with other size-related traits, such as aboveground biomass and rooting depth (Cornelissen *et al*. 2003).

Population density: I calculated population density by first selecting one focal plant, and then measuring the distance to its five nearest neighbours, if there were five. If a species formed crowns then I measured the distance between crowns. If not, then I measured the distance between individual fronds. I then inverted this distance measurement to estimate the density of a population, expressed in metres^{-1}. This trait signifies the spatial colonization patterns of each species, which again is associated with competitive vigour.

Photosynthetic area: I measured photosynthetic area by scanning the sampled fronds with a LI-COR 3100 Leaf Area Meter (LI-COR Lincoln, Nebraska, U.S.A.), ensuring that the fronds remained hydrated prior to scanning. I scanned the whole frond five times and then removed the stipe and rachis. Then I scanned the removed material five times. I subtracted the average value calculated for the stipe and rachis area from the average value of the whole frond area to obtain the final measurement of photosynthetic area. Photosynthetic area is a correlate of the plants potential relative growth rate (Cornelissen *et al*. 2003).

Leaf dry matter content (LDMC): I calculated LDMC for each frond by dividing the oven dried mass of its photosynthetic portion by its water-saturated fresh mass. Before I scanned each whole frond to obtain photosynthetic area, I weighed it on an electronic balance with a precision of three decimal places. I also weighed the stipe and rachis immediately after removing them from the frond. I subtracted the mass of the stipe and rachis from the mass of the whole frond to obtain the water-saturated fresh mass of the frond. After I removed the stipe and rachis, I dried each frond in a drying oven at 80°C for 48 hours. Once this time had passed, I quickly removed the dried material from the oven and placed into a plastic bag with a desiccant pack inside. This allowed the sample to cool down but not absorb any moisture in the meantime. Once cool, I weighed all the pieces of the dried material at the same time, rather than separately, to increase accuracy of the oven dried mass. The sample had to be cooled prior to being weighed to ensure that the mass readings were not made unstable by thermal currents. LDMC typically negatively correlates with potential growth rate, or mass-based photosynthetic rate, and positively with leaf toughness and leaf longevity. Species that have high LDMC values tend to be less productive and are found in infrequently disturbed environments (Cornelissen *et al*. 2003).

Leaf mass per area (LMA): I calculated LMA for each frond by dividing the oven dried mass of its photosynthetic portion (i.e. the frond with its stipe and rachis removed) by its photosynthetic area. LMA typically correlates with LDMC. It has a strong positive correlation with leaf lifespan because it is assumed that plants in resource-poor environments invest more carbon into their leaves (i.e. have higher LMA and LDMC values) as a defence mechanism to deal with resource stress (Cornelissen *et al*. 2003).

Leaf chlorophyll content (Chl): I measured leaf Chl by first using a SPAD-502 chlorophyll meter (Minolta Corp., Ramsey, N.J., U.S.A.). This instrument measures light transmittance through the frond and provides an output that can be converted into foliar Chl (Markwell and Levins 1999). I took twenty

SPAD readings from different areas of the sampled frond if possible. I recorded the average SPAD value and transformed it into a chlorophyll concentration value with the following conversion formula: Chl = 0.0885*SPAD reading*SPAD reading+1.9927*SPAD reading+32.78 (Monje and Bugbee 1992). I did not measure Chl for all of the 21 species in my study because I used data previously collected by Amanda Karst at the Gault Nature Reserve who made several such measurements on ferns for her research on correlations among fern foliar traits (Karst and Lechowicz 2006). I decided to simply add to Karst's data to ensure that I had at least five data points for each species. Chlorophyll content is directly related to photosynthesis; thus this trait provides another measure of the photosynthetic capacity of each species.

Leaf nitrogen content (N) and carbon to nitrogen ratio (C:N): I measured N and C:N by using an EA 1108 CHNS-O elemental analyzer (CE Elantech, Inc., Lakewood, N.J., U.S.A.) which is an instrument that can determine the concentration of carbon, hydrogen and nitrogen of sample. I created a standard calibration curve using acetanilide, which is recommended for measuring C and N of vascular plants. After calibrating the machine I encapsulated five ground and dried samples of fern fronds from each species and recorded the mass of each sample. I then performed a combustion analysis of the samples in the elemental analyzer. The machine burned the samples at high temperatures in a stream of oxygen flowing through a catalytic bed, causing the carbon and nitrogen in the samples to be oxidized to carbon dioxide and nitric oxide, respectively. These products were run through a gas chromatographic column in the analyser to estimate their respective amounts and this information was used in conjunction with the calibration curve and the weights of the samples before they were burned to determine the percentage weights of carbon and nitrogen in the sample per unit of dry leaf mass. I calculated the average C and N values from the five samples of each species. I determined C:N by dividing the C value by the N value for each sample, and then averaging the resulting values for each species. N is strongly correlated with mass-based maximum photosynthetic rate (Cornelissen *et* *al*. 2003). C:N is a measure of the trade-off between carbon and nitrogen as high values indicate a greater investment in leaf longevity than in productivity.

Maximum photosynthetic capacity (A_{max}): I used a LI-6400XT (LI-COR Lincoln, Nebraska, U.S.A.) portable photosynthesis system to measure A_{max}. I took A_{max} measurements between 7:30 am and 11:30 am (Eastern daylight saving time), because this is typically when photosynthesis is at its peak, and afternoon stomatal closure has not yet begun to affect A_{max} (Gildner and Larson 1992). As was the case with Chl, I also used A_{max} data obtained by Amanda Karst (Karst and Lechowicz 2006) to augment my own data collection. I added to Karst's data to ensure that I had at least five measurements for each species. I made sure to use the same settings as Amanda Karst did in her measurements of $_{Amax}$ with the LI-6400XT. These included: ambient humidity, a CO_2 concentration of 350 ppm, a temperature of 25°C, a stomatal ratio of zero and a photosynthetic photon flux density of 2000. Measurements were taken every ten seconds for a total of sixty seconds, and the average of these measurements used to determine A_{max}. The portion of the frond that was clamped in the chamber of the instrument was cut from the whole frond and later scanned so that its area could be determined and final A_{max} per unit area could be calculated.

Wintergreen: Whether or not an individual retains green fronds overwinter impacts the return on investment of resources over its lifetime. Species with overwintering fronds tend to have a high LMA, high C:N, and high N on an areal basis, as compared to species that do not overwinter (Karst and Lechowicz 2006). This trait was simply binary, and based on the published literature (Fleurbec 1993) and my own field observations..

Sporulation: Sporulation is relevant to the process of reproduction and its timing is an indication of both reproductive maturity and the timing of spore dispersal. Spores need to be released for haploid gametophytes to germinate, develop and mate. Therefore the timing of spore release affects the alternation of generations and production of new sporophytic individuals. I obtained information regarding the months in which each species typically sporulates in

Quebec from Lamoureux's (1993) Fleurbec field guide to identifying ferns, horsetails and clubmosses, and I made my own field observations to corroborate these literature values. I assigned a value from 1 to 6 to each species to represent their period of sporulation, with higher values coding later and longer periods of sporulation (Table 2).

Sporulation Period	**Assigned Category**
May-June	1
June-July	2
July-August	3
August-September	4
June-August	5
June-September	6

Table 2. Categories of fern sporulation periods that were assigned to the 21 species in my study, according to the information provided in Lamoureux's (1993) Fleurbec field guide to identifying ferns in Quebec.

Gametophytic drought tolerance: Fern gametophytes are small haploid organisms that precede the diploid sporophyte stage of the fern lifecycle. Therefore the growth and success of a fern sporophyte at a site is at the outset directly dependent on the survival of gametophytes at the site. To assess differences in the response of gametophytes to moisture stress, I sowed spores of my study species on nutrient agar in Petri dishes, and subjected them to ambient conditions that mimicked those found at the Gault Nature Reserve in the summer time. I had planned to transplant the gametophytes after spore germination into nutrient agar that was characterized by either having moisture stress or not, by using an osmoticum. I wanted to then let the gametophytes grow in these conditions and eventually measure gametophyte size in order to determine the drought tolerance of gametophytes for each species. This trial did not work because after spore germination it became clear that quantifying gametophyte size would unfortunately be impractical. Both the three dimensionality of the gametophytes and the fact that gametophytes for many species grew in large clusters prevented a simple surface area measurement. Furthermore, the fragility of these organisms prevented removing them from the agar to obtain a measurement of their mass, which in any case would have been extremely little and difficult to measure accurately.

Approach to data analysis

Once I fulfilled steps 1-3 of the data collection, I was able to move on to executing the rest of the steps in my test of the MaxEnt approach. In this section I will explain how I performed steps 4-7 in the previously presented outline summarizing my overall test of the MaxEnt approach:

4. Measure species abundance in plant communities located in varying environmental conditions and calculate community-aggregated traits for each study plot.
5. Decide on a prior distribution to incorporate in the MaxEnt approach.
6. Perform a circular test of the MaxEnt approach following established procedures.
    i. Predict SADs by inputting the *observed* CATs and the prior distribution for all the plots to the R code written for the MaxEnt approach in the 'FD' package in R (Laliberté and Shipley 2010).
    ii. Compare the predicted SADs with the observed ones using the inferential permutation test developed by Shipley (in press), which is available through the 'FD' package in R (Laliberté and Shipley 2010).
    iii. Work to improve the predictions by altering the different components of the MaxEnt approach.
7. Perform a non-circular test of the MaxEnt approach using independent calibration and test plots.
    i. Create an environmental gradient from measured environmental variables, along which the communities can be

placed and from which prediction constraints (CATs) can be estimated in test plots.

  a. Examine the environmental conditions in all of the plots and eliminate outliers if necessary.
  b. Divide the remaining plots into two subsets of “calibration” and “test” plots, along an environmental gradient.
  c. Define mathematical relationships linking different environmental gradients to the CATs calculated from observed data in the calibration plots.
  d. Use the mathematical relationships to estimate CATs in the test plots.

ii. Predict SADs by inputting the *estimated* CATs and the prior distribution for the test plots to the R code written for the MaxEnt approach in the ‘FD’ package in R (Laliberté and Shipley 2010).

iii. Compare the predicted SADs with the observed ones using the inferential permutation test developed by Shipley (in press).

iv. Work to improve the predictions by altering the different components of the MaxEnt approach.

4. MEASURE SPECIES ABUNDANCE IN PLANT COMMUNITIES LOCATED IN VARYING ENVIRONMENTAL CONDITIONS AND CALCULATE COMMUNITY-AGGREGATED TRAITS FOR ALL OF THE PLOTS

In addition to the raw trait data that I obtained, I needed fern abundance data in order to calculate community-aggregated traits for the 47 communities across the Gault Nature Reserve that I decided to study. As mentioned, abundance was measured in these 47 plots during two years (2002 and 2004). I performed a model II regression and determined that the total abundance of the 21 fern species I am studying observed in each plot between the two years significantly co-vary (adjusted R^2=0.885, $p<0.05$); the linear regression line is shown in Figure 2. This analysis highlighted one apparent outlier (coordinates of 0, -1.0). Upon closer examination of the raw abundance data, it became apparent that the plot contained only one species in both years (*Polystichum acrostichoides*), but the percent cover of that species decreased from 100% to 10% from 2002 and 2004. It is not apparent why this decrease occurred, so I did not exclude the plot from the analysis. I therefore took the mean of both years to weight the averaged trait data to calculate the CATs, in accordance with the methods previously described in the introduction.

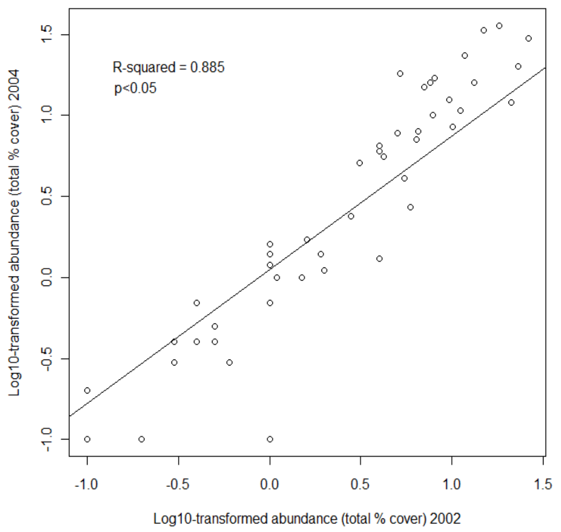

Figure 2. Model II regression of the log$_{10}$-transformed total abundance for the ferns in all 47 plots, as measured in 2002 and 2004. There is a significant correlation among the years (adjusted $R^2=0.885$, $p<0.05$)

Using weighted *means* rather than individual trait data in calculating the CATs has important implications for the level of biological organization that is being investigated. While each trait was measured from a specific part of the plant, averaging the trait data brought the information gained to a whole-plant level (Shipley 2009c). For example, I measured LMA at the frond level, so on its own each LMA data point is a reflection of the carbon investment into one specific frond, not the whole plant. The whole plant can contain multiple fronds as well as non-photosynthetic material, so it does not make sense to attribute one LMA measurement to the whole plant. However, by averaging the five to ten data points I obtained for each species, the trait now represents the carbon investment of each species at a whole-plant level reasonably well. Shipley (2009c) makes the point that because some functional traits can be measured at one level (e.g. the frond) and averaged to represent another level (e.g. the whole plant); these functional traits are really “plant-aggregated traits.” By weighting a plant-aggregated trait value by the relative abundance of the species it was measured for, the trait becomes a “community-aggregated trait.”

With the exception of the categorical wintergreen and sporulation traits, I log$_{10}$-transformed the trait data before averaging them to obtain plant-aggregated traits. Transforming the height data in this way essentially changed the data to an estimate of relative growth rate, as plant growth is logarithmic over time (Blackman 1919). Furthermore, logarithmically transforming the trait data emphasized relatively small trait differences among species, and is a common procedure in trait-analyses to obtain a normal probability distribution (e.g. Wright *et al*. 2004). I also had to adjust some of the data points to make them positive values because the logarithmic transform sometimes negative numbers, which the MaxEnt algorithm cannot handle. I did this by increasing the raw data values by an increment of one.

5. DECIDE WHICH PRIOR DISTRIBUTION TO INCORPORATE IN THE MAXENT APPROACH

The prior distribution is an important component in testing the MaxEnt approach and is necessary to fulfill the *relative* entropy maximization aspect of the MaxEnt approach. That is, using CAT constraints to inform predictions of species distributions requires that I start with a prior distribution that does not contain information about traits. The CAT information is used as a constraint to improve upon the prior distribution and develop a new distribution that in theory more accurately describes the relative abundance of species. The prior distribution cannot contain any information pertaining to the CAT constraints, but on the other hand does not necessarily have to be maximally uninformative. A maximally uninformative prior would involve each species being allocated an equal abundance so that there is uniformity among all of the species in the species pool. This uniform distribution is part of the default settings of the MaxEnt approach. While I did use this uniform prior distribution, I decided to also try another tactic in an attempt to further improve prediction results. I have information regarding the regional distribution of each species counted from an independent vegetation survey of the Gault Nature Reserve in 1996 (Bell *et al*. 2001), which can be used as a non-uniform prior distribution. For this survey the presence of each fern species was counted within each hectare of the Gault Nature Reserve (1014 hectares surveyed in total). To use the survey data in a non-uniform prior distribution I assigned each species a relative frequency based on the survey, and these values were taken to represent fern abundance in the regional pool relevant for all my 47 study plots (Table 1). These survey data satisfies the requirements of a prior distribution equally as well as the uniform prior, but contains more information than the uniform prior does. This extra information on the relative abundance of species in the regional pool can be used to complement the CAT constraints and potentially to improve predictions of species abundance using the MaxEnt approach.

Table 3. Relative frequency of the fern species in the species pool, as determined by a vegetation survey of each of the 1014 hectares in the Gault Nature Reserve on Mont-Saint-Hilaire, Quebec in 1996 (Bell *et al*. 2001). The survey scored presence or absence of fern species in each hectare of the reserve. Absolute frequency is the number of the hectares surveyed in the 10 km^2 reserve in which each species was found; relative frequency is the frequency of each species expressed as a proportion of the total number of the 21 fern species I am studying observed in the survey, a measure of a species contribution to the regional species pool of ferns.

Species	Relative Frequency (%)	Absolute Frequency
Dryopteris intermedia Muhlenberg ex Willdenow	14.02%	842
Dryopteris marginalis Linnaeus	13.79%	828
Polystichum acrostichoides Michaux	11.04%	663
Athyrium filix-femina Linnaeus	10.48%	629
Adiantum pedatum Linnaeus	8.68%	521
Dryopteris carthusiana Villars	6.76%	406
Botrychium virginianum Linneaus	4.71%	283
Polypodium virginianum Linnaeus	4.65%	279
Onoclea sensibilis Linnaeus	4.00%	240
Deparia acrostichoides Swartz	3.98%	239
Cystopteris fragilis Linnaeus	3.11%	187
Pteridium aquilinum Linnaeus	2.93%	176
Dennstaedtia punctilobula Michaux	2.50%	150
Gymnocarpium dryopteris Linnaeus	2.48%	149
Matteuccia struthiopteris Linnaeus	2.22%	133
Phegopteris connectilis Michaux	2.07%	124
Osmunda cinnamomea Linnaeus	1.18%	71
Woodsia ilvensis Linnaeus	1.03%	62
Phegopteris hexagonoptera Michaux	0.17%	10
Botrychium matricariifolium Döll	0.15%	9
Dryopteris clintoniana Eaton	0.05%	3

6. PERFORM A CIRCULAR TEST OF THE MAXENT APPROACH.

It is possible at this point to perform the same "circular" test that Shipley *et al*. (2006) did on their data. Shipley *et al*. (2006) computed community-aggregated trait values from observed species' abundances in their study sites, and then used these values to form predictions of species' abundances in the same sites. Testing the MaxEnt approach in this way involves three steps: i) predicting species abundance in all of the plots by fitting the MaxEnt approach to the CAT and prior distribution data using the "maxent" function, ii) comparing the predicted distributions with the observed ones using the inferential permutation test (maxent.test function) developed by Shipley (in press), and iii) working iteratively to improve the predictions by altering different components of the MaxEnt approach. The maxent and maxent.test functions are available through the 'FD' (functional diversity) package in R (Laliberté and Shipley 2010)

TEST 1: In my first attempt at the circular test I used the default settings that are included in the maxent function as well as the maxent.test function, as found in the 'FD' R package created by Etienne Laliberté and Bill Shipley (2010). These settings include a uniform prior distribution and a tolerance threshold of $1e^{-07}$ to determine convergence (maxent function), as well as 99 permutations for allowing inference at an alpha level of 0.05 (maxent.test function). I included 11 traits in this test (height, density, photosynthetic area, LMA, LDMC, Chl, N, C:N, A_{max}, wintergreen and sporulation) and the results showed that the MaxEnt approach did achieve a statistically significant prediction of species abundance (R^2=0.996, p=0.02; Figure 3a and 3b). Figure 3a illustrates the results on an arithmetic scale that suggests a useful predictive power; Figure 3b illustrates the results on a logarithmic scale that makes the weak predictions of low abundances more clear.

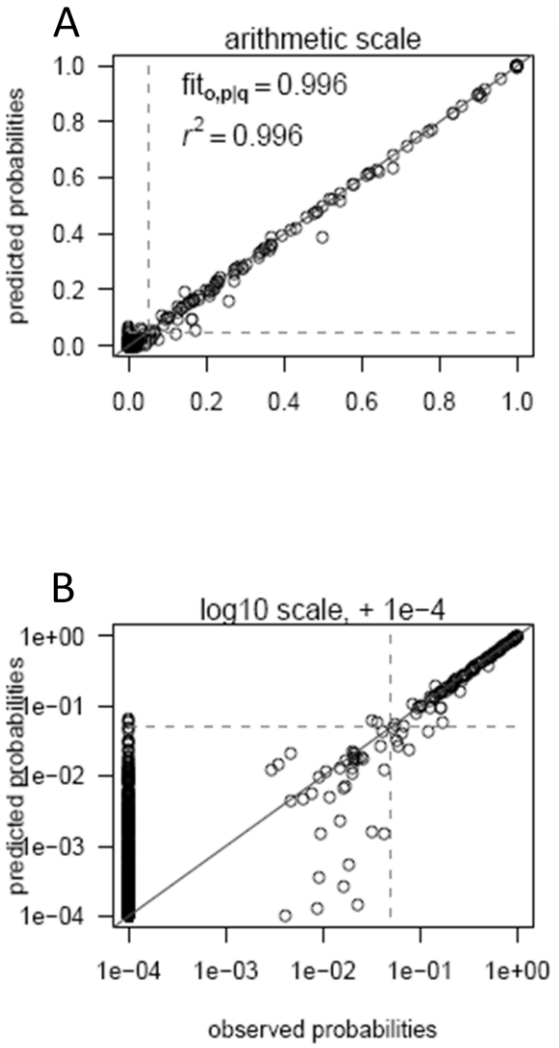

Figure 3a and 3b. Test 1 - results of a circular test using CATs of all 11 traits with a uniform prior and a convergence threshold of 1e^{-07} plotted on both arithmetic (top) and logarithmic (bottom) scales. Note that the logarithmic scale emphasizes the weak prediction of more rare species despite the overall significance of the prediction.

TEST 2: My second test was the same as the first except that I decreased the number of traits from 11 to 8, eliminating A_{max}, wintergreen and sporulation. I did this because I wanted to assess the degree to which the number of traits included had an effect on the results. I chose to exclude these three traits because A_{max} is a somewhat redundant trait in that similar information is conveyed through Chl, and the wintergreen and sporulation traits are categorical with the latter being based on a very broad description that might not be entirely accurate. The results of this test showed that the MaxEnt approach now did not accurately predict species abundance (R^2=0.878, p=1; Figure 4a and 4b)

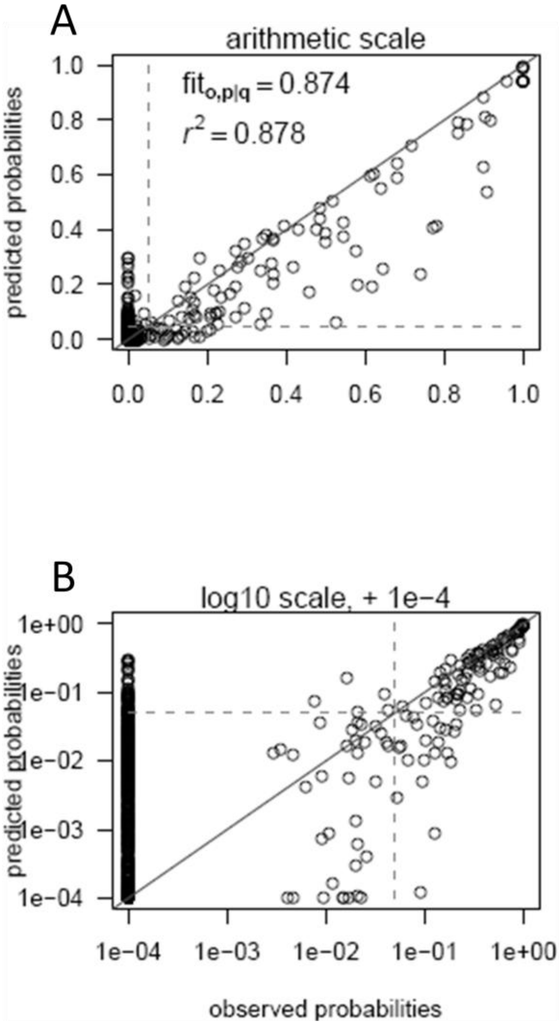

Figure 4a and 4b. Test 2 - results of a circular test using CATs of 8 traits (A_{max}, wintergreen and sporulation CATs were not included), a uniform prior and a convergence threshold of 1e^{-07}.

TEST 3: The maxent.test function includes an option to test if a subset of traits encodes information that is relevant to the prediction. This test showed that the three traits that I had eliminated on biological and methodological grounds were in fact very relevant to the abundance prediction (p=0.01), and the other eight traits combined less strongly so (p=0.06). This result led me to try testing the MaxEnt approach a third way, using only the three traits that I had originally removed (Amax, wintergreen, and sporulation), a tolerance threshold of e1^{-07}, and a uniform prior. The results proved to again be significant (R^2=0.748, p=0.01; Figure 5a and 5b), although with a good deal of scatter in the data.

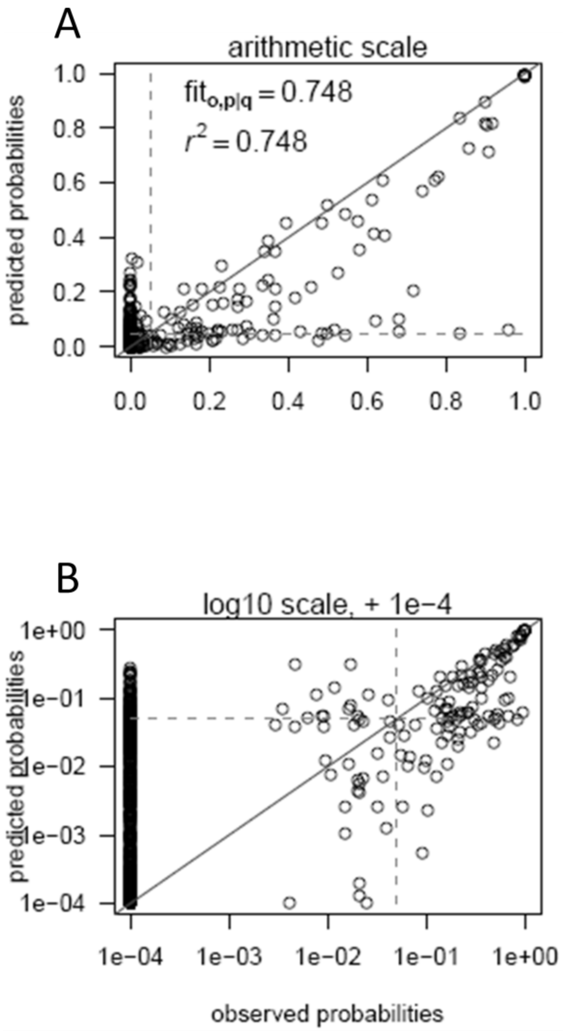

Figure 5a and 5b. Test 3 - results of a circular test using CATs of 3 traits (only A_{max}, wintergreen and sporulation CATs were included), a uniform prior and a convergence threshold of 1e^{-07}.

TEST 4: I also tried doing a test with only the two categorical traits (wintergreen and sporulation) and again the results proved to be significant (R^2=0.471, p=0.01; Figure 6a and 6b), although with a lot of scatter in the data.

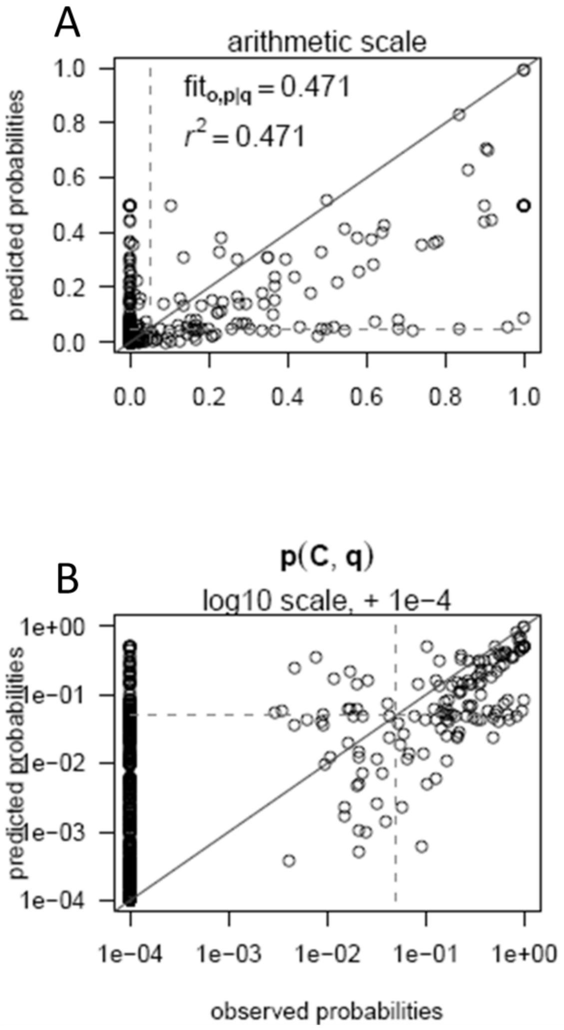

Figure 6a and 6b. Test 4 - results of a circular test using CATs of 2 traits (only wintergreen and sporulation CATs were included), a uniform prior and a convergence threshold of $1e^{-07}$.

TEST 5: My fifth test was the same as the second, for which I used CATs of eight traits and the results were not significant, except that I decreased the tolerance threshold to $1e^{-09}$. This had a dramatic effect on the results as the maxent.test function, which now showed that SADs were accurately predicted (R^2=0.962, p=0.02; Figure 7a and 7b).

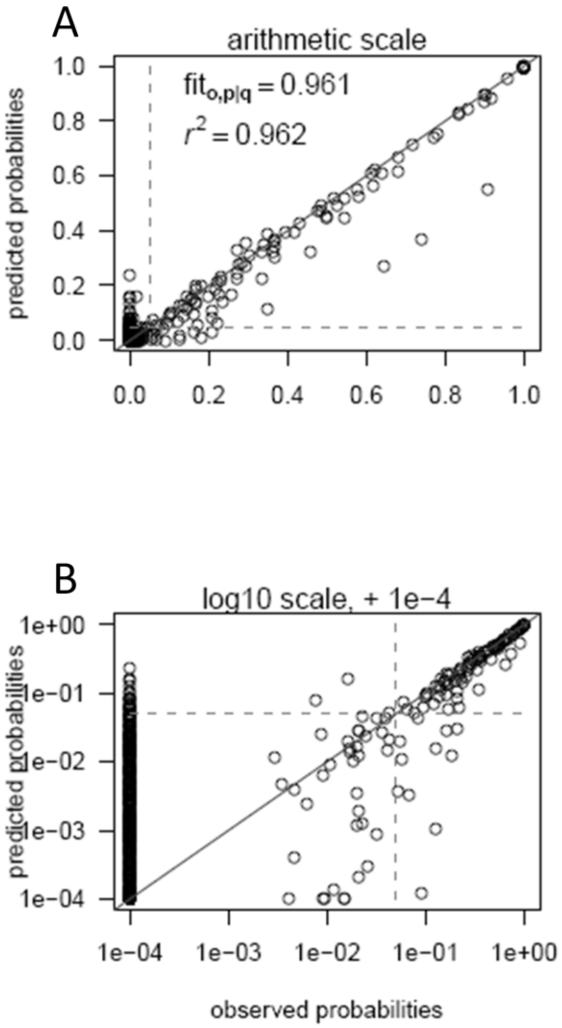

Figure 7a and 7b. Test 5 -- results of a circular test using CATs of 8 traits, a uniform prior and a convergence threshold of $1e^{-09}$.

TEST 6: In this circular test I decreased the tolerance threshold to 1e-^{09} and again included all 11 traits; the results were extremely significant (R^2=1, p=0.01; Figure 8a and 8b) and the apparent fit much better than in previous combinations of parameters, although the rarest species remain poorly predicted.

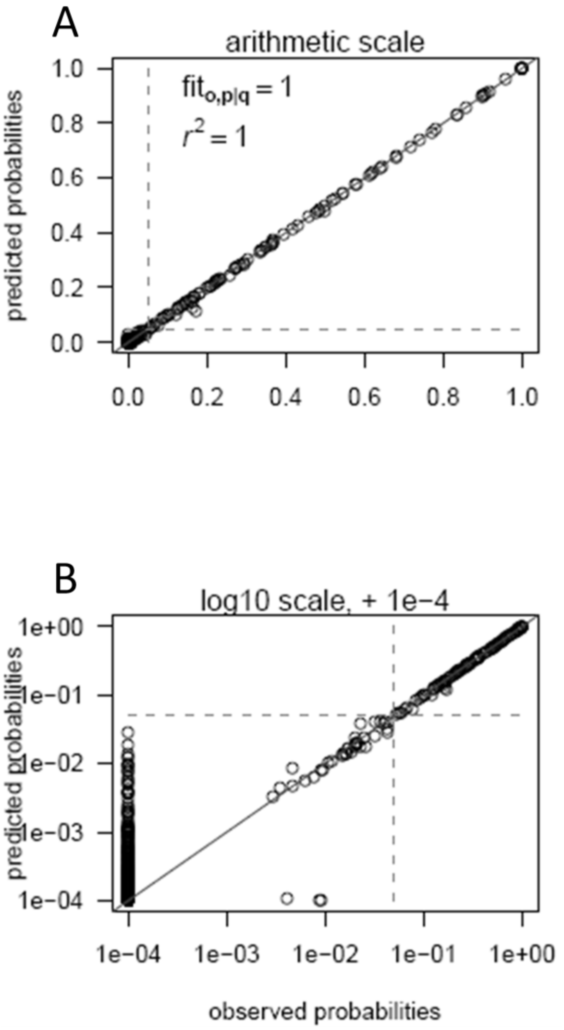

Figure 8a and 8b. Test 6 - results of a circular test using CATs of all 11 traits, a uniform prior and a convergence threshold of 1e^{-09}.

TEST 7: The seventh test was much like the sixth except that I used a non-uniform prior distribution based on the 1996 survey of fern distributions within the reserve (Bell *et al*. 2001). Again the results were extremely significant (R^2=1, p=0.017; Figure 9a and 9b), but predictions remain weak for the more rare species.

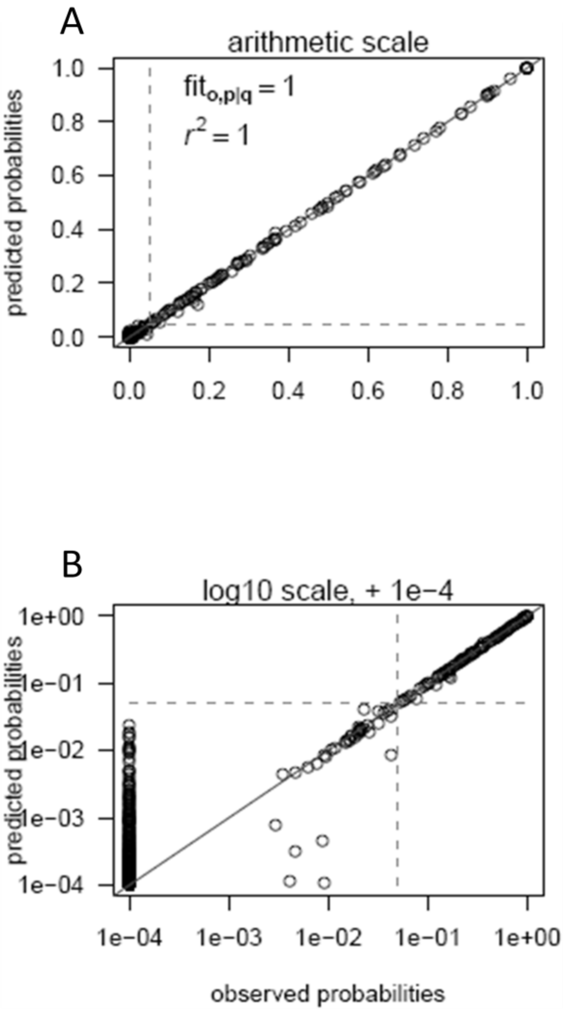

Figure 9a and 9b. Test 7 - results of a circular test using CATs of all 11 traits, a non-uniform prior and a convergence threshold of $1e^{-09}$.

The close to perfect predictive ability of these last two tests makes it unclear if using the non-uniform prior indeed makes any difference at all. Therefore I attempted one final circular test, using the same settings as in Test 2, which had not produced significant results (eight traits and a tolerance of $1e^{-07}$), but this time I used the non-uniform prior based on survey data. The results showed that while the prediction was still not accurate, it was improved by use of a more informative prior (R^2=0.965, p=0.857; Figure 10a and 10b)

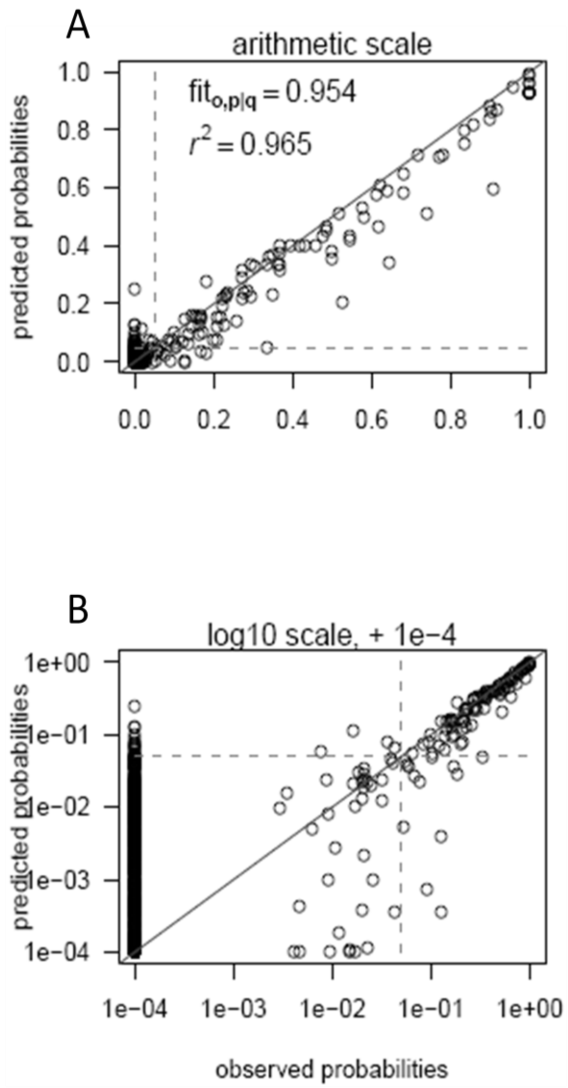

Figure 10a and 10b. Test 8 - results of a circular test using CATs of 8 traits (A_{max}, wintergreen and sporulation CATs were not included), a non-uniform prior and a convergence threshold of 1e^{-07}.

Through this series of circular tests I have confirmed that the MaxEnt approach can be fitted to my data. In other words, the circular application of the MaxEnt approach is useful in that it shows that trait-based environmental filtering plays a role in the assembly of fern communities in the 47 communities I am studying. The circular tests have also helped me decide how to optimize predictions by changing the various components of the MaxEnt approach. I am now ready to perform non-circular tests that are based on environmental gradients. These tests will determine if the MaxEnt approach can be used to predict relative abundance distributions in areas for which relative abundance has not already been measured, which is useful for application of the MaxEnt approach to conservation efforts.

7. PERFORM A NON-CIRCULAR TEST OF THE MAXENT APPROACH

Performing a non-circular test of the MaxEnt approach is very similar to performing a circular test, except extra measures had to be taken to avoid circularity when creating an environmental gradient from which CATs could be estimated.

I) CREATE AN ENVIRONMENTAL GRADIENT FROM MEASURED ENVIRONMENTAL VARIABLES, ALONG WHICH THE COMMUNITIES CAN BE PLACED AND FROM WHICH PREDICTION CONSTRAINTS (COMMUNITY-AGGREGATED TRAITS) CAN BE DEVELOPED IN TEST PLOTS.

In my test of the MaxEnt approach I created an environmental gradient underlying all of my study plots to circumvent the circularity that Shipley *et al*. (2006) were criticized for in the analysis of their data on a successional gradient (e.g. Marks and Muller-Landau 2007; Haegeman and Loreau 2008 and 2009). Although the circular application of the MaxEnt approach has some utility, the true predictive ability of the MaxEnt approach relies on its ability to be used to predict SADs from CATs that are estimated independently from environmental gradients, not observed relative abundance data. The environmental gradients I created to estimate CATs in test plots were based on observations made by Gilbert and Lechowicz (2004, unpublished data), whose study plots I am using in these analyses. The CAT values in my test plots were not based on observed data in those same plots but rather on estimates of trends in the CATs that were derived from a different set of calibration plots along the environmental gradient. The predicted SADs were not based on observed data, thereby circumventing the statistically questionable circularity involved when the same set of plots are used to both estimate the CATs and to test MaxEnt predictions of SADs in the plots. The process for creating an appropriate environmental gradient and using it in the estimation of a CAT has four parts: a) examine the environmental conditions in all of the plots and eliminate any outlying plots that might destabilize the analysis, b) divide the remaining plots into two subsets of "calibration" and "test" plots distributed along the selected environmental gradient, c) use GAMs to define mathematical relationships for any trends in the CATs along the selected environmental gradient in data for the calibration plots, and d) use these relationships to estimate CATs for the test plots distributed along the selected environmental gradient. The estimated CATs can then be used in a MaxEnt analysis to make statistically independent predictions of species abundance in the test plots.

a) Examine the environmental conditions in all of the plots and eliminate outliers if necessary

My first task in creating an environmental gradient was to examine the distribution of communities established by Ben Gilbert (Gilbert and Lechowicz 2004) in terms of habitat type and decide if outliers needed to be eliminated from my study. To do this I gathered the following environmental information that was measured in all 69 plots in 2002 and 2004: $\log_{10}$-transformed phosphorus, calcium, magnesium, potassium, and nitrate concentrations, pH, $\log_{10}$-transformed percentage of organic matter, median moisture level, and $\log_{10}$-transformed total light level. If environmental filtering does in fact drive the assembly of fern communities, then these environmental factors should be important to the process (Gilbert and Lechowicz 2004). After collating these data I had to eliminate 11 plots from my analysis because I combined the environmental data from both 2002 and 2004, and 11 of the 69 plots had only been surveyed in 2004. I took the median moisture value for each plot from the eight measurements that were taken in 2002 and 2004 combined (four measurements were made each year). I found the median value to be more informative than the mean value. I then averaged the environmental data from 2002 and 2004 to obtain single values for each of the other environmental factors I included, in each plot. I felt that the environmental conditions and species abundance data averaged over two years would provide a more robust database so I chose to work with the 58 plots sampled in both years rather than all 69 plots in only 2004.

I executed a principle component analysis (PCA) and identified ten of the remaining 58 plots as outliers (Figure 11). I decided to eliminate these ten outlying plots from the 58 surveyed in 2002 and 2004 on the grounds that they could cause a disjunct in any environmental gradient I created and they would lead me outside the limits of the alpha diversity level that the MaxEnt approach focuses on. With such a disjunct I would not be able to accurately relate the CATs to the environment. Fitting a model to the gradient and the observed CATs would be less accurate if I were to use the data as a whole rather fitting a model to the data *sans* outliers. Since I am relying on this mathematical relationship to estimate CATs in test plots and predict species' abundances in each plots, accuracy is very important.

Furthermore, I am testing if the MaxEnt approach is able to predict species abundance and distribution along environmental gradients that exist within one habitat, i.e. at the alpha diversity level. Although the Gault Nature Reserve is predominantly covered in an upland hardwood forest characterized by mineral soil, it does contain some small wetland areas that are characterized by more organic soils (Flinn *et al*. 2008). Seven of the outlying plots contained soil with a very high percentage of organic matter (>40% loss on ignition) and therefore represented organic soils more characteristic of a wetland habitat than an upland forest habitat. The definitions of organic and mineral soils are complex and involve knowledge of the thickness of the soil layer containing organic material, as well as the amount of time the soil is saturated with water; however soil material is considered to be organic if it contains more than 17% organic carbon, or approximately 30% organic matter, by weight (Canadian Soil Information Service, Chapter 2). As such, using a 40% organic matter cut-off point for distinguishing between organic and mineral soils and identifying outlying plots ensures that I only eliminate plots that are definitely not considered part of the upland forest habitat that the rest of the plots fall within.

Although the other three outlying plots contained less than 40 percent organic matter, they had very high or very low pH and/or nutrient concentrations relative to the rest of the plots (Figure 4). Including any of these extreme outliers could mean that I might end up examining species turnover across different habitat types, i.e at the beta rather than alpha diversity level. This would cause confusion regarding the drivers of community assembly since there can be differences in the environmental filtering process between alpha and beta diversity levels and the traits that define alpha and beta niches (Silvertown *et al*. 2006; Ackerly and Cornwell 2007; Morin and Lechowicz 2008). I therefore selected this subset of 48 plots to study because they arguably represent upland forest habitat and fall along a coherent, continuous axis of environmental variation.

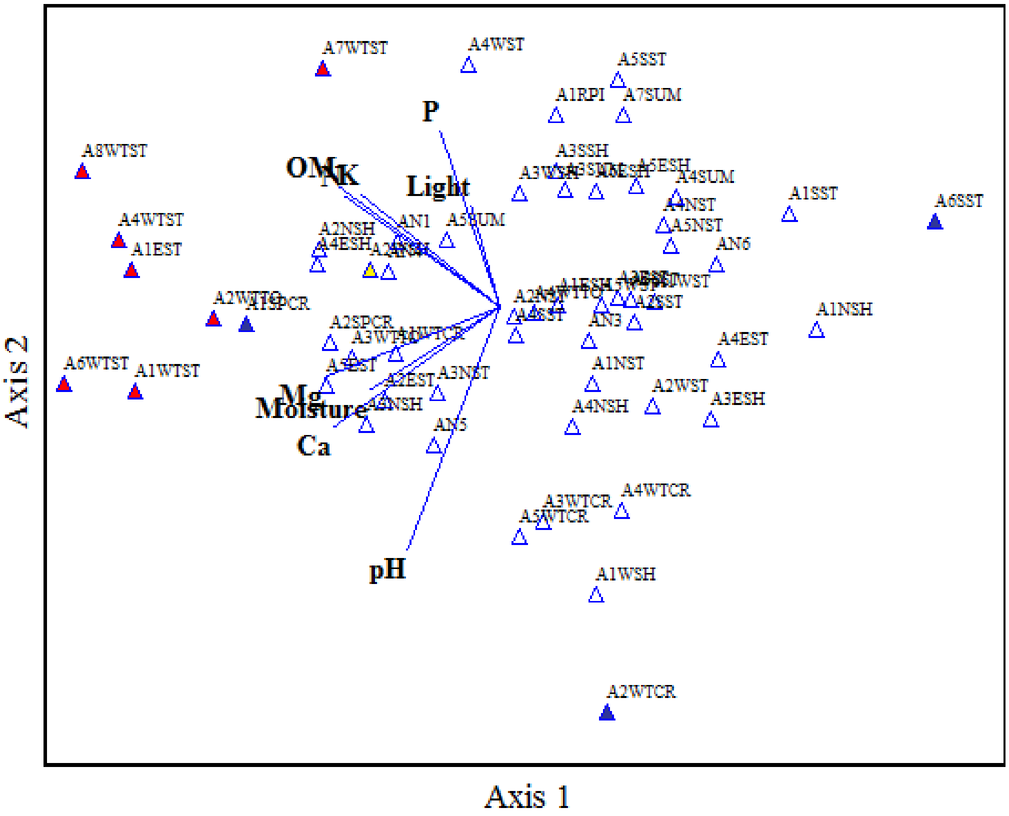

Figure 4. Principle component analysis of 58 plots surveyed by Ben Gilbert in 2002 (Gilbert and Lechowicz 2004) and resurveyed in 2004 in the Gault Nature Reserve, Mont-Saint-Hilaire, Quebec. The plots are characterized by the following environmental factors: $\log_{10}$-transformed phosphorus, calcium, magnesium, potassium, and nitrate concentrations, pH, $\log_{10}$-transformed percentage of organic matter, median moisture level, and $\log_{10}$-transformed total light level. Eliminated plots are filled (red=outlier with organic soil (>40% organic matter loss on ignition); blue=outlier with <40% organic matter; yellow=plot with no ferns present), while non-eliminated plots are not. The primary and secondary axes account for 47.78% and 20.27% of the variance, respectively.

Finally, I also had to eliminate one other plot because it did not contain any fern species at all (Figure 4). Although I would have liked to use it as a test plot to see if abundances would be predicted for this plot in a MaxEnt analysis, the R code written for the permutation test to determine the accuracy of predictions generated by the MaxEnt approach (Laliberté and Shipley 2010) includes restrictions against observed abundance values of zero. Hence, this "empty" plot had to be eliminated as well.

b) Divide the 47 plots into two subsets: "calibration" and "test" plots

With outlying plots removed, I had to divide the remaining 47 plots into a calibration group and a test group. The plots in the calibration group would be used to quantify trends between observed CATs and environmental gradients using GAMs. I would then use the relationships identified in the calibrations plots to estimate CATs and predict species abundance for the plots of the test group. I wanted to divide the plots in such a way that each group contained plots representing the full range of environmental conditions in all of the plots. I therefore had to array all of the plots along an environmental gradient before dividing them. In an attempt to reduce all of the environment data to a gradient with one dimension I did a principle component analysis for the 47 plots, as characterized by the nine environmental factors. The primary axis of this PCA accounts for 39.0% of the variance (Figure 12). I subsequently wrote a program in R that ordered all the plots in accordance to their place on the primary axis of this PCA (See Appendix 1 for the R code). My program then did a stratified random selection of plots across this gradient, making 35 of the plots (74.5%) calibration plots, and the other 12 plots (25.5%) test plots. I chose this distribution because I needed to ensure that the GAM I fit to the calibration plots was as accurate as the data would allow, while leaving sufficient test plots to assess the predictive power of the MaxEnt approach. If I used fewer calibration plots then the resulting relationship between the CATs and the environmental gradient would be less reliable, and estimations of CATs in the test plots would be less accurate. The random nature of this stratified selection of calibration versus test meant that the two groups were comprised of a different subset of plots each time I ran my program. Ideally I would have repeated these trials many times with different random sets of calibration and test plots, but limits on time and computational power forced me to limit the number of runs and to select representative examples to present and discuss.

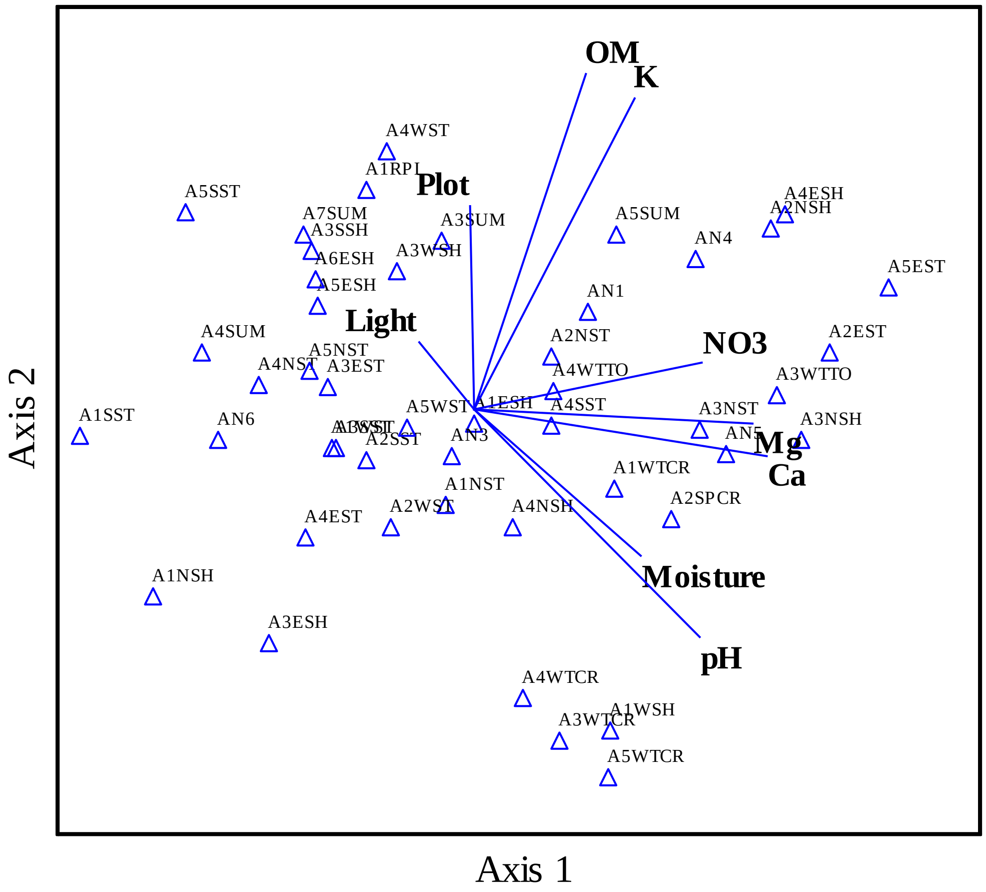

Figure 12. Principle component analysis of 47 permanent plots established by Ben Gilbert (Gilbert and Lechowicz 2004) across the Gault Nature Reserve in Mont-Saint-Hilaire, Quebec, as characterized by the following environmental factors: log10-transformed phosphorus, calcium, magnesium, potassium, and nitrate concentrations, pH, log10-transformed percentage of organic matter, median moisture level, and log10-transformed total light level. The primary axis accounts for 39.0% of the variance, and the secondary axis accounts for 23.0% of the variance.

c) Define mathematical relationships linking different environmental gradients to the CATs calculated from observed data in the calibration plots

In order to estimate CATs in the randomly selected subset of test plots so that I could predict SADs in these test plots, I had to define a relationship between

CATs and environmental data for the calibration plots. I chose to do so by fitting a generalized additive model (GAM) to the data. GAMs are quadratically penalized generalized linear models and the degree of smoothness they include is estimated as part of the fitting process (Wood 2010). A GAM is able to relate CATs and environmental data in a meaningful way because it strikes a balance between fitting the data closely and describing the general trend in the data. I wrote a program to create GAMs in R, using the gam() function in the mgcv package written by Wood (2010) (See Appendix 1 for the R code).

To accurately define a relationship between the CAT and environmental data in the calibration plots there has to exist a strong relationship between these variables in each plot, rather than a great deal of spread among the data points. The strength of this relationship is in part dependent on which environmental data I use, as each environmental factor is expected to have a different relevance to each trait. That is, changes among plant traits can be expected to correlate with changes in the environment in a manner that is unique to each combination of trait and environmental factor. I therefore struggled with choosing which environmental data I related to each CAT in my attempt to eventually develop a good estimate of the CATs in the test plots. I tried to resolve this problem via trial and error by generating GAMs relating the CATs of each trait to many different environmental gradients for all 47 plots. *A priori* I thought that the best GAM (i.e. the GAM showing the strongest relationship between the CATs and the environment) fitted to all 47 plots, would lead me to the best possible prediction of species abundance using the MaxEnt approach. To evaluate which GAM was best I looked at the adjusted R^2 and p-values that were generated, as well as the estimated degrees of freedom (EDF) and generalized cross-validation (GCV) score for each model. The adjusted R^2 takes account of the number and relative contributions of factors in fitting the GAM; a GAM may be fitted to a single environmental variable or to a number of multiple variables dependent on the number of calibration plots. I note that the adjusted R^2 for a GAM sometimes can be slightly negative if a multiple factor GAM is actually worse than a one parameter model.

Although it is possible in the MaxEnt approach to use a different environmental gradient to predict CAT values for each trait, I think it would be more useful from an application standpoint to use one gradient for all of the traits. Therefore in my first attempt to fit GAMs to the CAT and environment data I related CATs of each trait to all of the environmental factors at once by using the primary axis of the PCA I did for the 47 plots, which accounts for 39.0% of the variance in the environmental data (Figure 12). The observed trends illustrated by the GAMs varied in that the CATs of some traits had a relatively strong relationship with the environmental gradient (e.g. LMA: adjusted $R^2=0.247$, $p<0.05$; Figure 13), while others did not (e.g. height: adjusted $R^2=-0.0222$, $p>0.05$; Figure 14). Figures 1-13 in the Appendix 2 provide illustrations of all the GAMs based on univariate environmental gradients; I will only show selected examples in the main body of the text.

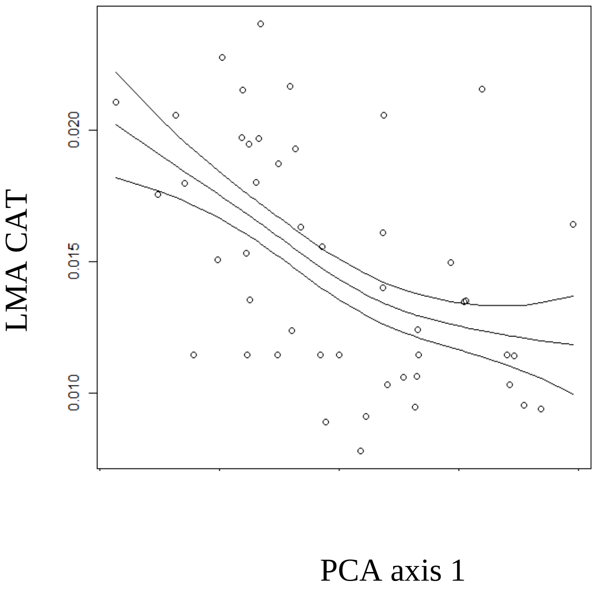

Figure 13. GAM fitted to the LMA community-aggregate trait and PCA1 data in all 47 plots, and the 95% confidence intervals. Adjusted $R^2=0.247$, $p<0.05$ EDF=1.93, GCV=$1.61e^{-05}$. There is a reasonable, although not particularly strong, trend in the LMA CAT on this synthetic environmental axis.

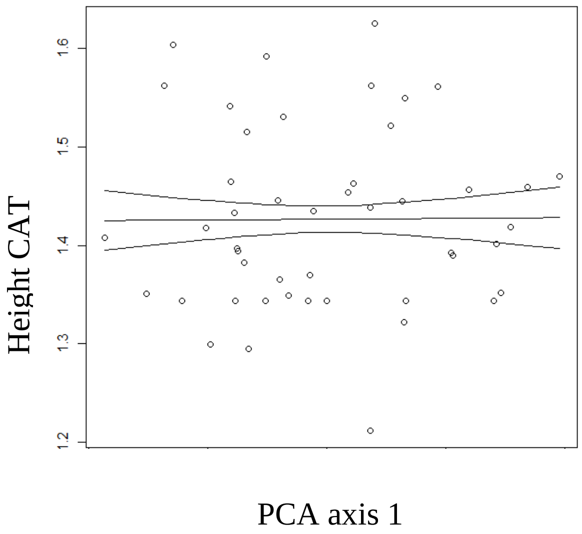

Figure 14. GAM fitted to the height community-aggregate trait and PCA1 data in the calibration plots, and the 95% confidence intervals. Adjusted R^2=-0.0222, $p>0.05$, EDF=1.00, GCV=0.009. There obviously is no trend in the height CAT on this synthetic environmental axis.

I then created GAMs using both the secondary and tertiary axes of the PCA (accounting for 23.0% and 15.7% of the variance, respectively) rather than the primary one, and noticed that the trends had potential to change drastically. For example, LMA did not have a significant relationship with either the secondary axis (adjusted R^2=0.0203, $p>0.05$) or the tertiary axis (adjusted R^2=-0.00904, $p>0.05$). Alternately, while height did not have a significant relationship with the secondary axis (adjusted R^2=-0.0198, $p>0.05$), it did have one with the tertiary axis (adjusted R^2=0.211, $p<0.05$). Table 4 summarizes all of the statistics regarding GAMs fitted to single PCA axes; yellow boxes indicate very significant relationships ($p<0.05$) and pink boxes indicate somewhat significant relationships ($0.5<p<0.1$); every table of the sort in this section follows this same color-coding scheme.

These results indicated that using one PCA axis as the environmental gradient to relate each CAT to may not be the best method. Any PCA axis I choose will not be significantly correlated to at least one of the CATs, and therefore fitting a GAM to the data will be meaningless in terms of using it to estimate CATs in test plots. This led me to attempt using a multivariate environmental gradient composed of all three PCA axes to fit the GAMs to all of the CATs (cf. Table 5 for summary statistics of these GAMs).

Table 4. Summary statistics for GAMs fitted to fern CATs and each axis of a PCA of the following environmental factors measured across the Gault Nature Reserve in Mont-Saint-Hilaire, Quebec: $\log_{10}$-transformed phosphorus, calcium, magnesium, potassium, and nitrate concentrations, pH, $\log_{10}$-transformed percentage of organic matter, median moisture level, and $\log_{10}$-transformed total light level.

CAT:		**LMA**	**LDMC**	**Chl**	**N**	**C:N**	**A_{max}**	**Height**	**Photosyn-thetic Area**	**Population Density**	**Wintergreen**	**Sporulation**
PCA1	**R^2**	0.247	0.311	0.214	0.297	0.364	0.270	-0.022	-0.019	0.138	0.213	-0.014
	p-value	0.002	0.002	0.007	0.001	0.000	0.002	0.957	0.719	0.049	0.032	0.559
	EDF	1.93	3.75	2.39	2.72	3.83	2.83	1.00	1.00	2.42	4.41	1.00
	GCV	0.000	0.002	0.008	0.001	0.002	0.007	0.009	0.040	0.001	0.135	1.640
PCA2	**R^2**	0.020	0.305	0.034	0.024	0.087	0.098	-0.020	-0.002	0.232	0.226	0.027
	p-value	0.410	0.001	0.458	0.456	0.085	0.190	0.744	0.343	0.003	0.000	0.140
	EDF	1.63	2.89	2.55	1.46	1.67	2.83	1.00	1.00	1.64	1.00	1.00
	GCV	0.000	0.002	0.010	0.002	0.002	0.008	0.009	0.039	0.001	0.123	1.570
PCA3	**R^2**	-0.009	-0.004	-0.013	-0.017	-0.021	-0.022	0.211	0.065	0.151	0.088	0.114
	p-value	0.447	0.366	0.515	0.631	0.847	0.985	0.001	0.047	0.004	0.025	0.012
	EDF	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	GCV	0.000	0.003	0.010	0.002	0.002	0.009	0.007	0.037	0.001	0.145	1.430
Maximum R^2:		**0.247**	**0.311**	**0.214**	**0.297**	**0.364**	**0.270**	**0.211**	**0.065**	**0.232**	**0.226**	**0.114**
Best Environmental gradient:		**PCA1**	**PCA1**	**PCA1**	**PCA1**	**PCA1**	**PCA1**	**PCA3**	**PCA3**	**PCA2**	**PCA2**	**PCA3**

Table 5. Summary statistics for GAMs fitted to fern CATs and a multivariate environmental gradient composed of the first three axes of a PCA of the following environmental factors measured across the Gault Nature Reserve in Mont-Saint-Hilaire, Quebec: $\log_{10}$-transformed phosphorus, calcium, magnesium, potassium, and nitrate concentrations, pH, $\log_{10}$-transformed percentage of organic matter, median moisture level, and $\log_{10}$-transformed total light level.

CAT:	**LMA**			**LDMC**			**Chl**		
GAM term:	PCA1	PCA2	PCA3	PCA1	PCA2	PCA3	PCA1	PCA2	PCA3
R^2:	0.249			0.478			0.176		
p-value:	0.003	0.522	0.302	0.001	0.000	0.324	0.009	0.478	0.716
EDF	1.430	1.469	1.000	1.446	1.952	1.658	1.489	1.000	1.230
GCV	0.000			0.002			0.008		
CAT:	**N**			**C:N**			**Amax**		
GAM term:	PCA1	PCA2	PCA3	PCA1	PCA2	PCA3	PCA1	PCA2	PCA3
R^2:	0.279			0.344			0.272		
p-value:	0.001	0.474	0.470	0.000	0.056	0.885	0.003	0.182	0.649
EDF	1.821	1.289	1.000	1.711	1.125	1.000	1.553	2.031	1.000
GCV	0.001			0.002			0.007		
CAT:	**Height**			**Photosynthetic Area**			**Population Density**		
GAM term:	PCA1	PCA2	PCA3	PCA1	PCA2	PCA3	PCA1	PCA2	PCA3
R^2:	0.368			0.325			0.498		
p-value:	0.765	0.122	0.000	0.791	0.015	0.040	0.001	0.000	0.000
EDF	1.000	7.497	1.331	1.000	8.394	1.234	1.000	1.000	1.000
GCV	0.007			0.034			0.001		
CAT:	**Wintergreen**			**Sporulation**					
GAM term:	PCA1	PCA2	PCA3	PCA1	PCA2	PCA3			
R^2:	0.429			0.139					
p-value:	0.004	0.000	0.005	0.542	0.257	0.010			
EDF	1.000	1.000	1.000	1.000	1.263	1.000			
GCV	0.095			1.464					

The results in Table 5 suggest that GAMs fitted to a multivariate PCA axis gradient may not be very accurate since not all of the terms in these GAMs show significant correlations between the CAT and environment data. Therefore I decided that I should also try using a different environmental gradient for each trait to see if that would improve the fit of the GAMs, despite the fact that this approach is more complicated than using a single environmental gradient in terms of the applying the MaxEnt approach. To choose which environmental gradient I used for each trait, I fit a GAM to each CAT and each environmental factor separately for all 47 plots and determined which univariate gradient had the best relationship with each CAT in terms of adjusted R^2, p-value, EDF and GCV. At this point I shifted my focus away from the PCA axes because I decided it would be more beneficial to those who wish to apply the MaxEnt approach to simply measure a few key environmental factors rather than a whole slew of them in order to create a PCA. I therefore separated the first three axes of the PCA from the rest of the environmental factors in this analysis (Table 6); see Figures 1-13 in the appendix for illustrations of these univariate GAMs for all CATs.

Table 6a. Summary statistics for GAMs fitted to fern CATs and environmental factors measured across the Gault Nature Reserve in Mont-Saint-Hilaire, Quebec.

CAT:		**LMA**	**LDMC**	**Chl**	**N**	**C:N**	**A_{max}**
NO_3^-	**R^2**	0.190	0.067	0.094	0.171	0.131	0.210
	p-value	0.001	0.044	0.021	0.002	0.007	0.001
	EDF	1.00	1.00	1.00	1.00	1.00	1.00
	GCV	0.000	0.003	0.009	0.001	0.002	0.007
P	**R^2**	0.153	0.055	0.132	0.044	0.068	0.038
	p-value	0.043	0.267	0.084	0.272	0.177	0.318
	EDF	2.33	1.95	2.43	1.83	1.98	1.89
	GCV	0.000	0.003	0.009	0.002	0.002	0.009
K	**R^2**	-0.007	0.322	-0.021	-0.020	-0.014	0.003
	p-value	0.725	0.012	0.784	0.736	0.888	0.605
	EDF	1.46	7.97	1.00	1.00	1.23	1.61
	GCV	0.000	0.002	0.010	0.002	0.002	0.009
Ca	**R^2**	0.261	0.292	0.198	0.280	0.327	0.278
	p-value	0.011	0.003	0.022	0.005	0.001	0.004
	EDF	4.76	3.36	3.06	4.22	4.03	3.77
	GCV	0.000	0.002	0.008	0.001	0.002	0.007
Mg	**R^2**	0.194	0.189	0.212	0.200	0.252	0.159
	p-value	0.001	0.001	0.001	0.001	0.000	0.007
	EDF	1.00	1.00	1.00	1.00	1.00	1.47
	GCV	0.000	0.002	0.008	0.001	0.000	0.008
pH	**R^2**	0.115	0.347	0.128	0.299	0.348	0.209
	p-value	0.054	0.000	0.079	0.007	0.001	0.005
	EDF	1.58	1.83	2.76	5.31	4.22	1.80
	GCV	0.000	0.002	0.009	0.001	0.002	0.007
Moisture	**R^2**	0.098	0.282	0.130	0.085	0.175	0.060
	p-value	0.018	0.000	0.007	0.053	0.002	0.054
	EDF	1.00	1.00	1.00	1.14	1.00	1.00
	GCV	0.000	0.002	0.008	0.002	0.002	0.008
% Organic Matter	**R^2**	0.105	0.220	0.068	-0.008	-0.022	-0.012
	p-value	0.286	0.051	0.449	0.431	0.858	0.498
	EDF	4.79	5.33	4.48	1.00	1.00	1.00
	GCV	0.000	0.003	0.010	0.002	0.002	0.009
Light	**R^2**	0.138	-0.011	0.067	0.005	-0.009	0.055
	p-value	0.140	0.488	0.459	0.276	0.448	0.473
	EDF	4.52	1.00	3.96	1.00	1.00	4.11
	GCV	0.000	0.003	0.010	0.002	0.002	0.009
Best Environmental gradient:		**Ca**	**pH**	**Mg**	**pH**	**pH**	**Ca**

Table 6a. continued

CAT:		**Height**	**Photosynthetic Area**	**Population Density**	**Wintergreen**	**Sporulation**
NO_3^-	**R^2**	0.049	0.004	-0.001	-0.020	0.014
	p-value	0.073	0.281	0.329	0.763	0.209
	EDF	1.00	1.00	1.00	1.00	1.00
	GCV	0.008	0.039	0.001	0.162	1.590
P	**R^2**	0.018	0.024	-0.019	0.021	0.225
	p-value	0.562	0.516	0.720	0.166	0.007
	EDF	2.45	2.64	1.00	1.00	2.51
	GCV	0.009	0.040	0.001	0.155	1.290
K	**R^2**	-0.022	-0.021	0.155	0.026	-0.003
	p-value	0.895	0.781	0.029	0.304	0.352
	EDF	1.00	1.00	2.23	1.20	1.00
	GCV	0.009	0.040	0.001	0.155	1.620
Ca	**R^2**	-0.022	-0.022	0.173	0.139	-0.020
	p-value	0.967	0.859	0.023	0.050	0.733
	EDF	1.00	1.00	2.71	2.73	1.00
	GCV	0.009	0.040	0.001	0.142	1.650
Mg	**R^2**	0.024	0.032	0.122	0.180	-0.021
	p-value	0.151	0.120	0.009	0.002	0.842
	EDF	1.00	1.00	1.00	1.00	1.00
	GCV	0.009	0.038	0.001	0.130	1.650
pH	**R^2**	-0.022	0.010	0.369	0.313	-0.019
	p-value	0.996	0.538	0.000	0.000	0.703
	EDF	1.00	1.63	2.67	2.19	1.00
	GCV	0.009	0.039	0.001	0.112	1.640
Moisture	**R^2**	0.059	0.046	0.334	0.247	-0.011
	p-value	0.078	0.080	0.000	0.000	0.488
	EDF	1.31	1.00	2.85	1.00	1.00
	GCV	0.008	0.037	0.001	0.120	1.630
% Organic Matter	**R^2**	0.077	0.029	0.241	0.119	0.064
	p-value	0.307	0.064	0.024	0.137	0.271
	EDF	3.29	2.99	4.76	3.53	2.67
	GCV	0.009	0.040	0.001	0.148	1.570
Light	**R^2**	0.234	0.172	0.022	0.056	0.105
	p-value	0.018	0.056	0.160	0.322	0.015
	EDF	4.07	3.53	1.00	2.66	1.00
	GCV	0.007	0.034	0.001	0.156	1.440
Best Environmental gradient:		**Light**	**Light**	**pH**	**pH**	**P**

Table 6b. Summary statistics for GAMs fitted to CATs and the first three axes of a PCA done of the 47 plots permanent plots established by Ben Gilbert (Gilbert and Lechowicz 2004) across the Gault Nature Reserve in Mont-Saint-Hilaire, Quebec, as characterized by the following environmental factors: $\log_{10}$-transformed phosphorus, calcium, magnesium, potassium, and nitrate concentrations, pH, $\log_{10}$-transformed percentage of organic matter, median moisture level, and $\log_{10}$-transformed total light level.

CAT:		**LMA**	**LDMC**	**Chl**	**N**	**C:N**	**A_{max}**
PCA1	**R^2**	0.247	0.311	0.214	0.297	0.364	0.270
	p-value	0.002	0.002	0.007	0.001	0.000	0.002
	EDF	1.93	3.75	2.39	2.72	3.83	2.83
	GCV	0.000	0.002	0.008	0.001	0.002	0.007
PCA2	**R^2**	0.020	0.305	0.034	0.024	0.087	0.098
	p-value	0.410	0.001	0.458	0.456	0.085	0.190
	EDF	1.63	2.89	2.55	1.46	1.67	2.83
	GCV	0.000	0.002	0.010	0.002	0.002	0.008
PCA3	**R^2**	-0.009	-0.004	-0.013	-0.017	-0.021	-0.022
	p-value	0.447	0.366	0.515	0.631	0.847	0.985
	EDF	1.00	1.00	1.00	1.00	1.00	1.00
	GCV	0.000	0.003	0.010	0.002	0.002	0.009
Best Environmental gradient:		**Ca**	**pH**	**PCA1**	**pH**	**PCA1**	**Ca**

Table 6b continued

CAT:		**Height**	**Photosynthetic Area**	**Population Density**	**Wintergreen**	**Sporulation**
PCA1	**R^2**	-0.022	-0.019	0.138	0.213	-0.014
	p-value	0.957	0.719	0.049	0.032	0.559
	EDF	1.00	1.00	2.42	4.41	1.00
	GCV	0.009	0.040	0.001	0.135	1.640
PCA2	**R^2**	-0.020	-0.002	0.232	0.226	0.027
	p-value	0.744	0.343	0.003	0.000	0.140
	EDF	1.00	1.00	1.64	1.00	1.00
	GCV	0.009	0.039	0.001	0.123	1.570
PCA3	**R^2**	0.211	0.065	0.151	0.088	0.114
	p-value	0.001	0.047	0.004	0.025	0.012
	EDF	1.00	1.00	1.00	1.00	1.00
	GCV	0.007	0.037	0.001	0.145	1.430
Best Environmental gradient:		**Light**	**Light**	**pH**	**pH**	**P**

The summary statistics of the GAMs based on univariate environmental gradients (Table 6) showed that certain gradients are significantly and consistently correlated to many CATs, e.g. NO_3^-, Ca, Mg, pH, and moisture. I decided to again try using one environmental gradient for all of the CATs in a GAM, based on these seemingly most important environmental factors. I first tried a univariate environmental gradient and chose which environmental factor to include based on previous research on the ecology of ferns. Edgar Wherry (1927) demonstrated that the level of acidity in soil is particularly important to the distribution of many ferns present in Eastern North America. This suggests that pH and calcium are important factors in the assembly of fern communities as calcium strongly buffers soil pH levels (Bache 1984). Furthermore, pH and calcium significantly correlate with the majority of CATs in my dataset. As such I performed a PCA to collapse pH and Ca into a single value estimating a gradient from less to more acid soils. I assigned this pH+Ca value to each plot according to the plot's location on the primary axis of this PCA, which accounted for 89% of the variance in the data. I fit GAMs to this gradient and the CATs of each trait (cf. Table 7 and Figure 14 in Appendix 2 for details on the summary statistics and illustrations of these GAMs).

Table 7. Summary statistics for GAMs fitted to each CAT and an environmental gradient composed of a single pH+Ca composite factor based on measurements made across the Gault Nature Reserve in Mont-Saint-Hilaire, Quebec.

CAT:	**LMA**	**LDMC**	**Chl**	**N**	**C:N**	**A_{max}**
R$^{2:}$	0.260	0.421	0.205	0.228	0.324	0.338
p-value:	0.018	0.000	0.023	0.000	0.001	0.003
EDF:	5.198	5.046	3.355	1.000	3.066	5.634
GCV	0.000	0.002	0.008	0.001	0.002	0.007
CAT:	**Height**	**Photosynthetic Area**	**Population Density**	**Winter-green**	**Sporu-lation**	
R$^{2:}$	-0.022	0.136	0.532	0.301	-0.019	
p-value:	0.980	0.158	0.000	0.004	0.702	
EDF:	1.000	4.811	7.518	4.648	1.000	
GCV	0.009	0.001	0.037	0.121	1.644	

It is apparent from Table 7 that not all of the CATs have a significant relationship with this composite measure of soil base status, i.e. the height, photosynthetic area and sporulation CATs are not significantly correlated to this pH+Ca gradient. Table 6 indicates that the height CAT is only significantly correlated to the light gradient (p=0.018), the photosynthetic area CAT is not very significantly correlated to any gradient, but has the strongest relationship with light (p=0.056), and the sporulation CAT is only correlated to the P and light gradient. As such I decided to use an environmental gradient composed of multiple environmental factors that are collectively significantly correlated to all of the CATs. Unfortunately it became apparent that the estimated degrees of freedom (EDF) available in my data set limited the number of environmental factors I could use to approximately three depending on the specific variables I incorporated into the GAM and the amount of “wiggliness” they introduced to the GAM. I therefore limited this environmental gradient to include a maximum of three environmental factors. I included the composite pH-Ca factor because of the well recognized ecological importance of soil acidity to fern distribution, as well as the apparent significant relationship of this composite factor to many of the CATs in my dataset (Table 7). I chose to include NO_3^- and total light as the other two environmental factors in the trivariate environmental gradient because NO_3^- significantly correlates with six of the eleven CATs, and light correlates with the CATs that do not significantly correlate with pH, Ca, or NO_3^- (Table 6). The summary statistics of the GAMs fitted to this trivariate environmental gradient and all of the CATs can be found in Table 8.

Table 8. Summary statistics for GAMs fitted to each CAT and a trivariate environmental gradient composed of the pH+Ca composite factor, total light and nitrate, which were measured across the Gault Nature Reserve in Mont-Saint-Hilaire, Quebec.

CAT:	**LMA**			**LDMC**			**Chl**		
GAM term:	pH+Ca	Light	NO_3^-	pH+Ca	Light	NO_3^-	pH+Ca	Light	NO_3
p-value:	0.187	0.153	0.158	8.43E-05	0.308	0.314	0.142	0.216	0.226
edf:	3.250	4.469	1.000	5.318	3.860	4.201	3.314	4.151	1.000
$R^{2:}$	0.363			0.527			0.302		
CAT:	**N**			**C:N**			**A_{max}**		
GAM term:	pH+Ca	Light	NO_3^-	pH+Ca	Light	NO_3^-	pH+Ca	Light	NO_3^-
p-value:	0.025	0.709	0.249	0.014	0.578	0.812	0.055	0.473	0.236
edf:	1.000	3.190	1.000	2.550	3.226	1.350	3.472	4.055	4.289
$R^{2:}$	0.257			0.333			0.414		
CAT:	**Height**			**Photosynthetic Area**			**Population Density**		
GAM term:	pH+Ca	Light	NO_3^-	pH+Ca	Light	NO_3^-	pH+Ca	Light	NO_3^-
p-value:	0.248	0.077	0.221	0.152	0.269	0.328	0.000	0.064	0.072
edf:	1.000	3.322	1.000	4.576	2.772	2.731	6.891	1.000	6.140
$R^{2:}$	0.210			0.267			0.645		
CAT:	**Wintergreen**			**Sporulation**					
GAM term:	pH+Ca	Light	NO_3^-	pH+Ca	Light	NO_3^-			
p-value:	0.000	0.281	0.032	0.306	0.017	0.541			
edf:	4.383	1.000	3.177	6.804	1.000	1.000			
$R^{2:}$	0.462			0.210					

The trivariate environmental gradient of pH-Ca, total light and NO_3^- appears to not be significantly correlated to many of the CATs (LMA, Chl, A_{max}, height and photosynthetic area), cf. Table 8. Closer examination of the summary statistics for the GAMs based on this environmental gradient reveals that the NO_3^- term is not significantly related to any of the CATs. This information led me to try one last type of GAM using a single multivariate gradient for all of the CATs that is based on the composite pH-Ca factor, total light, and the interaction between these two factors. Table 9 gives the summary statistics for these GAMs, and Figure 14 in Appendix 2 provides illustrations for the relationships.

Table 9. Summary statistics for GAMs fitted to each CAT and an environmental gradient composed of a pH+Ca composite factor, light, and the interaction between these two factors, based on measurements made across the Gault Nature Reserve in Mont-Saint-Hilaire, Quebec.

CAT:	**LMA**			**LDMC**			**Chl**		
GAM term:	pH+Ca	Light	Inter-action	pH+Ca	Light	Inter-action	pH+Ca	Light	Inter-action
R^{2}:	0.494			0.541			0.415		
p-value:	0.393	0.782	0.77	0.006	0.869	0.125	0.205	0.678	0.01
edf:	3.223	3.307	6.015	5.964	1	5.016	3.555	1.000	6.398
CAT:	**N**			**C:N**			**Amax**		
GAM term:	pH+Ca	Light	Inter-action	pH+Ca	Light	Inter-action	pH+Ca	Light	Inter-action
R^{2}:	0.618			0.560			0.540		
p-value:	0.985	0.487	0.019	0.616	0.341	0.023	0.859	0.513	0.217
edf:	1.742	1.957	16.46	1.000	1.000	15.65	1	5.031	6.451
CAT:	**Height**			**Photosynthetic Area**			**Population Density**		
GAM term:	pH+Ca	Light	Inter-action	pH+Ca	Light	Inter-action	pH+Ca	Light	Inter-action
R^{2}:	0.235			0.206			0.740		
p-value:	0.198	0.012	0.239	0.062	0.024	0.086	0.009	0.796	0.251
edf:	1.000	4.185	1.000	1	3.64	1	8.278	4.589	7.136
CAT:	**Wintergreen**			**Sporulation**					
GAM term:	pH+Ca	Light	Inter-action	pH+Ca	Light	Inter-action			
R^{2}:	0.333			0.117					
p-value:	0.036	0.465	0.564	0.147	0.007	0.114			
edf:	1.000	1.708	2.726	1.000	1.000	1.000			

At this point I have generated GAMs using one univariate environmental gradient for all of the CATs (cf. Table 4 and 7) as well as one multivariate environmental gradient for all of the CATs (cf. Table 5, 8 and 9). I have also used univariate environmental gradients that are unique to each CAT (cf. Table 6). The next logical step is to use multivariate gradients that are unique to each CAT, as some traits have strong relationships with multiple environmental factors. This approach is ecologically plausible as it is clear from my data that multiple environmental factors affect community-aggregated traits and that each trait is affected by different factors; this is also a statistical strategy to obtain the best possible MaxEnt predictions. By using different environmental gradients composed of multiple factors that are significantly correlated to each CAT I believe that I am maximizing the fit of the GAMs used to predict CATs in the test plots and thus I am maximizing the potential for an accurate SAD prediction. This approach is the most complicated in terms of applicability of the MaxEnt approach, but it also provides the best opportunity for good predictions. I therefore decided to perform a stepwise multiple linear regression using Akaike information criterion (AIC) to determine which combination of environmental factors have the greatest impact on each CAT. An AIC attempts to find a model that best explains the data with the fewest free parameters possible. Once the environmental factors most affecting each CAT were selected (Table 10) it became clear that I could not use this information to develop GAMs fitted to these data because I was again limited by the estimated degrees of freedom and forced to include only a few environmental factors. I therefore created environmental gradients comprised of the three best environmental factors, including PCA axes, based on the summary statistics shown in Table 6. Table 11 gives results for the best three environmental factors for each CAT, and the summary statistics of the associated GAMs.

Table 10. Results of a stepwise multiple linear regression using Akaike information criterion (AIC) to determine which combination of environmental factors have the greatest impact on each CAT. Checkmarks indicate environmental factors that were included in the final model for each CAT.

	NO_3	**P**	**K**	**Ca**	**Mg**	**pH**	**Moisture**	**% Organic Matter**	**Light**	**PCA1**	**PCA2**	**PCA3**
LMA	✓		✓			✓	✓	✓	✓	✓	✓	✓
LDMC	✓	✓		✓	✓			✓			✓	
Chl	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	
N	✓		✓	✓	✓	✓	✓	✓	✓		✓	✓
C:N	✓		✓	✓	✓	✓	✓	✓	✓		✓	✓
A_{max}			✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Height					✓					✓		✓
Photosynthetic Area	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
Population Density			✓			✓	✓	✓			✓	
Wintergreen	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Sporulation	✓		✓	✓	✓	✓	✓		✓	✓	✓	✓

Table 11. Summary statistics for GAMs fitted to each CAT and a trivariate environmental gradient composed of best three environmental factors for each CAT, which were measured across the Gault Nature Reserve in Mont-Saint-Hilaire, Quebec.

CAT:	**LMA**			**LDMC**			**Chl**		
GAM term:	Ca	PCA1	Mg	pH	K	PCA2	Mg	PCA1	Ca
R^2:	0.231			0.465			0.254		
p-value:	0.494	0.303	0.738	0.031	0.211	0.176	0.140	0.422	0.301
EDF	1.602	1.369	1.000	3.249	1.000	3.202	1.000	1.000	2.845
GCV	0.000			0.002			0.008		
CAT:	**N**			**C:N**			**Amax**		
GAM term:	pH	PCA1	Ca	PCA1	pH	Ca	Ca	PCA1	NO_3^-
R^2:	0.613			0.448			0.332		
p-value:	0.003	0.110	0.016	0.092	0.063	0.719	0.843	0.204	0.993
EDF	8.053	1.000	6.044	3.681	2.346	1.000	1.000	3.675	1.000
GCV	0.001			0.001			0.002		
CAT:	**Height**			**Photosynthetic Area**			**Population Density**		
GAM term:	Light	PCA3	Moisture	PCA3	Light	Moisture	pH	Moisture	OM
R^2:	0.250			0.197			0.571		
p-value:	0.387	0.306	0.544	0.711	0.124	0.119	0.001	0.032	0.202
EDF	3.310	1.000	1.000	1.000	3.532	1.000	1.000	2.551	5.001
GCV	0.007			0.035			0.001		
CAT:	**Wintergreen**			**Sporulation**					
GAM term:	pH	Moisture	PCA2	P	PCA3	Light			
R^2:	0.395			0.210					
p-value:	0.163	0.034	0.094	0.013	0.543	0.125			
EDF	1.827	1.000	1.000	1.000	2.619	1.000			
GCV	0.102			1.386					

The summary statistics in Table 11 indicate that this trivariate environmental gradient approach may not necessarily yield the best predictions using the MaxEnt approach, as there is an apparent lack of significant correlation between the gradients and the CATs. At this point and in light of approaching thesis submission deadlines, I concluded that I have tried enough different possible approaches to fitting GAMs to my data to provide a reasonably thorough test of the MaxEnt approach with my dataset.

So far I have only been describing my efforts to fit a suitable GAM to the data in all 47 plots for each approach, but the ultimate goal was really to fit a GAM to the data in only the calibration plots and then estimate CATs in independent test plots. I have been using all 47 plots to better assess the statistical behaviour of the GAMs, but to actually test the MaxEnt approach I must fit a GAM to the selected CAT and environmental data in randomly selected calibration plots for each of the approaches that I outlined above. With the resulting independently estimated CAT-GAM relationships I can move forward to the next step in my attempt to circumvent circularity in the MaxEnt approach: estimating CATs in the test plots. Although the preceding results suggest the GAM-CAT relationships may be too weak in my data set to support good noncircular predictions, I nonetheless proceeded to complete the program I initially had planned to fully test the predictive capacity of the MaxEnt approach.

iv) Use the GAMs fitted to the CAT and environmental data in the calibration plots to estimate CATs in the test plots.

After going through the process of choosing which environmental gradients to use and fitting GAMs to the data in the calibration plots, I used the calibration GAM in conjunction with the environmental data in the test plots to estimate what the CATs were expected to be in the test plots. This procedure was written in R, using the predict.gam() function in the mgcv package written by Wood (2010) (See Appendix 1 for the R code). I estimated CATs for the test plots using all seven of the environmental gradients I described in the previous section: 1) the primary axis of the PCA, 2) the first three axes of the PCA, 3) the best environmental factor for each CAT, 4) a pH+Ca composite factor, 5) pH+Ca, total light and nitrate, 6) pH+Ca, total light and the interaction of these two terms, and 7) the best three environmental factors for each CAT. By developing CATs based on an environmental gradient rather than on observed data, I was prepared to move to steps ii, iii, and iv in performing a non-circular test of the MaxEnt approach.

7. PERFORM A NON-CIRCULAR TEST OF THE MAXENT APPROACH (CONT'D)

A true test of the MaxEnt approach is a non-circular one in which SADs are predicted from CATs estimated from an environmental gradient, rather than from CATs entirely based on observed data. After the step of estimating CATs in the test plots I was able to perform this non-circular test in a manner similar to the circular test by executing steps ii, iii, and iv in the procedure I outlined earlier: ii) predict SADs in the test plots by using the maxent function in R to fit the MaxEnt approach to the estimated CATs and the prior distribution for the test plots, iii) compare the predicted distributions with the observed ones using the inferential permutation test (maxent.test function) developed by Shipley (in press), and iv) work to improve the predictions by altering different components of the MaxEnt approach.

In the previous section I came up with seven different ways of creating environmental gradients to estimate CATs in the test plots, which I used to perform non-circular tests using all 11 traits. I had already discovered that a low tolerance threshold to determine convergence was important to the significance of the results, so I used a tolerance threshold of $1e^{-11}$ in all of my tests. I also used the prior distribution based on the vegetation survey data rather than the uniform prior distribution for these tests.

TEST 1: The first non-circular test used test plot CATs that were estimated from an environmental gradient composed of the primary axis of the

PCA of all 47 plots. In this test the MaxEnt approach proved unable to accurately predict species abundance in the test plots (R^2=0.249, p=1; Figure 15). The statistics are non-significant and fit to the data clearly deficient.

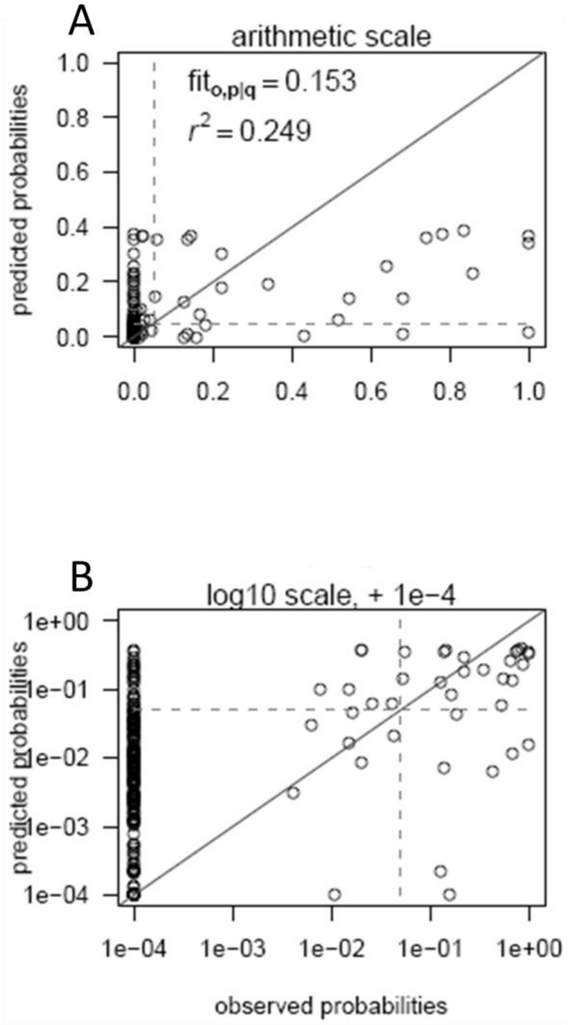

Figure 15. Test 1 - Results of a non-circular MaxEnt test that used an environmental gradient composed of the primary axis of the PCA of all of the environmental factors measured in my study plots. All 11 traits were included.

TEST 2: The summary statistics for the GAMS made with an environmental gradient composed of the primary axis of the PCA of all 47 plots indicate that the height, photosynthetic area and sporulation CATs are not significantly correlated to the primary axis of the PCA (Table 5). As such, I performed this test again after removing those CATs. The results were improved, but still not significant (R^2=0.335, p=1; Figure 16). This improvement led me to perform the rest of the tests with only the CATs that are significantly correlated to the environmental gradient being used.

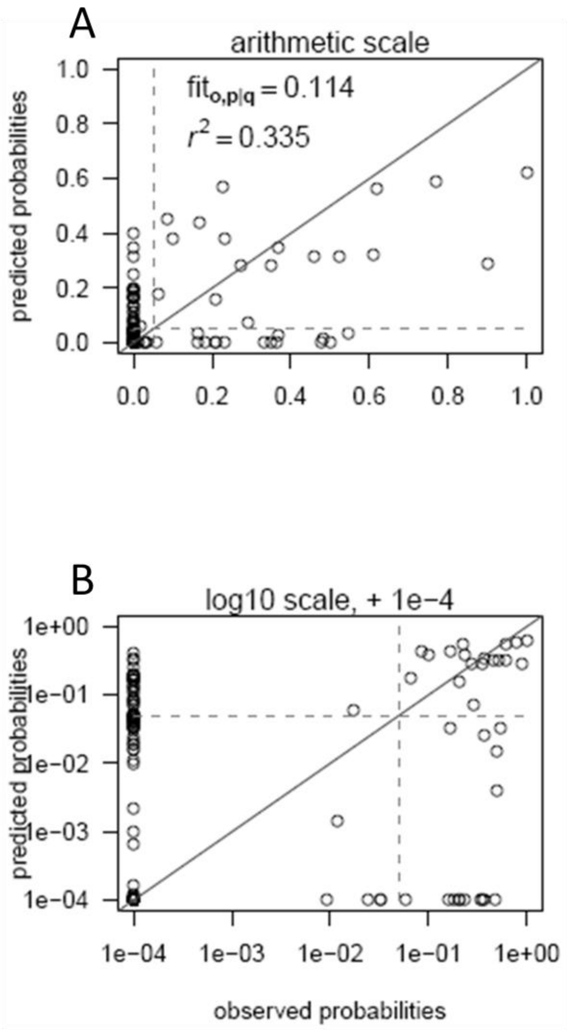

Figure 16. Test 2 - Results of a non-circular MaxEnt test that used an environmental gradient primary axis of the PCA of all of the environmental factors measured in my study plots. Height, photosynthetic area and sporulation CATs were not included because they were not significantly correlated to the environmental gradient.

TEST 3: My third non-circular test used CATs that were estimated from an environmental gradient comprised of the primary, secondary, and tertiary axes of the PCA I created for the environmental data in all 47 plots. No CATs were eliminated for this test because they all showed a significant relationship with the environmental gradient (Table 5). These results also showed that the MaxEnt approach was unsuccessful in predicting species abundance in the test plots (R^2=0.183, p=1; Figure 17).

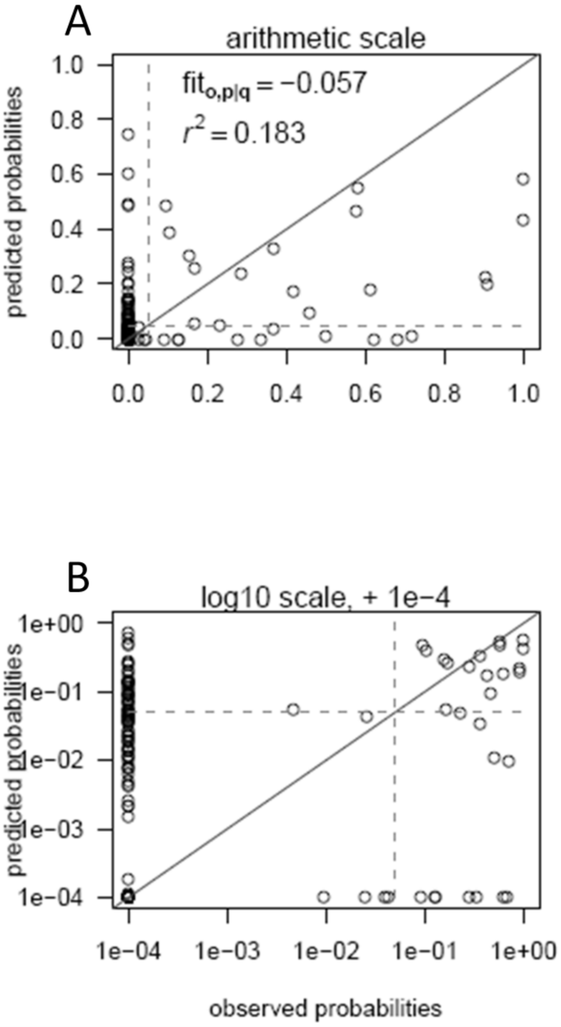

Figure 17. Test 3 - Results of a non-circular MaxEnt test that used an environmental gradient comprised of the primary, secondary, and tertiary axes of the PCA I created for the environmental data in all of my study plots. All 11 traits were included.

TEST 4: The fourth test I performed used CATs that were estimated from different environmental gradients. These gradients were based on the single "best" environmental factor for each trait, i.e. the environmental factor that the CATs had the strongest relationship with. The results of this test were also insignificantly correlated with the observed data. I performed this test five times (each result was different due to a random selection of test plots each time), and while the R^2 value ranged from 0.147 (Figure 18) to 0.344 (Figure 19), the p-value was 1 every time.

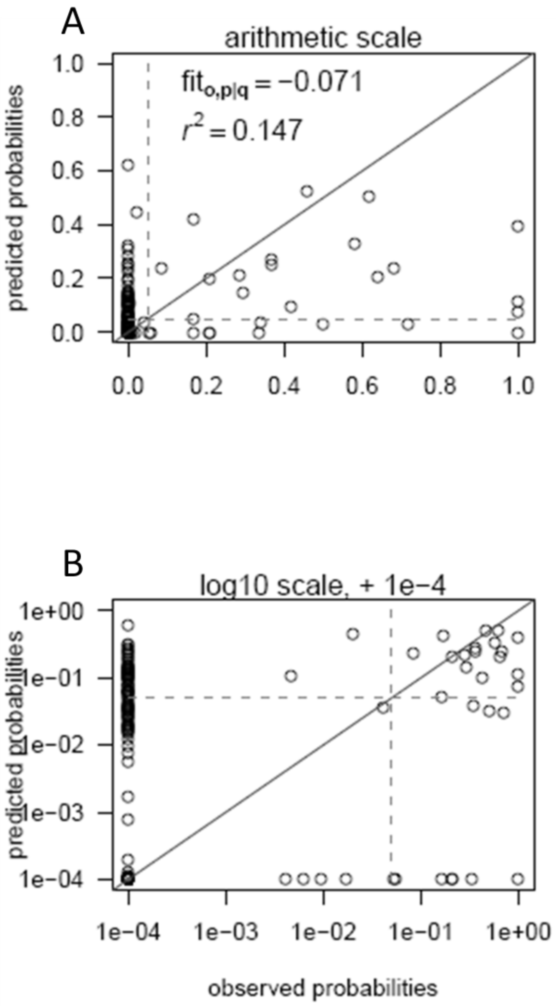

Figure 18. Test 4 - One example of the results of a non-circular MaxEnt test that used environmental gradients composed of the single “best” environmental factor for each of the 11 traits.

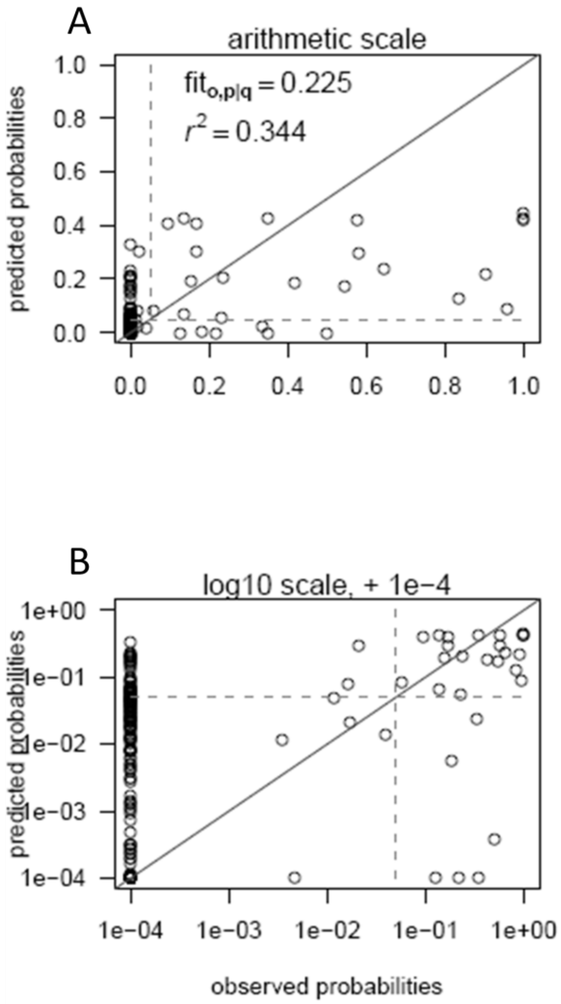

Figure 19. Test 4 - One example of the results of a non-circular MaxEnt test that used environmental gradients composed of the single "best" environmental factor for each of the 11traits.

TEST 5: My fifth test involved using an environmental gradient combining pH and Ca as a composite factor derived via PCA. I eliminated the height, photosynthetic area, and sporulation CATs because they were not significantly correlated to this gradient. It should be noted that while I used the primary axis of the PCA (Figure 12) to order the 47 plots so that I could perform a random stratified selection of test plots for all of the other tests, I was able to order the plots along the pH+Ca gradient in this case. The results showed that the prediction of species abundance in the test plots was not accurate (R^2=0.191, p=1; Figure 20)

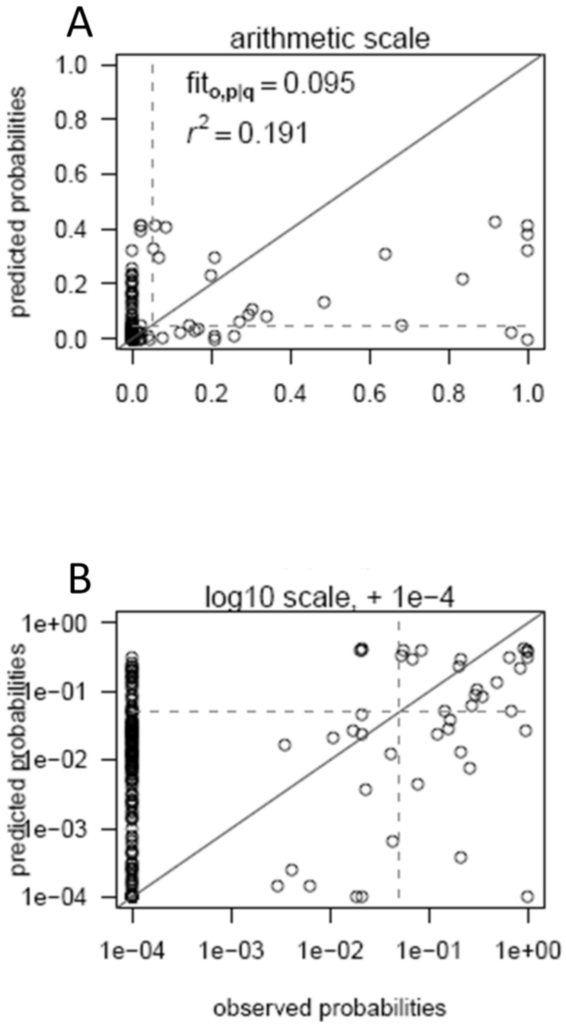

Figure 20. Test 5 - Results of a non-circular MaxEnt test that used an environmental gradient composed of a pH and Ca composite factor. Height, photosynthetic area, and sporulation CATs were not included because they were not significantly correlated to this gradient.

TEST 6: For my sixth test I used a multivariate environmental gradient that included the pH+Ca composite factor, as well as total light and nitrate. I eliminated the Chl, A_{max}, height, and photosynthetic area CATs because they did not significantly correlate with this gradient. The results of this test also proved insignificant (R^2=0.211, p=1; Figure 21).

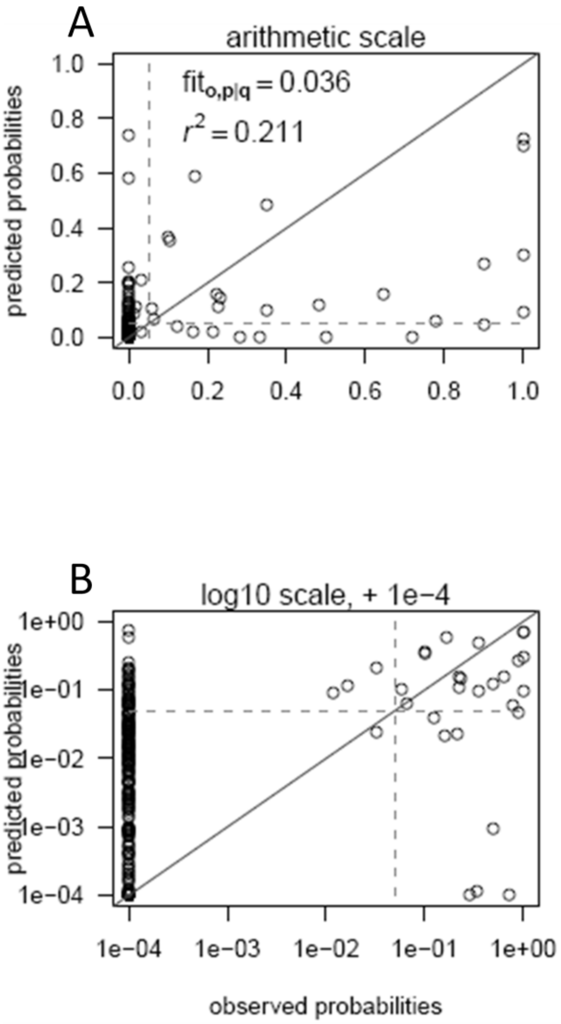

Figure 21. Test 6 - Results of a non-circular MaxEnt test that used an environmental gradient composed of a pH and Ca composite factor, total light, and nitrate. Chl, A_{max}, height, and photosynthetic area CATs were not included because they were not significantly correlated to this gradient.

After the sixth test I attempted a test using a multivariate environmental gradient that included the pH+Ca composite factor, as well as total light and the interaction between these two factors. I was not able to perform this test because there were not enough estimated degrees of freedom when I tried to fit a GAM to the data in the calibration plots, despite there having been enough when I fit a GAM to the data in all 47 plots.

TEST 7: For my seventh test I used CATs that were estimated from different multivariate environmental gradients. These gradients were based on the three “best” environmental factors for each trait, I eliminated the LMA, Chl, A_{max}, height, and photosynthetic area CATs because they did not significantly correlate with their unique multivariate gradients. The results of this test proved insignificant (R^2=0.082, p=1; Figure 22)

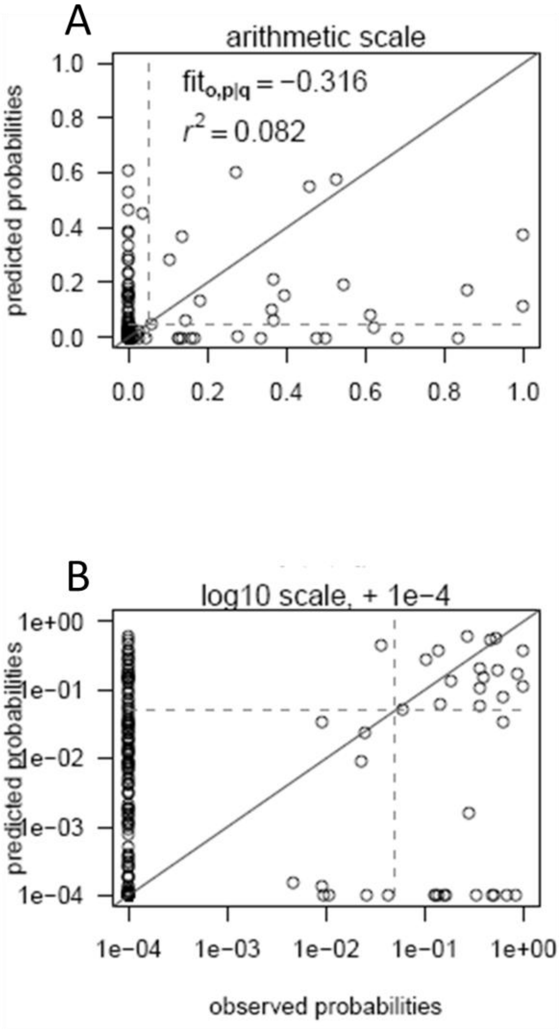

Figure 22. Test 7 - Results of a non-circular MaxEnt test that used a different multivariate environmental gradient for each CAT. These gradients were based on the three "best" environmental factors for each trait. I did not include the LMA, Chl, A_{max}, height, and photosynthetic area CATs because they did not significantly correlate with their unique multivariate gradients

TEST 8: I performed one final test using the same CAT estimation method as in my fourth non-circular test, i.e. CATs for each trait were estimated from the single "best" environmental factor, but this time I reduced the number of traits from to 11 to 3. I decided to perform the test only using A_{max}, wintergreen and sporulation CATs because I was able to produce accurate predictions when I used only these three traits in a circular test. Unlike that circular test, the results of my fifth non-circular test proved unable to accurately predict the species abundance in the test plots (R^2=0.253, p=1; Figure 23)

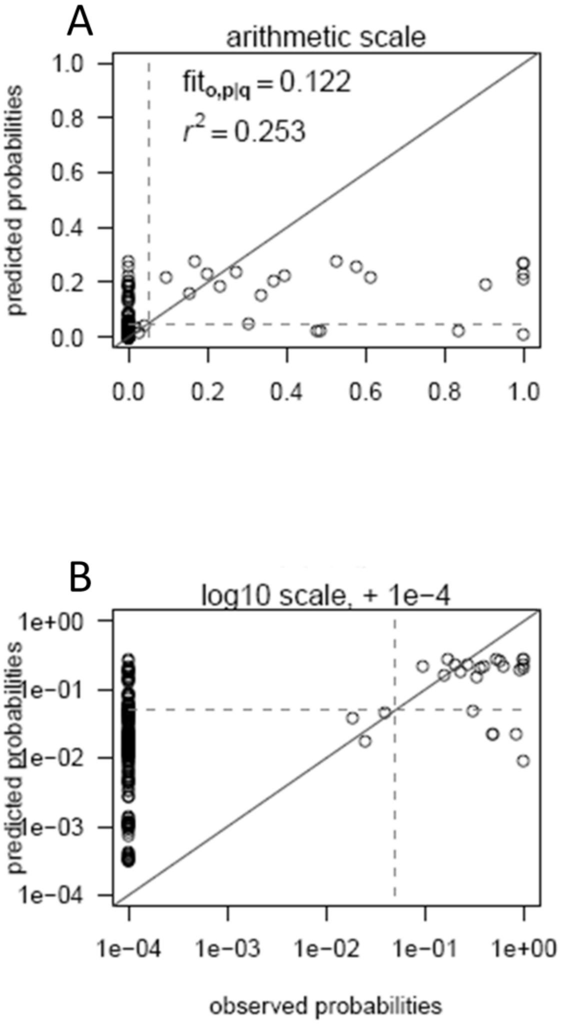

Figure 23. Test 8 - Results of a non-circular MaxEnt test that used a different univariate environmental gradient for each CAT. These gradients were based on the single "best" environmental factors for each trait. I only included the A_{max}, wintergreen and sporulation CATs.

The results of my non-circular tests overwhelmingly indicate that the MaxEnt approach cannot be used to predict SADs from CATs estimated from an environmental gradient. If these results are definitive then the MaxEnt approach cannot be used to predict the affects of changes in environmental conditions on plant communities, nor can it be used to predict the ability of invasive species to colonize an area. In other words, the apparent lack of predictive ability of the MaxEnt approach strips it of its potential utility for conservation purposes. As such, I must analyze the results of my non-circular tests to determine if my non-significant results are definitive, or if there is still a chance that the MaxEnt approach can be used to predict SADs from environmental gradients.

Discussion

The MaxEnt approach attempts to predict the relative abundances of species in communities that occur along an environmental gradient by incorporating both stochastic (neutral) and deterministic (niche) processes. The prior used in the MaxEnt approach implicitly invokes a neutral perspective, either through the equality of species suggested by a uniform prior or through the stochastic process of dispersal limitation suggested by a prior defined by abundance in the regional species pool. Species with high abundances that are in close proximity to a locality are more likely to colonize that locality and become abundant there than species that have low regional abundances and have to disperse to that locality from a greater distance (Gilbert and Lechowicz 2004; Hubbell 2005; Jones *et al*. 2006). Alternatively, the deterministic process that the MaxEnt approach is based on is a niche perspective rooted in the trait-based environmental filtering inherent in the constraints in a MaxEnt approach. The idea behind environmental filtering is that all the species in a regional species pool have the potential to enter a given community, but the environment acts as a filter that only permits entrance to species that have certain combinations of functional traits conducive to survival in that locality (Keddy 1992; Weiher and Keddy 1995; Belyea and Lancaster 1999; Booth and Swanton 2002). It is hypothesized that this filtering process causes variation in the distribution and abundance of species and drives community assembly. Species with traits better suited to a particular environment are more likely to become abundant than species with traits that are less conducive to survival in that environment, even if the latter species are less affected by dispersal limitation.

From a statistical viewpoint, the MaxEnt approach to community assembly is Bayesian in that it is first assumed that communities are randomly assembled according to neutral processes, but then CATs that encode information about the environmental filtering process are included as constraints to inform predictions of a abundance distributions in these communities. These constraints shift the relative abundances of species that were set by neutral processes to ones set by niche processes. Additionally, it should be reiterated that the abundance distributions that are predicted by the MaxEnt procedure only contain the information input as constraints, and are otherwise randomly assembled. In other words, the abundance distributions that MaxEnt selects are random (neutral framework), except with regards to the imposed community-aggregated trait constraints (niche framework). If the MaxEnt approach works, then the apparently opposing frameworks of niche and neutral theory will be linked and the central question in community ecology – how communities assemble from a species pool – will have a more complete answer. Intriguing as the MaxEnt approach is in these respects, it has yet to be thoroughly tested empirically.

My study of fern community assembly was designed to provide such a test and thereby assess the value of the MaxEnt approach in describing and predicting community structure. To test the MaxEnt approach I first needed to select communities to study. I chose to focus on a set of forty-seven 50 m^2 plots established by Ben Gilbert (Gilbert and Lechowicz 2004) across the upland hardwood forest of the Gault Nature Reserve. The 21 fern species present in these plots determined the species pool. I was fortunate that the abundances of these species were previously measured in each of the plots that I chose to study (Gilbert and Lechowicz 2004, unpublished data). I personally obtained average trait data for all of the species in the species pool using samples collected throughout the Gault Nature Reserve, and was then able to calculate community-aggregated trait values (CATs) for each plot. I did so by weighting the average trait values by the abundance of each species in each plot and summing the values for each species to obtain a single CAT for each plot. With all of this information in hand I was able to test if the MaxEnt approach fit my data, i.e. if trait constraints can predict the relative abundance of species in the fern communities described by Gilbert and Lechowicz (2004). Once I established that a MaxEnt model could indeed be fitted to these data, I then attempted to test the predictive abilities of the MaxEnt approach using CATs independently estimated from environmental data for the Gilbert and Lechowicz (2004) study plots .

Fitting the observed data in a MaxEnt approach

I was able to test if a MaxEnt model fit my data by using the calculated CATs in the Gilbert and Lechowicz (2004) plots to constrain the predicted relative abundance of each species in each of the plots. Since the calculated CATs were derived from the observed relative abundances of fern species in each study plot that were measured by Ben Gilbert, in this instance the relative abundances produced by the MaxEnt algorithm are essentially only a measure of how well a MaxEnt model can be fitted to the observed data, not true predictions. I have called this test of fit a "circular test" since the final abundance values generated by MaxEnt are so closely linked to the observed abundances. In my attempts at fitting the MaxEnt approach to my data using circular tests I made incremental changes to the following components of the MaxEnt approach to improve the fit to the observed data: 1) the combinations and number of traits included, 2) the tolerance threshold used and 3) the prior distribution used. I made changes to one component at a time in this order to observe if the alterations affected the quality of the fit of the MaxEnt approach to my data, independently of alterations in each of the other components.

1) Trait selection: My first circular tests used the default settings of the R-code (Laliberté and Shipley 2010) implementing a MaxEnt model, which included a uniform prior distribution and a tolerance threshold of $1e^{-07}$ to determine convergence. The number of traits, however, had no default value as it was up to me to decide which traits to include. The ecological literature supports the notion that all 11 of the traits that I measured (height, density, photosynthetic area, LMA, LDMC, Chl, N, C:N, A_{max}, wintergreen and sporulation) hold biologically relevant information about community assembly processes at the alpha scale (e.g. Westoby *et al*. 2002; Lebrija-Trejos *et al*. 2010). These alpha-traits are associated with factors involved in the ability of a plant to acquire and use resources in a specific environment (Morin and Lechowicz 2008). I therefore included all 11 traits in my first circular test. The relative abundances predicted in this test were significantly correlated to the observed abundances in all of the communities, i.e. the test showed that a MaxEnt model could be fitted to these data. The CATs of these 11 traits could be used to predict relative abundances that were significantly similar to the observed relative abundances.

There exists a trade-off between the number of traits I include and the statistical power of the test, as the degrees of freedom in a MaxEnt analysis are equal to the number of species minus the number of traits. This trade-off prompted me to decrease the number of traits I used to determine if the fit of the MaxEnt approach could be improved. Although all of these 11 traits are hypothesized to be relevant to community assembly, I eliminated three traits (A_{max}, wintergreen and sporulation) that I did not think were as important as the others due to being redundant (A_{max}) or being categorical rather than ordinal (wintergreen and sporulation). When I removed these traits, I found that the fit of the MaxEnt approach actually worsened and was no longer significant.

This decrease in the fit of the MaxEnt approach when using only 8 instead of all 11 traits at first led me to believe that having more statistical power was less important than having more information in the form of constraints for fitting the MaxEnt approach. A very recently published study by Mokany and Roxburgh (2010) supports my initial decision to include all 11 traits because CATs of alpha traits similar to mine were shown to improve the fit of the MaxEnt approach at the alpha scale. Their research also showed that at smaller spatial scales more traits appear to be significant, i.e. information on more traits produces a better fit of the MaxEnt approach at the alpha scale. When I tested the importance of the A_{max}, wintergreen and sporulation CATs (combined) in comparison to the combination of CATs for the other eight traits in the first circular test, I was surprised to see that the combination of traits I removed in fact was more important than the combination of the ones I kept in the MaxEnt approach!

The apparent significance of A_{max}, wintergreen and sporulation led me to attempt another test in which I only included the CATs of these three traits, and the fit of the MaxEnt approach was again significant. I then tried another circular test with only wintergreen and sporulation CATs, and while the fit of the MaxEnt approach declined from the previous test, it was significant as well. The fact that using three traits and two traits improved the significance of the fit over using eight traits suggests two possibilities: 1) perhaps there is a critical point at which increased power trumps increased information and the MaxEnt approach will fit the data regardless of the traits used, or 2) perhaps A_{max}, wintergreen and sporulation CATs combined indeed contain more information about how fern communities are constrained than the other combination of traits does. If the latter possibility is true, then perhaps other combinations of traits are even more important than the ones I tested. I was not able to go through each combination individually as there were too many possibilities; however in their recently published paper, Mokany and Roxburgh (2010) developed a stepwise selection procedure that can identify which traits/combinations of traits are the most important constraints. This procedure can be used on my data in the future to elucidate the importance of each of the traits I measured in my study.

2) Setting the convergence tolerance threshold: In addition to the number of traits included in the MaxEnt approach, it became clear in my circular tests that the convergence tolerance was important to the significance of the predicted abundances produced by a MaxEnt model. Although the fit of the 8-trait model in which I eliminated A_{max}, wintergreen and sporulation was not significant at the default convergence tolerance of $1e^{-07}$, a lower tolerance of $1e^{-09}$ improved the fit of the MaxEnt approach dramatically, making it significant. Roxburgh and Mokany (2010) discuss how to choose a convergence tolerance for MaxEnt to ensure that a satisfactory solution is found. They comment on how more stringent tolerance levels generally improve fits in numerical optimization procedures, but that each dataset is unique in the relationships among species abundances and trait values that it contains. Therefore care has to be taken when setting the convergence tolerance as this component of the MaxEnt approach should change according to the specific dataset being used. My dataset contains a great number of low abundance values. As such, a relatively low convergence tolerance is required to observe the small changes in predicted relative abundance that occur between successive iterations when executing the MaxEnt approach. A more stringent tolerance limit, however, can also substantially increase the time required to iteratively solve the MaxEnt algorithm.

3) Choosing a prior distribution: To employ the MaxEnt approach I had to decide on a prior abundance distribution to which CAT constraints could be added so that the MaxEnt algorithm could come up with a predicted abundance distribution. I used the default parameters in my initial circular tests, which included a maximally uninformative uniform prior distribution in which all of the species were equally abundant in all of the plots. This prior is part of the neutral aspect of the MaxEnt approach as it implies that all species have an equal ability to enter the communities that I am studying if traits are not taken into consideration.

Shipley (2009c) indicated that a different prior distribution could be used if other information regarding species abundances was available. A non-uniform prior distribution essentially acts as an additional constraint on the predicted relative abundance estimates that the MaxEnt approach generates. I started off using a uniform prior because I wanted to see the effects of changing the traits as well as the tolerance threshold independently of the effects of adding information in the form of a non-uniform prior distribution. Once I established the effects of trait selection and tolerance threshold, and determined how to obtain optimal results by changing these components, I decided to incorporate a non-uniform prior comprised of the relative abundances of each species in the regional pool of fern species on Mont Saint Hilaire. I had the preconceived notion that the non-uniform prior would improve the fit of the MaxEnt approach from that based on a uniform prior because it could add information more directly relevant to the assembly of fern communities at Mont Saint Hilaire. I obtained this non-uniform prior distribution from a comprehensive vegetation survey of Mont Saint Hilaire made in 1996 (Bell *et al*. 2001). I was concerned that the non-uniform prior might act as a misleading constraint since it is possible for a species to be regionally rare and locally abundant, but implementing the non-uniform prior did indeed improve the fit of the MaxEnt model to my data.

Neutral processes acting at the alpha level are still incorporated in the MaxEnt approach with this non-uniform prior, although in a different way than with the uniform prior. Using the non-uniform prior implies that each species' ability to enter a community is directly proportional to its regional abundance so long as the environment is not filtering species based on their traits at the alpha scale. The regional abundance of each species may still encode constraints, but these constraints would be acting at the regional scale (i.e. beta-traits).

The non-uniform prior approach that I took is similar to one taken by Sonnier *et al*. (2010) in a recent study using the MaxEnt approach to assess if traits are good predictors of relative abundance at very large spatial scale – all of England. Sonnier *et al*. (2010) describe three different scenarios of local community assembly: 1) "pure local trait-based assembly" in which the prior is maximally uninformative, and CATs are added as constraints, 2) "pure local neutral assembly" in which a non-uniform distribution obtained from the relative abundance of each species at the landscape level is used and CATs are not incorporated to constrain predicted relative abundances, and 3) a "hybrid model" in which the prior is the non-uniform regional species abundance distribution (scenario two) and CATs are included to constrain the predicted abundance distribution (scenario one). Sonnier *et al*. (2010) compared the predicted abundances they obtained with the MaxEnt approach for each of the three scenarios, and the hybrid model produced the best results. The pure local trait-based assembly scenario produced good predictions as well, but unlike with the hybrid model the fit of the MaxEnt approach declined dramatically when a greater number of species were included in the species pool. Nonetheless, permutation tests showed that the traits were still significant constraints on the MaxEnt predictions in all of their tests. The pure local neutral assembly scenario produced worse predictions than both of the other scenarios, but the traits were also determined to significantly predict the observed relative abundance.

My use of a regional abundance distribution as the prior distribution and CATs to constrain the predicted relative abundance generated by the MaxEnt algorithm is very similar to scenario three, the hybrid model, described by Sonnier *et al*. (2010). Furthermore, the results of the study by Sonnier *et al*. (2010) coincide with my results, as my use of the non-uniform prior also produced more accurate predictions than my use of the uniform prior, although the circular tests using both of these priors showed that the CATs significantly constrain the predicted abundance generated by MaxEnt. The results obtained by Sonnier *et al*. (2010) in addition to my results alleviated my concern that the non-uniform prior would act as a misleading constraint.

Utility of fitting the observed data in a MaxEnt approach

All of the progressive changes I made to the MaxEnt approach in my circular tests helped me understand the conditions necessary to fit an effective model to my data. The important outcome of all of these tests is that I can conclude that a MaxEnt model can indeed be fitted to my data, i.e. that community-aggregated traits can be used to predict the abundance distribution of ferns in the communities I sampled in the Gault Nature Reserve. Although some researchers criticize this sort of circular implementation of a MaxEnt model and claim that it has neither validity nor utility (e.g. Marks and Muller-Landau 2007; Haegeman and Loreau 2008 and 2009), others defend the use this MaxEnt approach for assessing and describing the role of community-aggregated traits as measures of a filtering process affecting the relative abundance distributions of species in a community (e.g. Shipley 2009a; Shipley 2009b; He 2010; McGill and Nekola 2010; Mokany and Roxburgh 2010; Roxburgh and Mokany 2010; Sonnier *et al*. 2010). I agree with the latter camp and have myself demonstrated that MaxEnt models can be fitted to community data, but I acknowledge that the real utility of the MaxEnt approach ultimately hinges on its predictive ability. In other words, to apply a MaxEnt approach to predict the vegetation structure of communities that have not been surveyed, the MaxEnt approach must be able to predict relative abundance from CATs that are not derived not from observed abundances in a set of surveyed plots but from independently measured environmental conditions.

Exploring uncharted territory with environmental gradients

I therefore have derived CATs from environmental gradients to assess and test the potential predictive ability of the MaxEnt approach. I want to emphasize that the predictive as opposed to simply descriptive ability of a MaxEnt model is entirely dependent on the ability to estimate CATs from an environmental gradient. The circular tests did not employ this method, as environmental data associated with the Gilbert and Lechowicz (2004) plots were not considered in fitting MaxEnt models to their data on the relative abundance of species. Testing the predictive ability of MaxEnt involves using GAMs to quantify the relationship between the CATs and the environmental gradient in a subset of calibration plots so that CATs can be predicted in test plots from which relative abundance predictions can be made for the test plots. I am calling this predictive test "non-circular" to emphasize the fact that in this method the CATs used to constrain relative abundance predictions are independent of the observed relative abundances, unlike in the circular tests I executed initially.

I know of only one other, still unpublished study using environmental gradients to test the predictive ability of the MaxEnt approach (Shipley, Laughlin, Sonnier and Otfinowski, in press). I therefore had to develop the non-circular test method on my own and struggled with how to incorporate the trait and environment components of the test. As previously discussed, there is a trade-off between the number of traits I include in a MaxEnt model and the power of the test of that model. Although I had already discovered that different combinations and numbers of traits have differential impacts on the significance of the prediction through my circular tests, I had to deal with this issue again in performing non-circular tests. I fitted GAMs to the environment and CAT data in all of the plots, rather than just the calibration plots, to identify whether or not the CATs significantly correlated to each environmental gradient that I used, as well as what the characteristics of the correlation trend was, if there was a trend. If the CATs of a trait were significantly correlated to the environmental gradient that I used then I included that trait in the subsequent non-circular test; if it was not then I excluded it from that particular test. As previously discussed, I had no other basis for excluding traits, although in the future the stepwise selection procedure recently developed by Mokany and Roxburgh (2010) can be used to determine if my correlation criterion caused me to only include the traits most important to the MaxEnt relative abundance distribution prediction, or if other combinations of traits should have been included in my non-circular tests.

In addition to the statistical power vs. number of traits trade-off, there is also a trade-off between statistical power and the number of environmental factors I can include in the environmental gradients when I fit GAMs to these data. Using more test plots would increase the power of the test; however it would also take away from the number of calibration plots that I use. Fewer calibration plots would mean that I had fewer degrees of freedom available when fitting the GAMs that the CATs would be estimated from in the test plots. That translates into being able to include fewer environmental factors in the GAM fitted to the calibration plots, as each environmental factor decreases the estimated degrees of freedom available by an amount that is related to the wiggliness of the fit of the GAM. I felt that using 25% of the plots as test plots and 75% of the plots as calibration plots was the best balance between power and number of environmental variables I was able to include. I thought that using fewer calibration plots would cause any patterns observed among the CATs and the environmental gradients picked up by the GAMs to degrade, causing poor estimations of CATs from the environmental gradient. It appears that Shipley *et al*. (in press) used the same percentage of their 96 plots as test plots and calibration plots, lending support to my decision to use this 25/75 split.

Within the scope of this M.Sc. research, I have only been able to complete an initial exploration of the predictive ability of the MaxEnt approach, and the results so far suggest that successfully predicting species abundance distributions from CATs that are estimated from an environmental gradient will not be straightforward, if it is possible at all. None of the non-circular tests that I tried based on insights gained in fitting MaxEnt models to my data yielded significant predictions. It is important to analyze the possible reasons for these non-significant results in order to interpret if they are definitive, or if there is still potential that the MaxEnt approach can not only describe but also predict relative abundance in communities along environmental gradients. This analysis includes examining if the CATs were accurately estimated from an environmental gradient, as well as questioning if there are other more fundamental issues with the MaxEnt approach to community assembly that prevented me from obtaining significant predictions of species abundance.

Were CATs well estimated in the test plots?

It is necessary to investigate how good the CAT estimation process is to determine why the non-circular tests did not produce good predictions, as this process is the only difference between the non-circular tests and the circular tests, which did significantly describe the patterns of species abundance in my study plots. For this investigation I compared the observed CATs in a randomly selected set of test plots to the estimated CATs in the same plots. I did this for two of the estimation methods I tried in my non-circular tests: the one that involved using the single “best” environmental factor as the environmental gradient for each CAT, and the one in which I used the three best environmental factors. I chose to examine these methods because the GAMs fitted to these data in all of the plots illustrate what I believe to be strong statistical relationships between the CATs and the environment that are consistent with expectations for the ecological roles of traits as filters in community assembly. For example, LDMC strongly correlated with the univariate gradient of pH (R^2=0.347, p=7.00e^{-05}; Table 6a; Figure 6 in the appendix) and foliar nitrogen strongly correlated with the multivariate gradient comprised of pH, PCA1, and Ca (R^2=0.613, p=0.003, 0.110 and 0.016 for each environmental factor, respectively; Table 11).

I assumed that GAMs fitted to data exhibiting such strong relationships would produce good estimations of CATs in test plots. I used a model II regression to determine whether or not the CATs in a randomly selected subset of test plots that were estimated from an environmental gradient significantly co-vary with the observed CATs in the same test plots. The results indicate that the null hypothesis of zero correlation cannot be rejected for the majority of CATs ($p>0.05$), i.e. that the estimated CATs are significantly different from the calculated CATs for the test plots for most of the traits (Table 12 and 13). To illustrate this incongruity I plotted the observed and estimated LDMC CATs for a subset of test plots along a pH environmental gradient (this was the best univariate gradient for LDMC) against each other ($R^2=0.0004$, $p=0.953$; Figure 24).

It must be reiterated that the calibration and test plots are different each time I perform a non-circular test, as they are randomly selected each time. As such, the correlation between the estimated and observed CATs in the test plots will be different if the plots are randomly drawn in a different way. In the time available for my M.Sc. research, I did not have the computing power to run these tests enough times to statistically verify that the CATs would always be poorly estimated using environmental gradients in my non-circular tests; however the experience I have in executing multiple non-circular tests leads me to believe that the results I outlined above are not anomalous.

CAT	Environmental Gradient	R^2	p-value
Wintergreen	pH	0.404	0.026
Height	Light	0.330	0.051
N	pH	0.202	0.143
Photosynthetic Area	Light	0.191	0.155
Sporulation	P	0.164	0.191
C:N	pH	0.139	0.233
LMA	Ca	0.116	0.279
Chl	Mg	0.082	0.366
LDMC	pH	0.047	0.497
A_{max}	Ca	0.009	0.771
Population Density	pH	0.006	0.806

Table 12. Results of a model II regression performed on calculated and estimated community-aggregated traits (CATs) for a randomly selected subset of test plots. The CATs were generated from one of my non-circular tests of the MaxEnt approach by Shipley et *al*. (2006), using a different environmental gradient for each CAT; each gradient was composed of the single environmental factor most strongly related to each CAT.

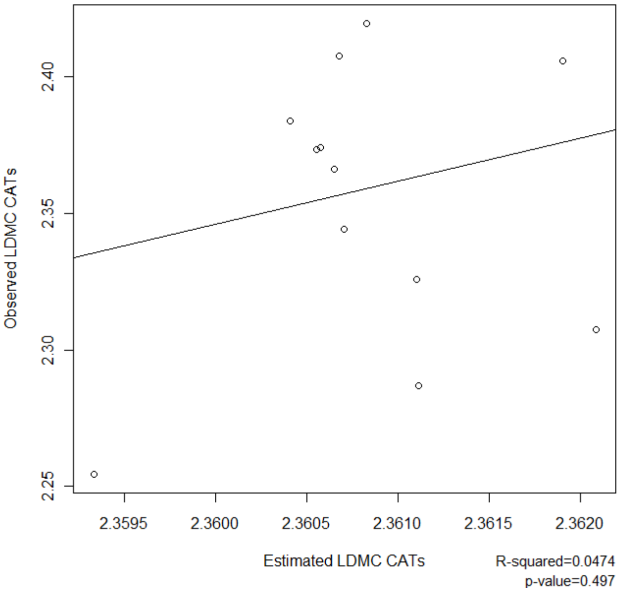

Figure 24. Regression line and results of a model II regression for observed and estimated LDMC community-aggregated traits (CATs) in a randomly selected subset of test plots. A pH gradient was used as this environmental factor was the most strongly related to the LDMC CATs of all the factors.

Table 13. Results of a model II regression performed on calculated and estimated community-aggregated traits (CATs) for a randomly selected subset of test plots. The CATs were generated from one of my non-circular tests of the MaxEnt approach by Shipley et *al*. (2006), using a different environmental gradient for each CAT; each gradient was composed of the three environmental factors most strongly related to each CAT.

CAT	**Composite Environmental Gradient**			**R^2**	**p-value**
LMA	Ca	PCA1	Mg	0.475	0.013
LDMC	pH	K	PCA2	0.009	0.771
Chl	Mg	PCA1	Ca	0.220	0.124
N	pH	PCA1	Ca	0.061	0.439
C:N	PCA1	pH	Ca	0.055	0.463
A_{max}	Ca	PCA1	NO_3^-	0.132	0.246
Height	Light	PCA3	Moisture	0.255	0.094
Photosynthetic Area	PCA3	Light	Moisture	0.093	0.335
Population Density	pH	Moisture	Organic Matter	0.053	0.471
Wintergreen	pH	Moisture	PCA2	0.154	0.206
Sporulation	P	PCA3	Light	0.120	0.270

Possible Type II error

This evidence strongly suggests that using a GAM to estimate CATs from an environmental gradient is not effective for my data, as the estimated CATs were significantly different from the observed CATs in the test plots in the two examples I present here. Therefore it is possible that the negative results of my non-circular tests are in fact a reflection of a Type II error. This error could have been caused by either a weak environmental gradient, the GAM being a poor choice of model to fit to the data, or a lack of power in my test of the MaxEnt approach. In the following text I will explain why I believe that the latter possibility is the most plausible of these three possibilities

In the conclusions of their article, Shipley *et al*. (2006) state that testing their model will require quantifying major environmental gradients and demonstrating general patterns of community-aggregated traits over such environmental gradients. Shipley *et al*. (2006) were unable to fulfill these requirements because they had no environmental data characterizing their study sites. Although Shipley *et al*. (2006) did not have any environmental data, they did attempt to predict CATs by using a cubic-spline regression to smooth the observed CAT data over the successional age gradient along which their study sites were distributed. This successional gradient, however, was not well-suited to the theory of environmental filtering that the MaxEnt approach is based upon because time does not cause vegetation to change, but rather environmental variables cause vegetation to change over time. Furthermore, vegetation changes differentially in different habitats over time (Shipley 2007, Shipley 2009c). To better test the predictive ability of the MaxEnt approach I attempted to create environmental gradients using variables that do indeed directly affect vegetation patterns and that therefore could determine CATs through a filtering process during community assembly.

I fulfilled the requirements to test the MaxEnt approach by quantifying major environmental gradients and demonstrating general patterns of CATs over these gradients as best as I could using my data. I have tried a multitude of ways to create environmental gradients with the environmental data available in order to demonstrate the relationships between them and the CATs, such as collapsing the environmental data into a few factors with PCAs, as well as by determining which environmental factors are most relevant to the different CATs by fitting univariate and multivariate GAMs to the data and performing stepwise linear regressions. It is clear that environmental gradients can form complex and often nonlinear relationships with CATs, even if these gradients are comprised of only one environmental factor. Using GAMs to capture this complexity is a legitimate method; Shipley *et al*. (in press) used GAMs to estimate CATs from an environmental gradient, and this produced accurate predictions in their study. Using GAMs, I have indeed shown that meaningful relationships do exist in my data (cf. Figures 1-13 in Appendix 2 for examples). I believe I have exhausted my options in quantifying an environmental gradient underlying the communities spread around the diverse temperate forest of Mont Saint Hilaire, and that the GAMs have shown how CATs trend along these environmental gradients. It is expected that these strong trends would translate into good estimations of CATS in the test plots. Since this is not the case, I believe that the possible Type II error in my tests is instead the result of a lack of power in the analyses.

Lack of statistical power

Not having enough statistical power in testing the MaxEnt approach is a serious problem that I think has led to a Type II error in my results. Statistical power determines the probability that a statistical test will correctly reject a false null hypothesis, i.e. not make a Type II error (Thomas 1997). Power analyses can be done before statistical tests to determine the sample size that is necessary to have enough power, and similarly, *post hoc* power analyses are common. These tests require information regarding the sample size, α-level, sampling variance and effect size (Thomas 1997). Due to the complicated nature of the MaxEnt approach, obtaining this information and performing a power analysis before beginning my study, as well as after, was not possible. Nonetheless, I know that the power of my test is dependent on the number of plots, the number of traits and the number of species that I included. I can use this knowledge to speculate on possible causes of a lack of power and hence the risk of a Type II error in my results.

Effect of the number of plots on power

Since statistical tests use samples to make conclusions about populations, the number of samples used directly effects the power of a test, with more samples increasing power (Thomas 1997). I already explained that there is a trade-off between power and number of plots, but I did so in the context of determining how to split up all of the plots into calibration and test plots so to fit GAMs to multivariate environmental gradients. Since my data set is fixed at 47 study plots, including more test plots in an analysis meant that I had fewer calibration plots to fit the GAM to, and thus could include fewer environmental factors in the gradient. Here I am determining if I had enough plots to use GAMs to quantify the relationship between the CATs and the environmental gradient at all. A lack of power jeopardizes my ability to use a GAM, or any type of model, to quantify the relationships between the CATs and environmental gradients in order to estimate CATs in test plots and predict abundances in these plots. If I do not have enough calibration plots then fluctuations in the relationship between the CATs and the environment that are clear when I fit a GAM to all of the plots might not be reflected in the GAM fitted to only the calibration plots. Therefore even if the CATs in the test plots behave similarly to those in the calibration plots over the environmental gradient, the CAT estimations in the test plots and the subsequent SAD predictions may be highly inaccurate. Furthermore, it is possible that relatively subtle relationships may not be noticed because there are not enough plots to illustrate them.

In retrospect, I believe that I do not have enough plots, and therefore statistical power, to provide a robust, noncircular test of a MaxEnt model. When I fit GAMs to the CAT and environmental data in all of the plots, clear patterns arise yet when I fit GAMs to the data in the subset of 35 calibration plots, these patterns appear to break down. Figures 25-28 show examples of the contrast between GAMs fitted to all of the plots and GAMs fitted to only the calibration plots. The fact that each non-circular test that I performed displayed very similar results (i.e. p=1) also supports this notion of lack of statistical power. It did not matter which environmental gradient I used because the relationship between the gradients and the CATs was not being accurately defined by the GAMs due to my having too few calibration plots.

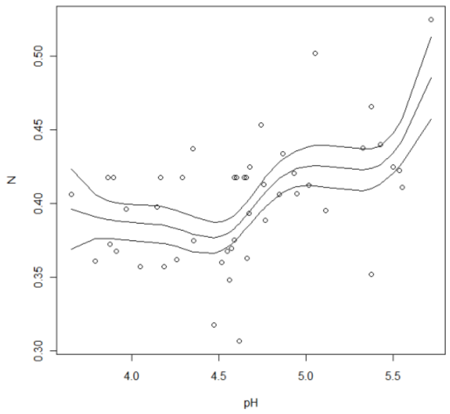

Figure 25. GAM fitted to the N community-aggregated trait and pH gradient data in all 47 plots established by Ben Gilbert (Gilbert and Lechowicz 2004) on the Gault Nature Reserve in Mont-Saint-Hilaire, Quebec, that I included in my study, including the 95% confidence intervals. **R^2=0.299, p=0.00653**

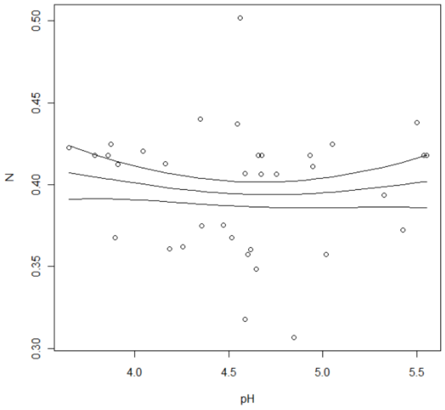

Figure 26. GAM fitted to the N community-aggregated trait and pH gradient data in 35 randomly selected calibration plots established by Ben Gilbert (Gilbert and Lechowicz 2004) on the Gault Nature Reserve in Mont-Saint-Hilaire, Quebec, that I included in my study, including the 95% confidence intervals. **R^2=-0.00251, p=0.700**

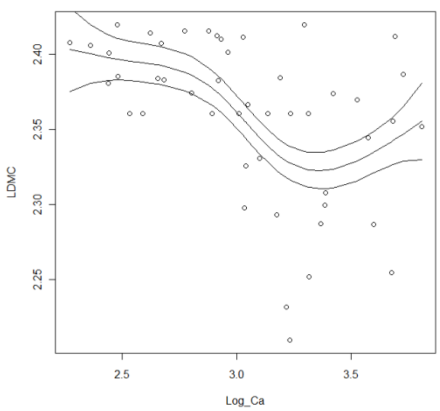

Figure 27. GAM fitted to the LDMC community-aggregated trait and $\log_{10}$–transformed Ca gradient data in all 47 plots established by Ben Gilbert (Gilbert and Lechowicz 2004) on the Gault Nature Reserve in Mont-Saint-Hilaire, Quebec, that I included in my study, including the 95% confidence intervals. **R^2=0.292, p=0.00261**.

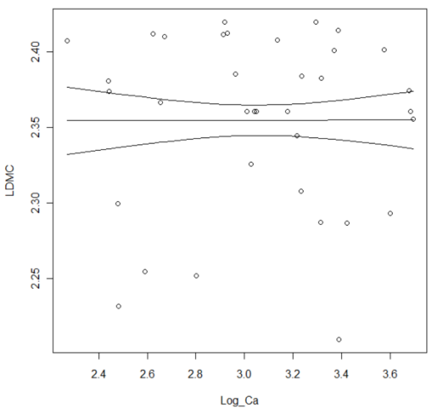

Figure 28. GAM fitted to the LDMC community-aggregated trait and $\log_{10}$–transformed Ca gradient data in 35 randomly selected calibration plots established by Ben Gilbert (Gilbert and Lechowicz 2004) on the Gault Nature Reserve in Mont-Saint-Hilaire, Quebec, that I included in my study, including the 95% confidence intervals. **R^2=-0.0303, p=0.987**.

Lack of power is a difficult problem to overcome because the best way to minimize the risk of it and the resulting Type II error is to increase the sample size (Thomas 1997). This was not feasible in my study because in the scope of an M.Sc. thesis I did not have the time or resources available to expand upon these plot data by establishing and surveying more plots to be used for calibrating the GAMs, and was therefore limited to using the plots that were established by Ben Gilbert (Gilbert and Lechowicz 2004) in my research. *A priori* these permanent plots seemed ideal for a test of the MaxEnt approach because they had already undergone a careful vegetation survey from which I could determine species' relative abundance, and many environmental variables had already been measured in them as well. The only information therefore left for me to obtain was trait data required to characterize the various fern species in Ben Gilbert's (Gilbert and Lechowicz 2004, unpublished data) plots in the Gault Nature Reserve. Furthermore the number of test plots (versus calibration plots) in my study (12 plots) is equal to the number of sites used in the initial MaxEnt study (Shipley *et al.* 2006) and is in the same order of magnitude as the number of test plots used in the recent study by Shipley *et al*. (in press) during their cross-validation procedure (24 plots), which is the only other study I know of that used environmental gradients to estimate CATs. Hence it was difficult for me to have known *a priori* that having only 47 plots in total might cause my noncircular tests to have a lack of power, potentially causing a Type II error in the results.

One could argue that I should not have eliminated the outlying plots before beginning my analysis in order to provide more statistical power; however including these plots would probably have caused the GAMs to fit less well due to there being a break in the distribution of plots along the organic matter gradient and perhaps in other environmental variables as well. Since the distinction between organic and mineral soil is also associated with significant changes in habitat, including Ben Gilbert's plots with high organic matter might have changed the focus of my analysis from a spatial scale associated with alpha diversity to one involving some degree of beta diversity. I wanted to, and am, using the MaxEnt approach as a tool to discover how alpha diversity is constrained along an environmental gradient. Including the outlying plots would introduce multiple environmental gradients, thereby confusing alpha and beta scale processes and confounding the results. Nonetheless a future direction for this study could be to include the outlying plots, perhaps resurveying the existing plots and adding additional plots as well. It is likely, however, that this also would increase the species pool, so trait data would have to be obtained for these new species, which could be a significant task. These options are well beyond the scope of an M.Sc. thesis.

Effect of the number of traits and species on power

As previously discussed, the number of traits and species I incorporated in my tests of the MaxEnt approach is also associated with the statistical power of my tests as these determine the degrees of freedom available in my analysis. In a MaxEnt analysis, the degrees of freedom are equal to the number of species in the species pool minus the number of traits (Shipley 2009c). I was limited to including 21 species in the species pool as these were all of the species present in the 47 permanent plots that I used. As such there was a cap of at most 20 degrees of freedom (if I only incorporated one trait) in this analysis. I did have room to adjust the number of traits I included in my test of the MaxEnt approach; however as mentioned earlier in the discussion, I had no ecological basis to do so. I do not believe that the number of traits I included caused a Type II error in my tests of the MaxEnt approach because the maximum number of traits that I included was 11, meaning that I had at least ten degrees of freedom in all of my tests. Furthermore, excluding some of these traits did not affect the significance of the results of any of my non-circular tests, and removing or adding certain traits in my circular tests had the effect of both increasing and decreasing the significance of the results, depending on what the traits were. As such, I believe that the number of traits I included does not have much bearing on the results, at least not as much as which specific traits I included.

More importantly, I think that the number of plots I used in my study had the greatest impact of all of the components determining the power of my analysis, and perhaps caused a Type II error. It therefore would be premature to conclude that the non-significant species abundance predictions produced by my non-circular tests indicate that the MaxEnt approach cannot work. That said, neither can I discount the possibility that there was no Type II error and the MaxEnt approach in fact cannot yield useful predictions of community assembly along environmental gradients. The situation merits some additional consideration of reasons for the lack of significant results in all of my non-circular tests.

Might the MaxEnt approach have worked to some degree?

Although the results were not significant as a whole, it is possible that some species' abundances were accurately predicted in my non-circular tests. The results generated by the permutation test used to assess the fit of the MaxEnt approach indicated that the overall quantitative predictive ability of the MaxEnt approach was not good for my data; however Shipley *et al*. (in press) recently devised a method to determine the qualitative predictive ability of the MaxEnt approach, i.e. to determine if predicted abundances were differentially accurate depending on the observed abundances of each species in each plot. Indeed there has been speculation that a MaxEnt model may predict the relative abundances of dominant species better than those of rare species because the CATs are weighted in favour of these dominant species (Marks and Muller-Landau 2007; Haegeman and Loreau 2008). Additionally, although I have not determined which relative abundance values were best predicted in my circular tests, a visual examination of the permutation test plots for the circular tests suggest that the rarer species were less well predicted than the dominant species (See Figures 3-10). A recent study by Roxburgh and Mokany (2010) provides a method to modify the permutation test to give greater weight to rarer species so that the measure of fit of predicted abundances to observed abundances will not be over-determined by the most dominant species. This newly published method can be applied to my data in the future and the results of the subsequent tests can be compared to the tests I have already performed to determine if the abundance distribution of the dominant species were in fact better predicted than that of the rare species.

This new development by Roxburgh and Mokany (2010) aside, a qualitative analysis method developed by Shipley *et al*. (in press) involves first classifying all of the species as either dominant, rare, or absent in each plot, based on observed relative abundances. After this is established, the number of plots in which the predicted relative abundances were accurate for all or some of the dominant species is counted. The median, 2.5% and 97.5% quantiles of the predicted relative abundances can then be determined for the rare and absent species in each plot. Shipley *et al*. (in press) found that the predicted relative abundances of dominant, rare and absent species varied in accuracy depending on the number of traits they used. Due to the recent development of this qualitative analysis of the MaxEnt results by Shipley *et al*. (in press), I was not able to identify if the MaxEnt approach did in fact work in predicting such categorical abundances for some of the species in my dataset before completing my thesis analyses. This method can be used in the future, however, and will not only be useful in determining if the MaxEnt approach at least partially worked with my data, but also in deciphering the effects of trait constraints on the predictions that MaxEnt generates.

Other speculations about why species abundances were not well-predicted

It is possible that the overall non-significant results I obtained were not due to a Type II error caused by a lack of statistical power, but rather to more fundamental flaws in the MaxEnt approach and/or the way I applied the approach to fern communities in the Gault Nature Reserve. In this section I consider the fact that the MaxEnt approach is unable to predict abundance values equal to zero, as well as the fact that my dataset contains many observed abundances equal to zero, or near zero. Additionally, I will reflect on whether or not the traits I chose to include are indeed the most critical traits in the assembly of fern communities. Finally, I will discuss the possibility that stochastic processes involved in fern community assembly have not been accounted for in the MaxEnt approach and have caused inaccurate SAD predictions.

Zero abundance

Most of the 47 plots I included in my study contain very few species; the median species richness was 3 out of a total of 21 species, and 11 plots only contained one species. As a result the majority of my observed relative abundance values are zero. It is not possible for a MaxEnt model to predict zero abundance for a species because according to Bayesian statistics, that would mean it is not logically possible for that species to occur, and therefore it should not be in the species pool to begin with (Shipley 2009c). Although it is unclear as of yet whether or not the relative abundances of the rarer species were less well predicted than those of the more dominant species, if the criticisms claiming the MaxEnt approach is unable to predict low species abundances (Marks and Muller-Landau 2007; Haegeman and Loreau 2008) are based in reality, then the high number of zero abundance values in my dataset could be the cause of the overall poor predictions of species abundances in my test plots.

Nonetheless, I do not think that having so many abundance values equalling zero caused the MaxEnt approach to produce inaccurate predictions because this was not the case in the circular tests I performed. It became clear in my circular tests that a relatively low convergence tolerance was required to obtain accurate results because I had so many low abundance values, and once this low tolerance was set the MaxEnt approach accurately predicted relative abundances overall (see figures 11-16). I performed the non-circular tests with low convergence tolerances as well, but the results were that the MaxEnt approach did not accurately predict relative abundances overall. I am therefore not convinced that the low abundance values in my data are not conducive to the MaxEnt approach working.

Other critical traits in fern community assembly

It is possible that I did not include the most critical traits in fern community assembly in my assessment the MaxEnt approach. Although there is great support for my choice of traits in the trait-based community ecology literature (e.g. Weiher *et al*. 1999; Westoby *et al*. 2002; Wright *et al*. 2004; Violle *et al*. 2007; Lebrija-Trejos *et al*. 2010), fern ecology literature (e.g. Page 2002; Watkins *et al*. 2007) suggests that perhaps I missed some key traits in my study.

Early in my thesis I stated that there were five categories of plant functional traits that affect community assembly: 1) growth form, 2) spatial colonization patterns, 3) productivity, 4) phenology, and 5) reproductive capacity. I included the timing of sporulation as a phenological measure associated with reproduction, and discussed the gametophyte drought tolerance experiment that I attempted but failed to complete. In retrospect it may well be that for ferns, gametophytic traits are more important to the success of fern species over a range of environments than are reproductive traits involving spore production. Therefore omitting gametophytic traits in my analysis of the MaxEnt approach may have been to its detriment.

The logic behind this supposition is that all ferns are able to disperse great numbers of spores over large distances (Dassler and Farrar 2001; Page 2002; Moran 2008), thus reducing the impact of any variation in spore production across species. Moreover, sporophyte recruitment would not be possible without the completion of the gametophyte life stage. In fact, it is apparent that while not all spores will develop into gametophytes, an even smaller number of gametophytes will develop into sporophytes (Flinn 2007). Thus, differential success of gametophytes due to the traits they possess (e.g. drought tolerance) has a large impact on sporophyte recruitment rates. Unfortunately there are few studies examining the ecology of fern gametophytes, so there is very little known about them in comparison to fern sporophytes (Flinn 2006; Watkins *et al*. 2007). Trait-based approaches to fern ecology would benefit greatly from the development of experimental protocols for measuring gametophytic traits. The information made available from such research would allow a new analysis of my data.

Alternatively, perhaps gametophytic traits are in fact beta-traits, and thus do not necessarily constrain species abundance distributions at the alpha-scale. Although there is a lack of information on gametophyte tolerances to various environmental conditions, it is apparent that correlations exist between gametophyte growth form and habitat (Cooke and Racusen 1988; Dassler and Farrar 1997; Watkins 2007). Growth form may affect the drought tolerance of a gametophyte since it affects the amount of moisture that a gametophyte can hold (Dasslar and Farrar 1997). There are five basic growth form types of fern gametophytes: cordiform, strap, ribbon, gemmiferous strap and gemmiferous ribbon; the species I am studying are of the first two forms, which comprise most of the terrestrial fern species in the orders Polypodiales and Osmundales, respectively (Shorina 2000; Farrar *et al*. 2008).

Since my study was focused on the upland hardwood forest of the Gault Nature Reserve, which is characterized as one type of habitat, and the species I studied were of only two types of gametophyte growth form, it can be assumed that a regional environmental filter acted on fern gametophyte form to constrain the beta diversity of ferns. As such, perhaps gametophytic traits do not contain very much information regarding the differential success of fern species along local environmental gradients. Nonetheless, research into the ecology of gametophytes is greatly needed to develop a deeper understanding of fern community assembly processes.

Do predicted abundances reflect where a species <u>can</u> grow rather than where it <u>actually</u> grows?

The importance of gametophytes in fern community assembly may extend past the affects of their traits on constraining SADs at the alpha and beta scales. It is possible that stochastic factors associated with gametophytes that are not explicitly incorporated in the MaxEnt approach cause there to be a difference between the potential and realized relative abundances of fern species in the communities I studied. I am suggesting this because although dispersal limitation is greatly reduced for ferns in comparison to seed-bearing plants, fern gametophyte establishment and survival, and therefore fern sporophyte survival is in part based on stochastic factors and may not be frequent even if fern spore dispersal is good. Spore banks containing extremely high numbers of spores exist for virtually all fern species (Dyer and Lindsay 1992; del Ramirez-Trejo *et al*. 2004). These banks usually contain very large amounts of spores from several species, with higher densities in the upper soil layers, and may persist for decades (Lloyd and Klekowski 1970; Dyer and Lindsay 1992). Disturbance of the top soil layer (e.g. by erosion, animal activity) allows spores from deep within the bank to be exposed to the environmental conditions required for germination. As such, gametophyte germination can be dependent on chance disturbance events, and there is potential for species that are not present in a community at one point in time to establish at a later date and survive in that area. Furthermore, fern gametophytes rely on water films to transfer sperm from the antheridia of male gametophytes to the archegonia of female gametophytes (Klekowsi 1969); the sequence of weather conditions favourable to an episode of potential fertilization has high stochasticity. It is therefore possible that a species may occur in a community but remain unseen as a gametophyte until fertilization requirements are met and a sporophyte grows. This latent establishment of fern sporophytes could have the potential to cause inaccurate predictions of species' abundances, even if the theory behind the MaxEnt approach, and the MaxEnt approach's execution, are correct.

It is possible to test if the stochastic nature of gametophyte establishment and fertilization misled the MaxEnt algorithm and cause inaccurate SAD predictions. In the future a planting experiment could be executed in which species that were not found to occur in a community were planted in that locality. If that species is able to survive and persist in its planted location then it is true that there are other mechanisms other than trait-based environmental filtering driving the assembly of fern communities at the alpha scale.

Conclusions

The MaxEnt approach is a significant step forward in community ecology. It has provided a novel way that to approach the question of how plant communities are assembled from a species pool that draws from both niche and neutral theory. My research, in addition to other recent studies, confirms that community-aggregated traits calculated from observed relative abundances can be used to fit species abundance distributions. My study of the MaxEnt approach therefore supports the idea that trait-based environmental filtering at least in part drives the assembly of communities at a local scale.

While confirming that the MaxEnt approach fits my data and the data of others is an important step in understanding the process of community assembly, the utility of the MaxEnt approach depends on its ability to make predictions in areas in which species' abundances have not already been measured. Such predictive ability would make the MaxEnt approach valuable for conservation efforts, as it could be used to determine the ability of an invasive plant species to colonize an area, as well as the impact of changing environmental conditions on plant communities. My study is one of the first to attempt to go beyond simply predicting species abundance distributions from CAT data that are based on observed abundances; I have used the MaxEnt approach to try to make predictions from CATs that were estimated from data describing environmental gradients.

My research indicates that executing a MaxEnt model in this manner is not straightforward. Despite my trying many different strategies to characterize environmental gradients that might control species abundance, none of the predictions I generated based on the environmental gradients I evaluated were significant. It is difficult to ascertain which traits are most important to constraining community assembly, as well as which environmental factors are most important to filtering species based on their traits. Nonetheless, I have completed an initial exploration of the predictive ability of the MaxEnt approach, and believe that there is still a possibility that the MaxEnt approach to predicting community assembly can work. There have been a number of very recent developments in the MaxEnt literature that can be used to determine how to alter the components of the MaxEnt approach to optimize predictions and provide a more thorough test of the approach. While these new developments can be incorporated into a doctoral thesis extending analysis of my data, they exceed the scope and prescribed time limits of my Master's degree.

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Appendix 1

```
##Code for a circular test of the MaxEnt approach using a uniform prior and
CATs of all 11 traits.  The basic code for this test can be found in the ‘FD’
package in R (Laliberté and Shipley 2010).

require(mgcv)

require(FD)

rm(list=ls())

setwd("C:/Users/Monica/Documents/M.Sc./My Data Analysis/R code for testing
MaxEnt")

monica.abund<-read.table("observed abundances.txt",header=T,row.names=1)

monica.traits<-read.table("average trait values.txt",header=T,row.names=1)

constr<-functcomp(x=monica.traits,a=as.matrix(monica.abund)) # “constraint
means vector” = observed CATs for all 47 plots

states=t(monica.traits) # transposed matrix of average trait values

obs=monica.abund # the observed probability distribution for all of the species in
all 47 plots

mtest<-maxent.test((model=maxent(constr, states, tol = 1e-11, lambda =
FALSE)), obs, nperm = 99, quick = FALSE, alpha = 0.05, plot = TRUE)
#executes the MaxEnt model and the permutation test
```

```
##Code for a non-circular test of the MaxEnt approach using a non-uniform prior, CATs of all 11 traits, and a synthetic environmental gradient defined by the primary axis of a PCA analysis of all of the other univariate environmental gradients.  Eric Pedersen helped me write parts of this code.


require(mgcv)

require(FD)

rm(list=ls())


setwd("C:/Users/Monica/Documents/M.Sc./My Data Analysis/R code for testing MaxEnt")

env.matrix<-read.table("environmental matrix.txt",header=T,row.names=1)

monica.abund<-read.table("observed abundances.txt",header=T,row.names=1)

monica.traits<-read.table("average trait values.txt",header=T,row.names=1)

cat.matrix<-functcomp(x=monica.traits,a=as.matrix(monica.abund)) #calculates CATs for all of the plots based on the observed abundances and average trait values


 temp.data = data.frame(plot.name = 1:47, enviro=env.matrix$PCA1, cat.matrix) #assigns plot names to the plots in their original order

 temp.data = temp.data[order(temp.data$enviro),] #order the plots according to their place along PCA1 environmental gradient

 random.index = c(sample (1:8,2), sample(9:16,2), sample (17:24,2), sample (25:32,2), sample (33:40,2), sample(41:47,2)) #random stratified selection of 12 test plots


test.matrix = temp.data[random.index,]  #12 randomly selected test plots are assigned to a test plot matrix

calib.matrix = temp.data[-random.index,]  #the other 35 plots will be used to fit a calibration GAM

test.cats=test.matrix[,3:13]  #identifies the 11 CATs for each trait in the test plots
```

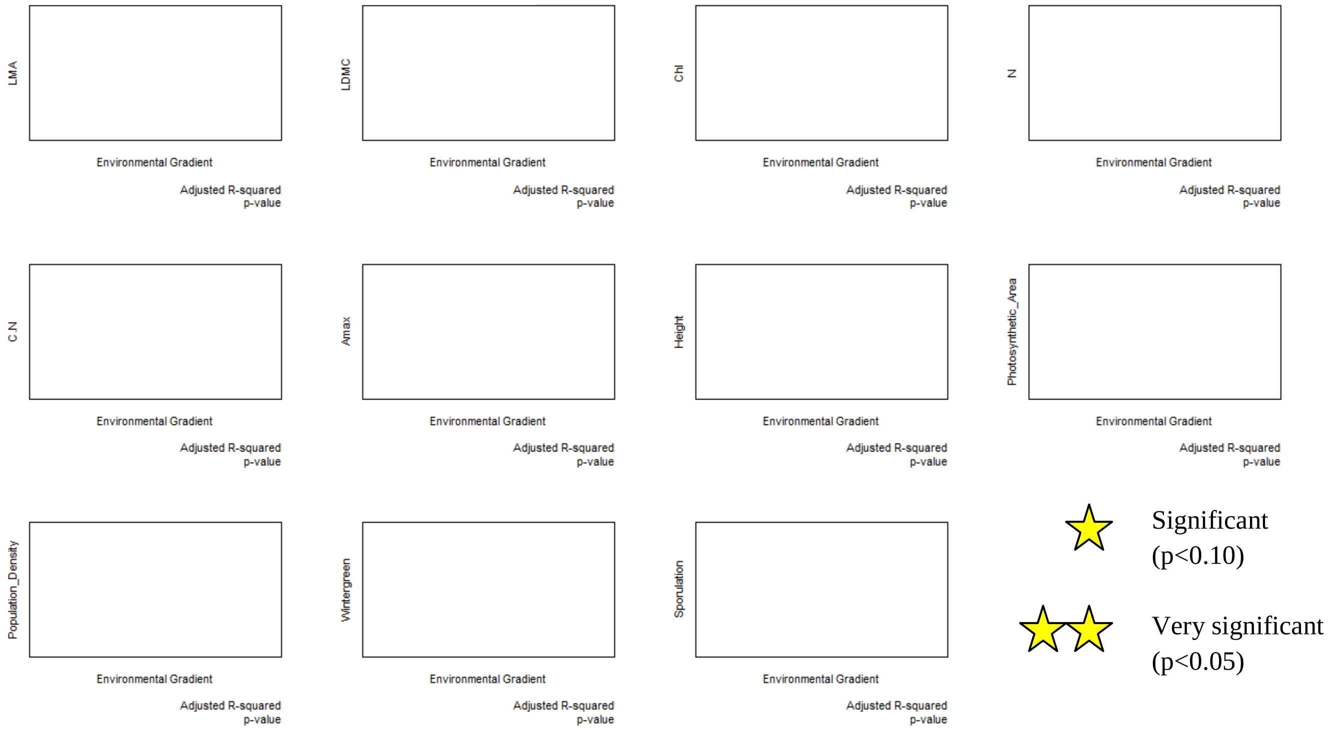
```
calib.cats=calib.matrix[,3:13] #identifies the 11 CATs for each trait in the calibration plots


all.envs=data.frame(plot.name=1:47,env.matrix)

calib.env.plots=all.envs$plot.name%in%calib.matrix$plot.name

calib.envs=env.matrix[calib.env.plots,] #identifies the environment data for the calibration plots


   for (j in 1:11)   #run through each of the 11 CATs in cat.matrix

  {

CAT=calib.cats[,j] #identify the calibration CATs for trait j

env=calib.envs$PCA1 #assigns the environmental gradient as PCA1

gam.model = gam(CAT~s(env)) #fit a GAM to the CAT and environmental gradient data for the 35 calibration plots

test.new = data.frame(env=env.matrix[random.index,"PCA1"],CAT = test.matrix[,j+2]) #new data for predicting CATs in the test plots using the GAM fitted to the calibration plots

constr=predict(gam.model,test.new)  #"constraint means vector" = predicted CATs for the 12 test plots

test.matrix<-cbind(test.matrix,constr) #combines the predicted CATs for the test plots with the observed CATs for the test plots

  }


states=t(monica.traits) #transposed matrix of average trait values

constr<-(test.matrix[,14:24]) #identifies the predicted CATs for the test pots for each of the 11 traits


prior_probabilities_relative_frequency<-read.table("non-uniform prior.txt",header=T,row.names=1)

prior_probabilities=data.frame(plot.name=1:47,prior_probabilities_relative_frequency)
```

```
prior_plots=prior_probabilities$plot.name%in%test.matrix$plot.name

prior=prior_probabilities_relative_frequency[prior_plots,] #identifies the prior probability distribution for all of the species in all of the test plots


observed_probabilities=monica.abund

observed= data.frame(plot.name = 1:47, observed_probs)

observed_plots=observed$plot.name%in%test.matrix$plot.name

obs=observed_probs[observed_plots,] #identifies the observed probability distribution for all of the species in all of the test plots


mtest<-maxent.test((model=maxent(constr, states, prior, tol = 1e-11, lambda = FALSE)), obs, nperm = 99, quick = FALSE, alpha = 0.05, plot = TRUE) #executes the MaxEnt model and the permutation test
```

APPENDIX 2

Appendix GAM figure template. The following pages will include illustrations of GAMs fitted to each CAT along univariate environmental gradients in the page format summarized below. The layout of CATs for each respective trait is fixed and the legend applies ot all the pages in this format.

Figure number and title



CAT Legend: LMA = Leaf mass per area (kg/m^2); LDMC = Leaf dry matter content (mg/g); Chl=Leaf chlorophyll content (mg/m^2); N = Leaf nitrogen content (percentage weight per gram of dry mass); C:N = ratio of leaf carbon to nitrogen content; A_{max} = Maximum photosynthetic capacity (µmol CO_2/m^{2}s); Height (cm); Photosynthetic area (cm^2); Population density (distance to nearest neighbour^{-1}); Wintergreen = Overwintering fronds (binary trait); Sporulation = Timing of spore release (cf. Table 2 for categories).

Figure 1. GAMs fitted to each CAT along a NITRATE GRADIENT with 95% confidence intervals.

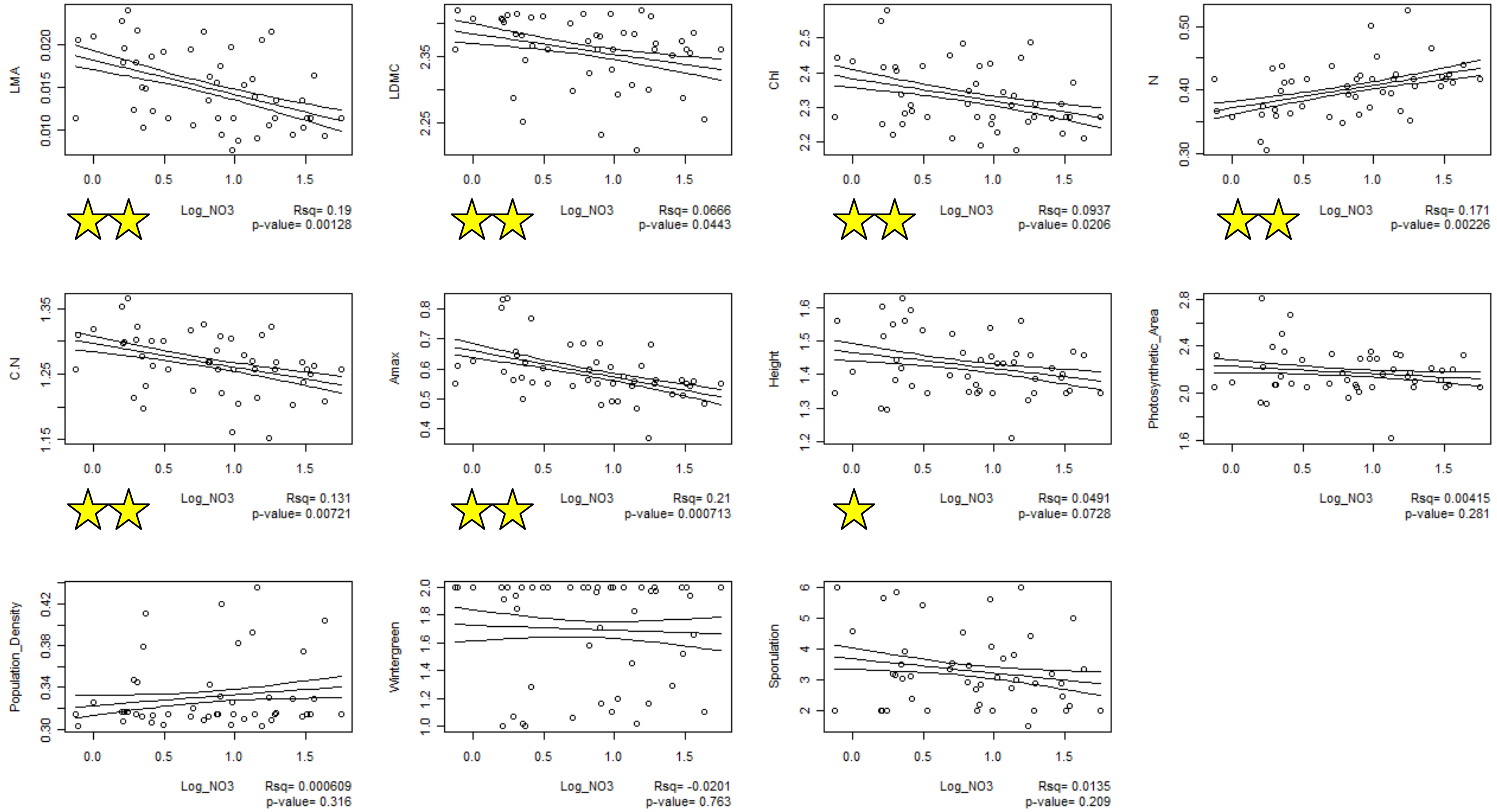


Figure 2. GAMs fitted to each CAT and a PHOSPHORUS GRADIENT with 95% confidence intervals.

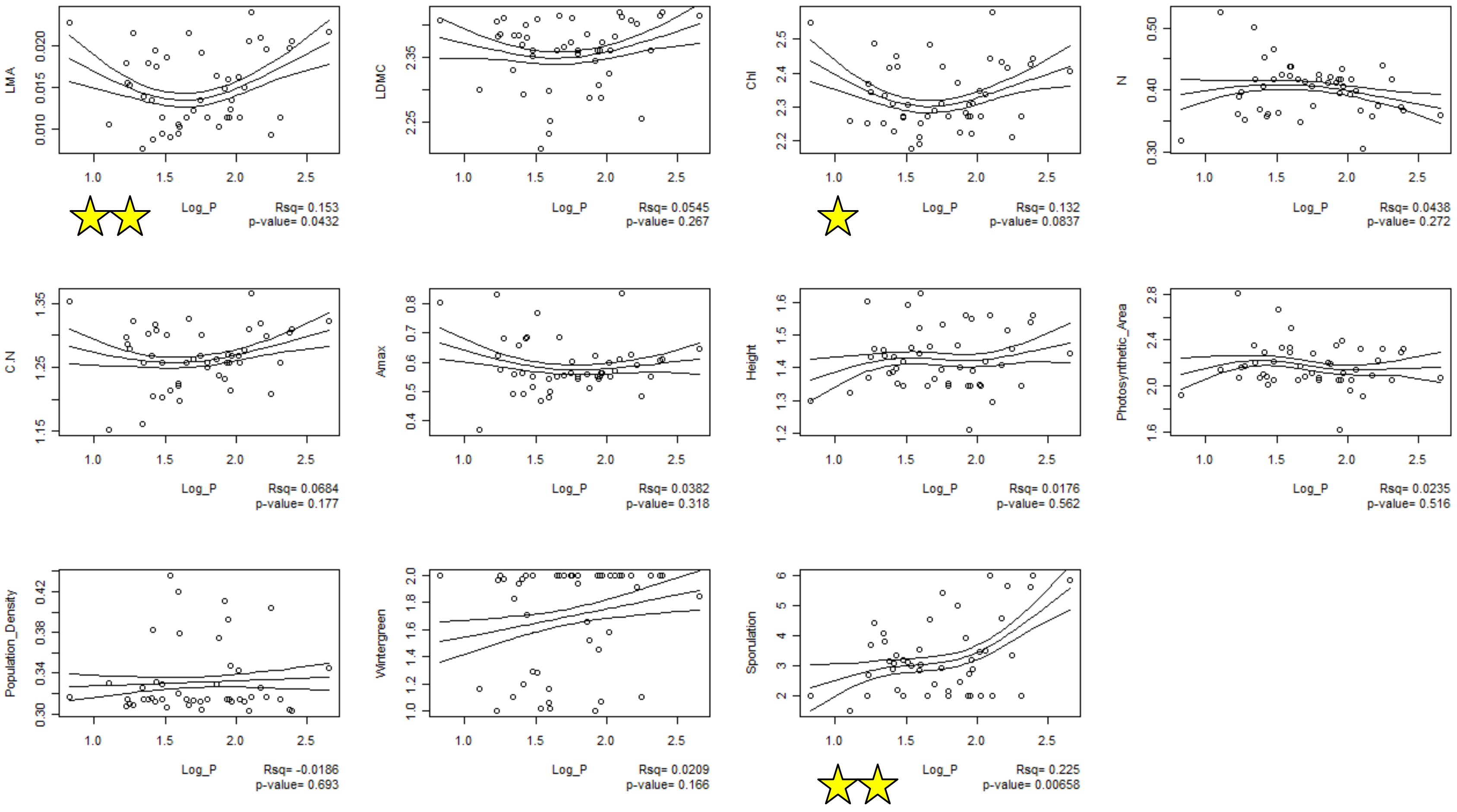


Figure 3. GAMs fitted to each CAT and a POTASSIUM GRADIENT with 95% confidence intervals.

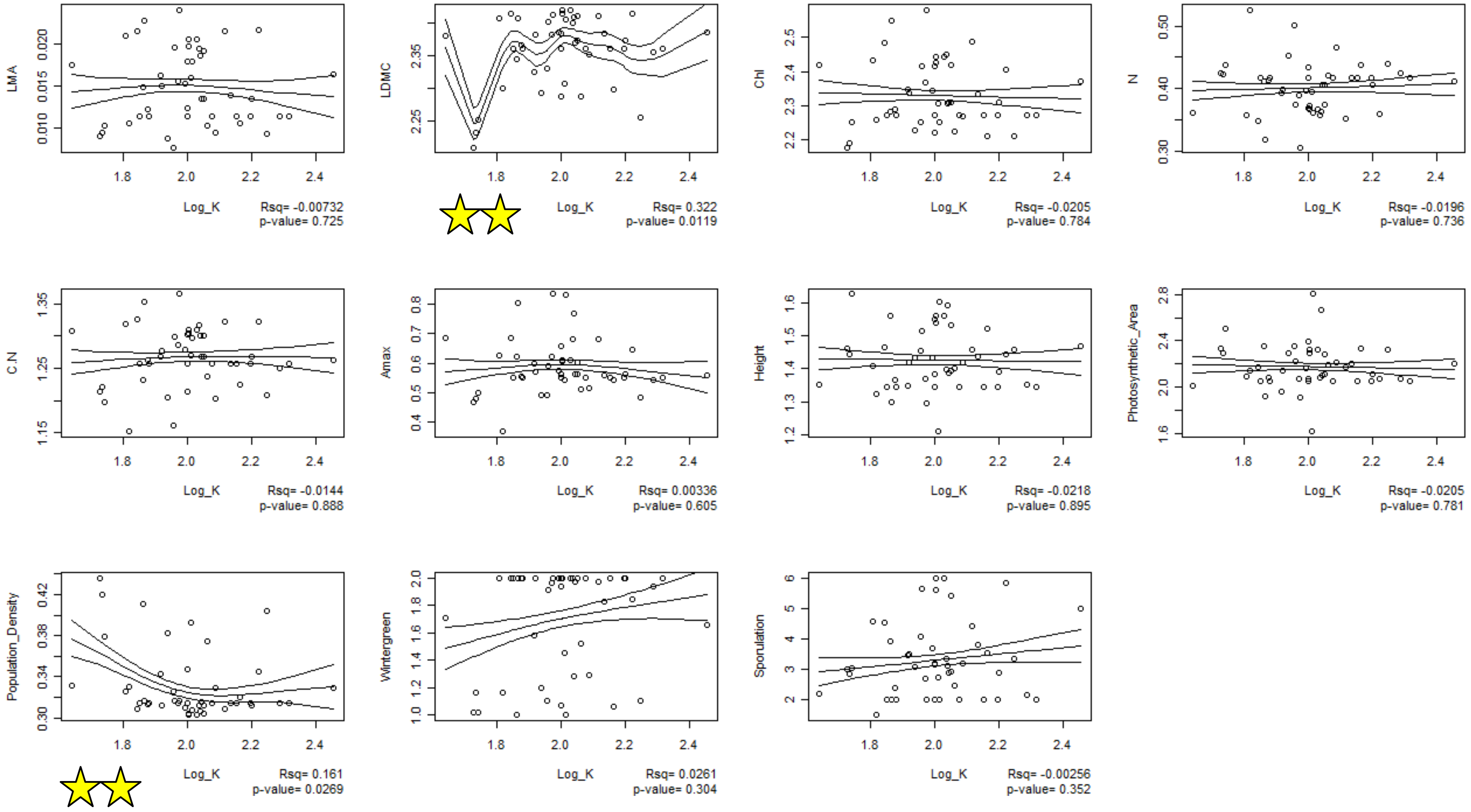


Figure 4. GAMs fitted to each CAT and a CALCIUM GRADIENT with 95% confidence intervals.

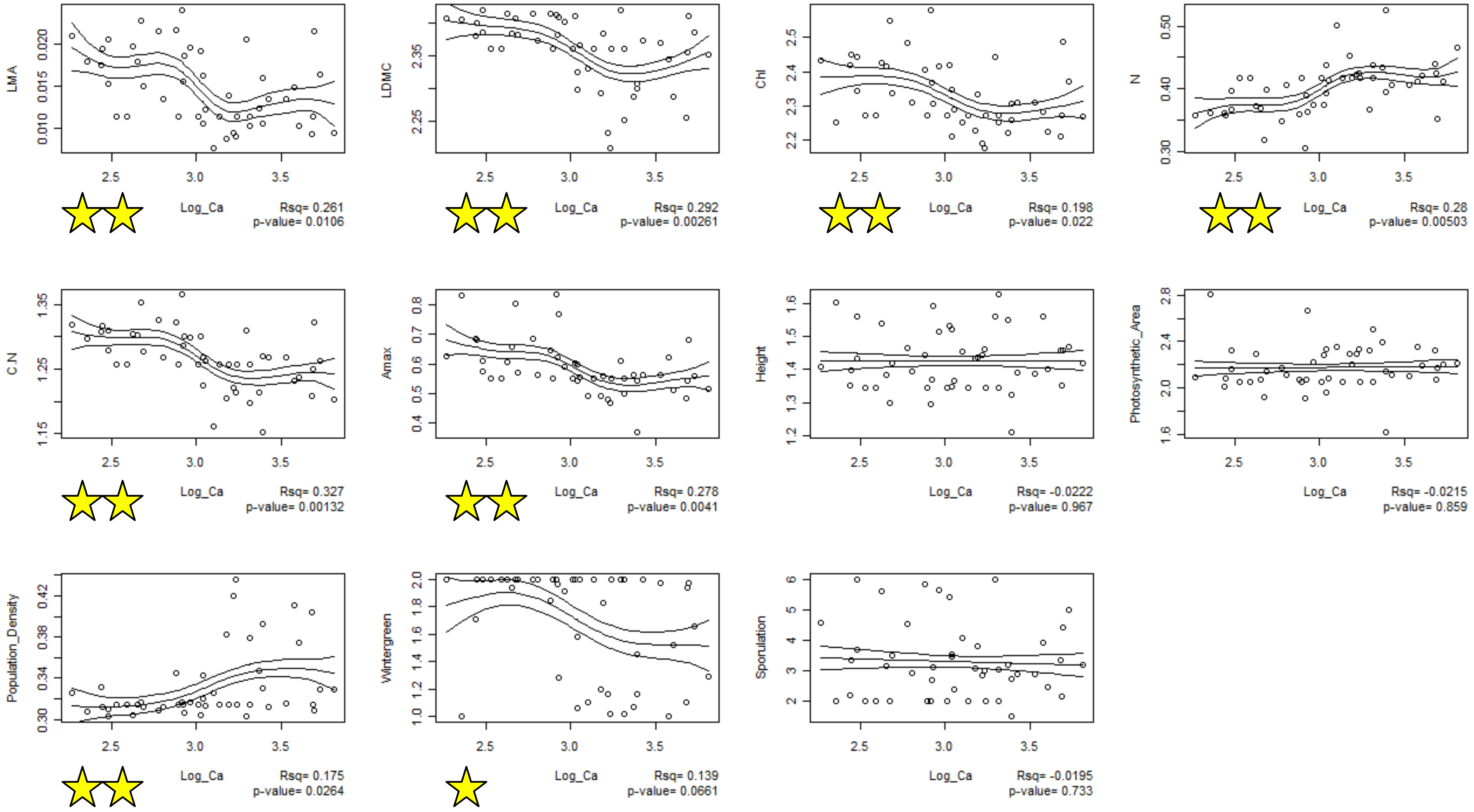


Figure 5. GAMs fitted to each CAT and a MAGNESIUM GRADIENT with 95% confidence intervals.

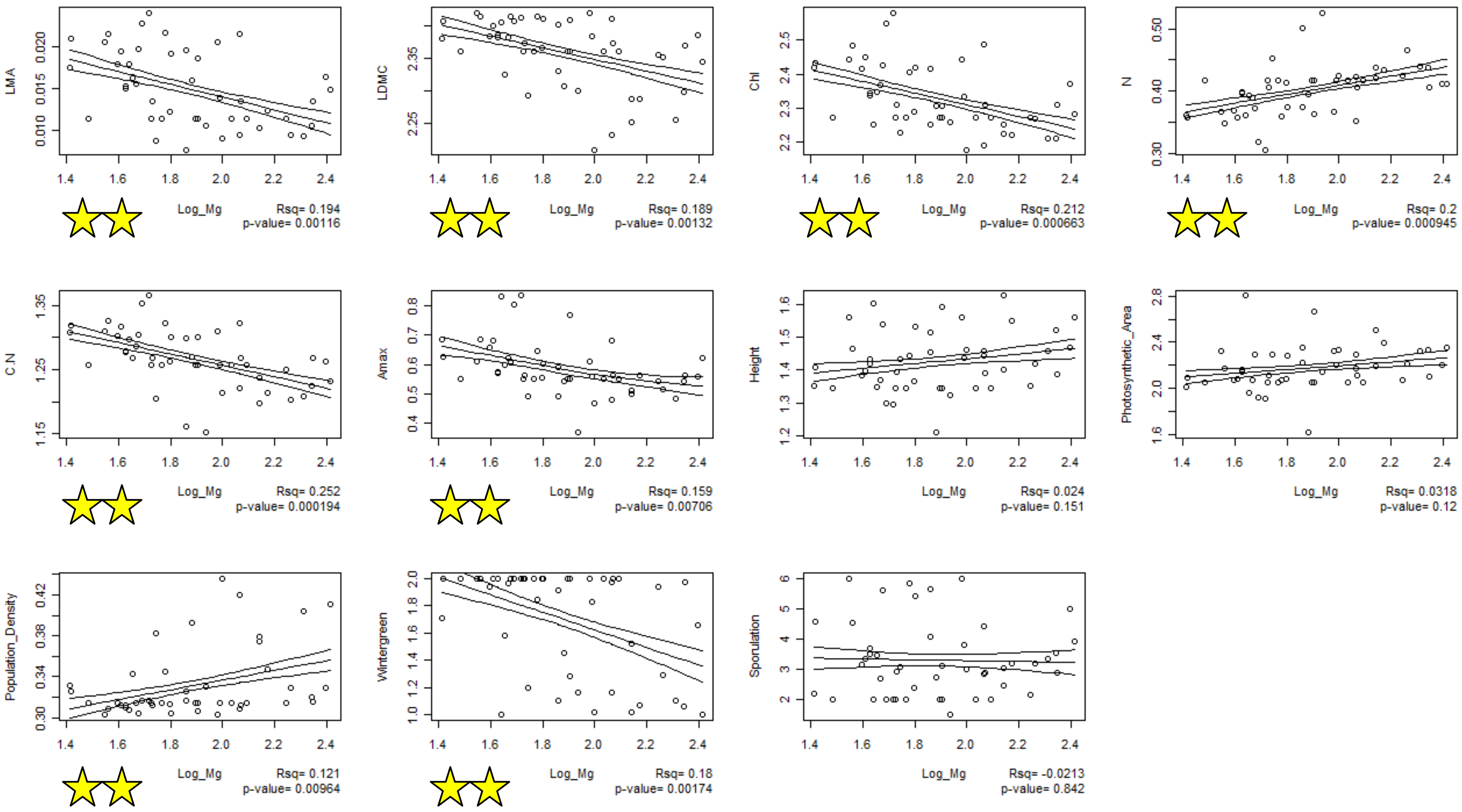


Figure 6. GAMs fitted to each CAT and a pH GRADIENT with 95% confidence intervals.

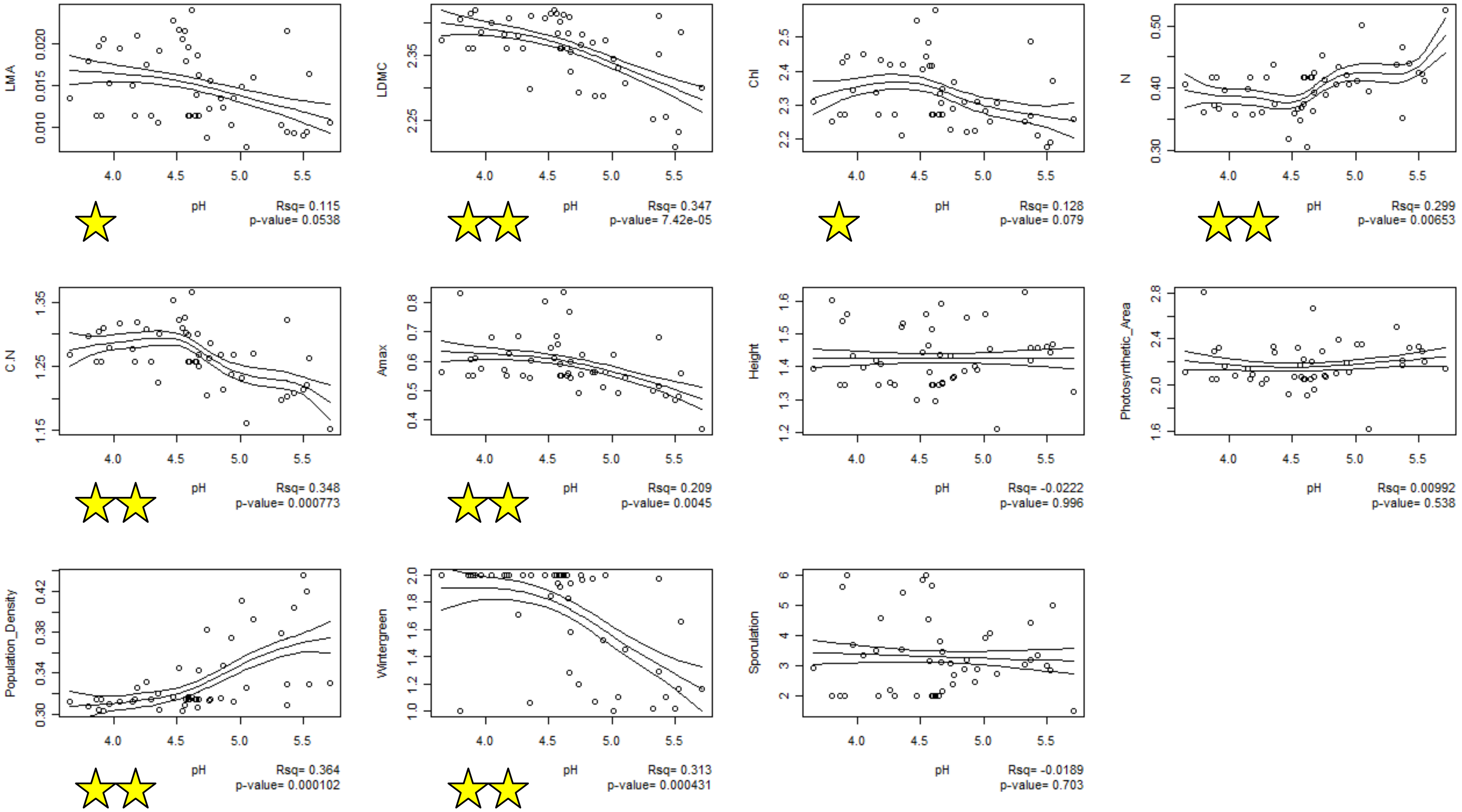


Figure 7. GAMs fitted to each CAT and a MOISTURE GRADIENT with 95% confidence intervals.

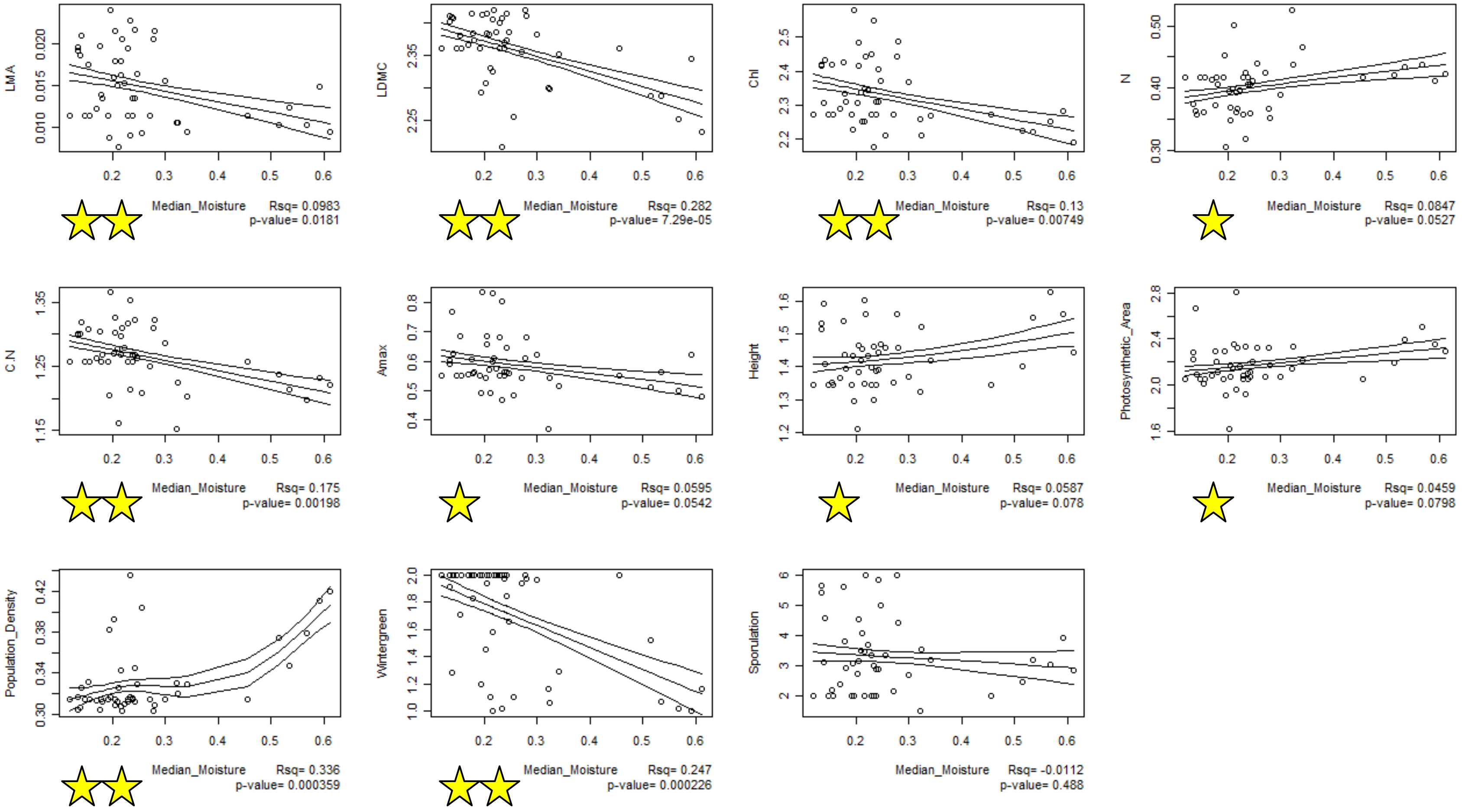


Figure 8. GAMs fitted to each CAT and a %ORGANIC MATTER GRADIENT with 95% confidence intervals.

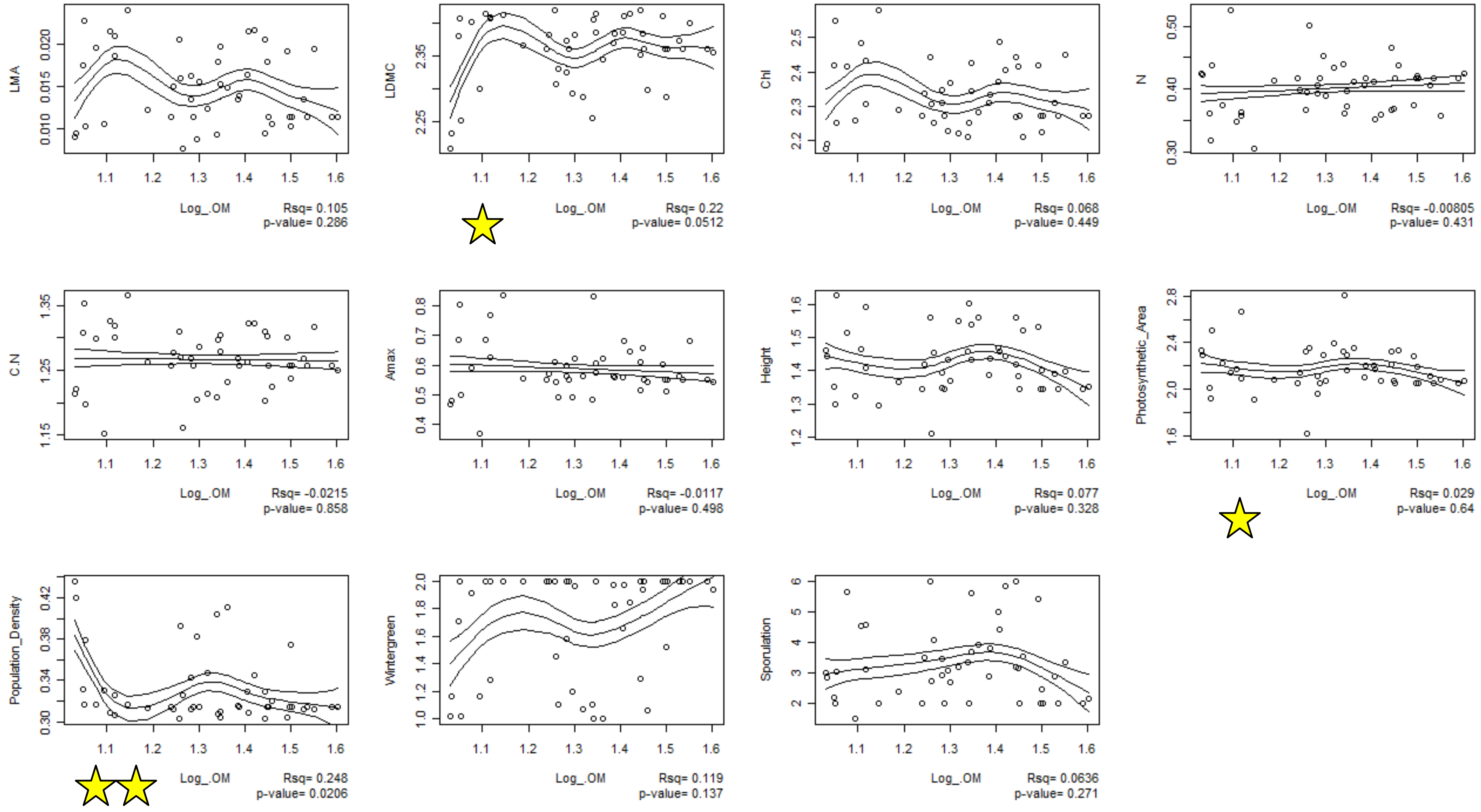


Figure 9. GAMs fitted to each CAT and a TOTAL LIGHTGRADIENT with 95% confidence intervals.

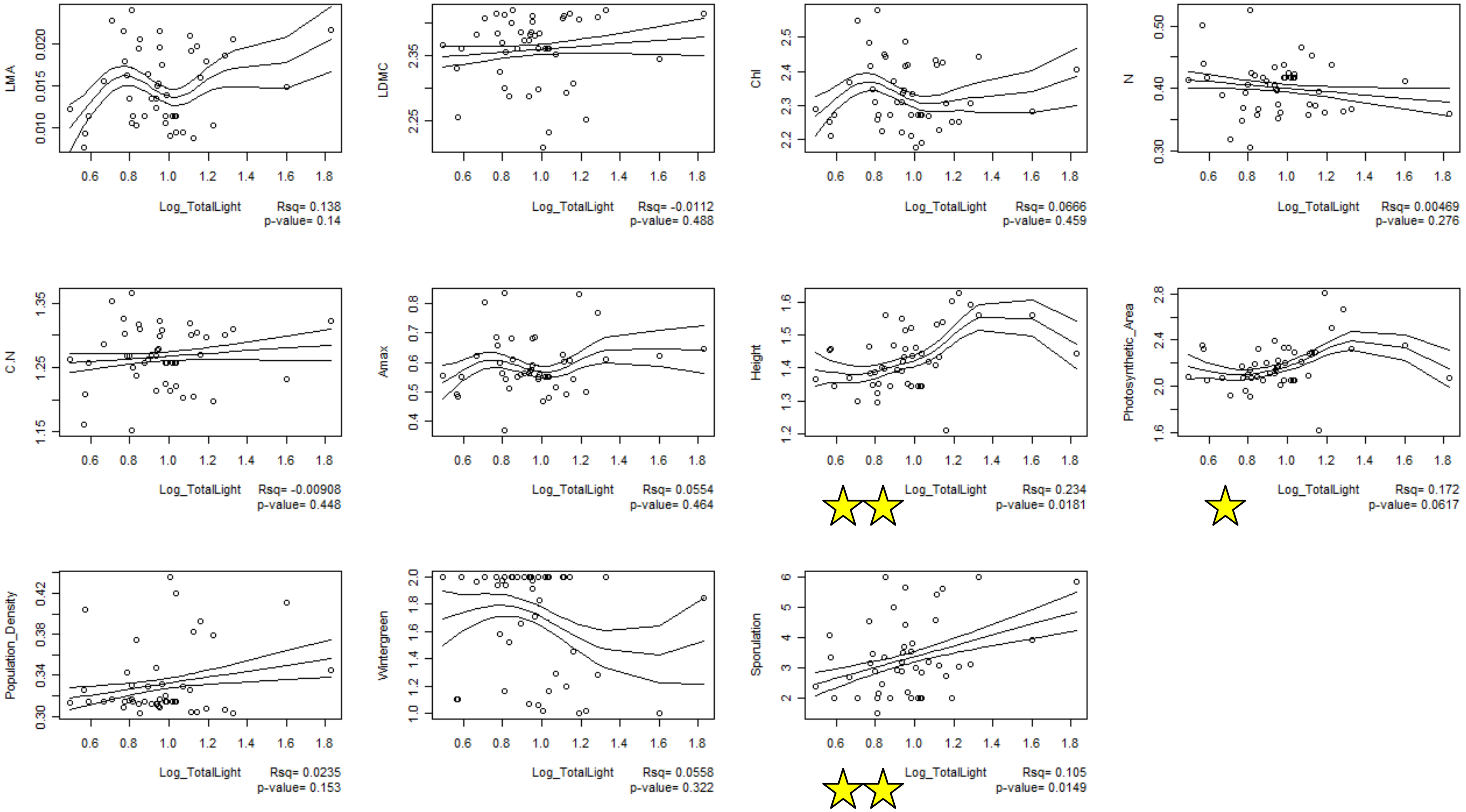


Figure 10. GAMs fitted to each CAT along a synthetic environmental gradient defined by the first axis of a PCA analysis of all of the other univariate environmental gradients, with 95% confidence intervals. The axis accounts for 39.0% of the variance in the data.

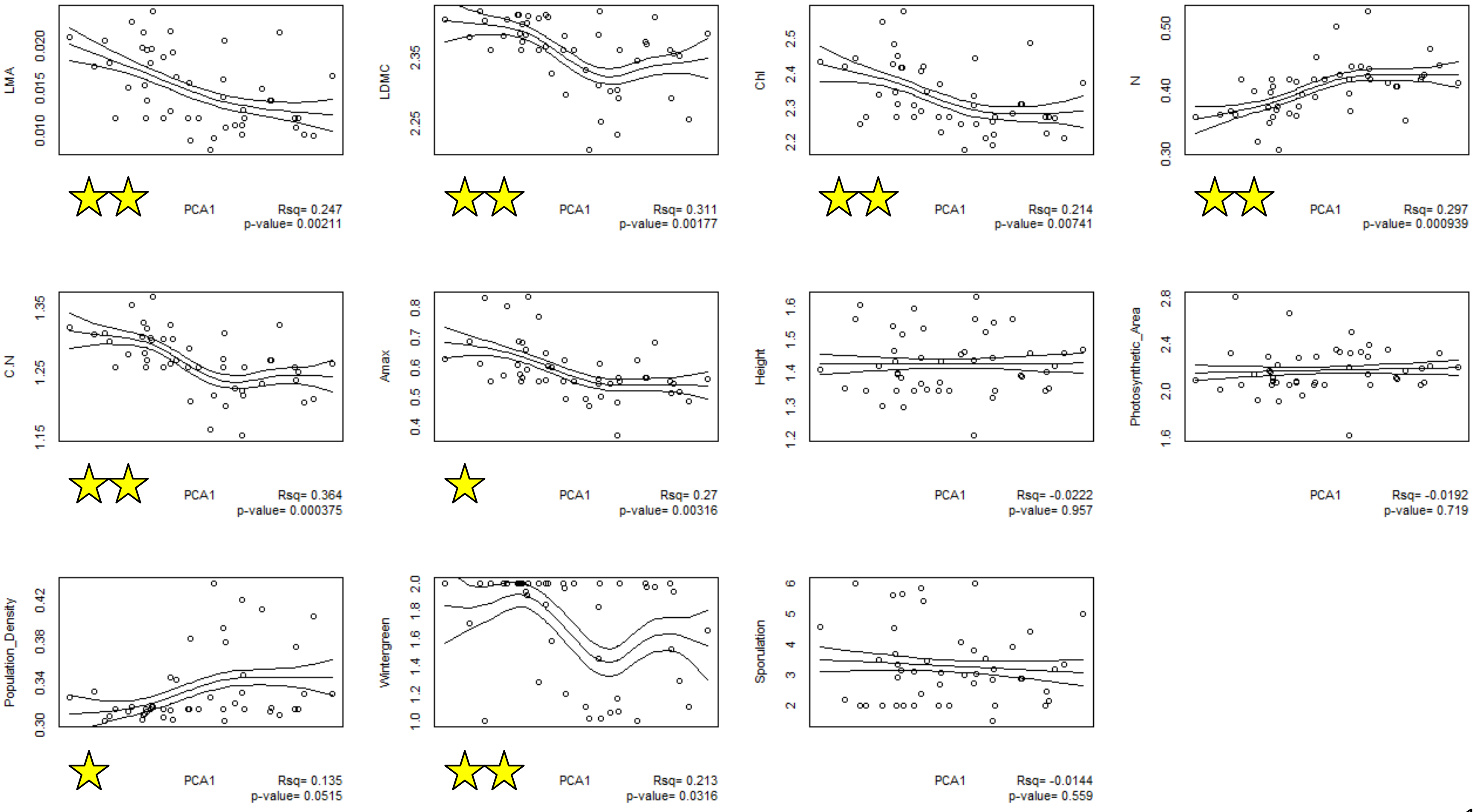


Figure 11. GAMs fitted to each CAT along a synthetic environmental gradient defined by the second axis of a PCA analysis of all of the other univariate environmental gradients, with 95% confidence intervals. The axis accounts for 23.0% of the variance in the data.

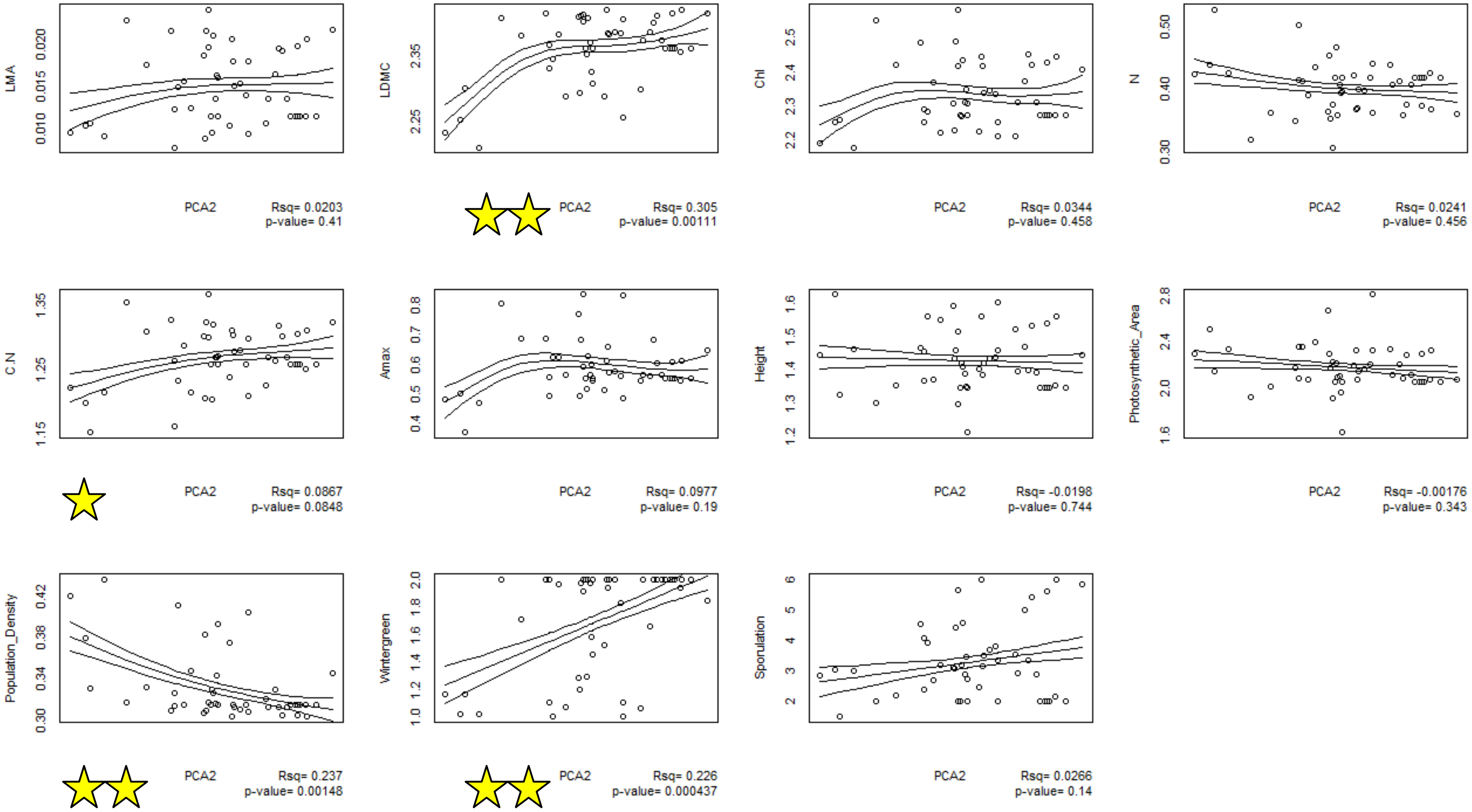


Figure 12. GAMs fitted to each CAT along a synthetic environmental gradient defined by the second axis of a PCA analysis of all of the other univariate environmental gradients. The axis accounts for 15.7% of the variance in the data.

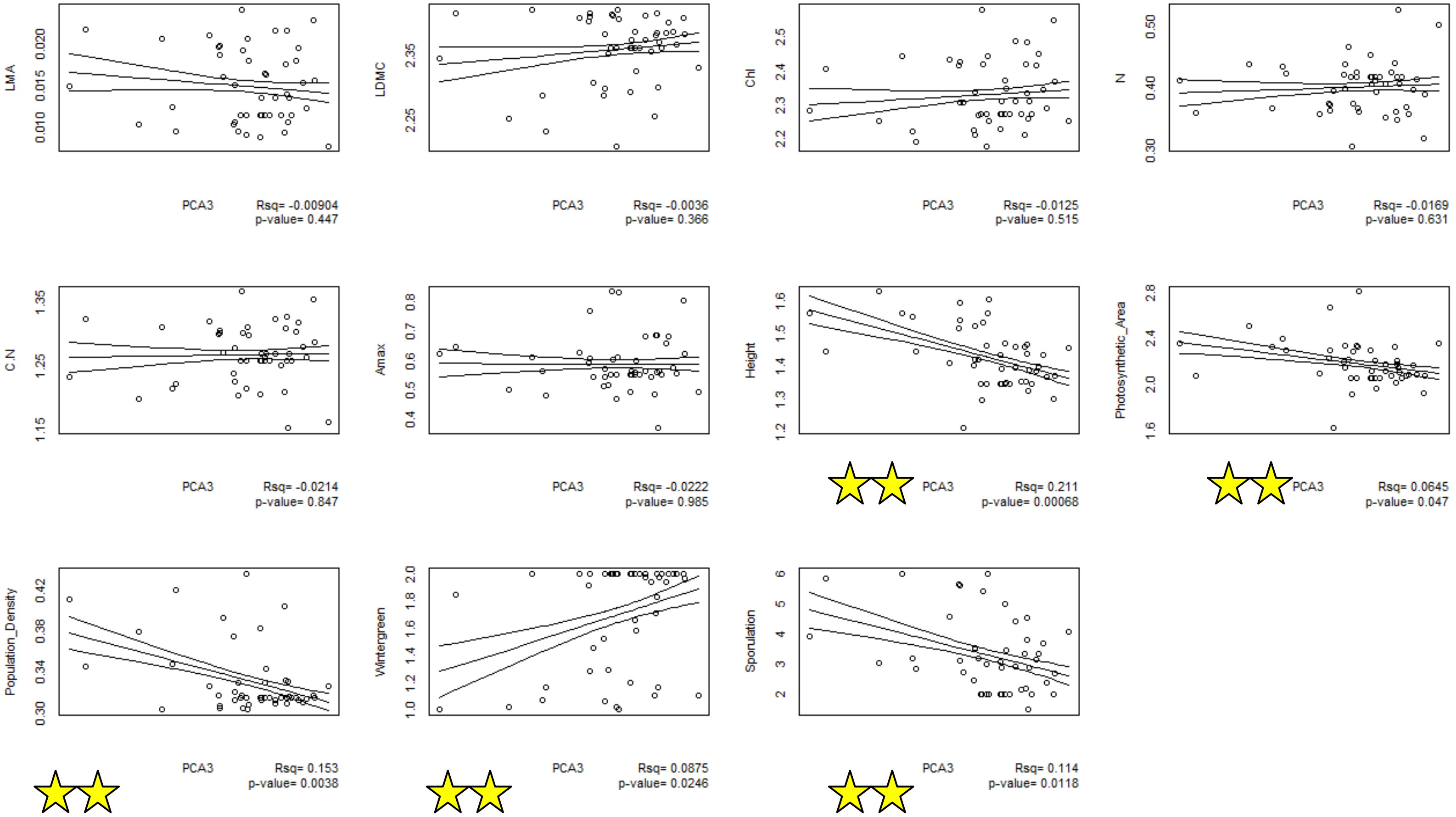


Figure 13. GAMs fitted to each CAT along a composite environmental gradient, the primary axis from a PCA for just the pH and calcium gradients, with 95% confidence intervals; the axis accounts for 89.29% of the variance in the data.