

Linking plant strategies and ecosystem function: an assessment of the contribution of
biodiversity to neotropical grassland productivity

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ABSTRACT

To understand the diversity of strategies of resource use at the species and community level of co-existing species in semi-natural grasslands, I used three different approaches: a) the comparison of above-ground net primary productivity (ANPP) in semi-natural grasslands and sown pastures common to silvopastoral systems in Central Nicaragua and their relationships with rainfall seasonality; b) quantitative ordination of a set of primary foliar traits and another set of traits with less well-documented evidence about their correspondence with the trade-off between resource acquisition and conservation, across various life histories and life forms; c) the determination of community weighted means of plant traits following seasonal shifts in species relative cover, over a year. These issues were investigated in a range of paddocks with two types of cover: semi-natural grassland and sown pastures within an altitudinal range of 200 to 400 m. In nine paddocks, 11 sequential biomass harvests after 22-day periods of grazing exclusion were conducted approximately every month for each paddock. In eight different paddocks 14 morpho-phenological traits were measured in the 36 dominant species. This information was complemented with: i) species cover estimations from five different cover plots in 1 x 1 m² quadrats, located in each of these areas during the period July 2nd 2007 and May 26th 2008, totalling 440 records; ii) soil chemical and physical properties; iii) daily rainfall data. The main axis of species differentiation was identified based on primary or key foliar traits, which provided evidence of a predominant resource economy axis in these grasslands, and a second axis which differentiated life-history and life forms among species. The aggregated morpho-phenological traits showed that the dominance of plants with high foliar concentrations of P, N, Ca, K and Mg, high specific leaf area, long lateral spread by clonal growth, low leaf dry matter content and short leaf lifespan increased in the rainy season and declined towards the dry season. These results suggested the relevance of rainfall seasonality to community functional properties in these grassland assemblages and that species shift their dominance according to the general species level trade-offs between resource acquisition and conservation, in this case based on the seasonal gradient of water and, indirectly, resource supply. There were no differences in annual ANPP between sown pastures and grasslands with both sown pastures and grasslands having low productivity in the late dry season. In contrast, the temporal stability of ANPP was higher in semi-natural grasslands than in sown pastures. Overall, I interpreted that the gains of replacing grasslands with sown pastures are questionable, and need to be weighed against increased costs and the ecological and environmental risks of reducing the diversity of these grasslands.

Keywords: Ecosystem processes, comparative ecology, plant functional traits, rainfall seasonality, resource use gradients, Central America.

DECLARATION

This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree.

Signed.....(Candidate)

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This thesis is a result of my own investigations, except where otherwise stated. Other sources are acknowledged by footnotes giving explicit references. A reference list is appended.

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DEDICATION

To my parents; Saúl, Marleny and my brother,

Who are exceptional workers, inimitable and simple people, the builders of my feelings
and my personal life

To Wojciech Simon Waliszewski,

The landlord, the student, the friend, my “pan” and my future husband

To William Villegas, in Marquetalia, Caldas, Colombia,

My first teacher, the inspiration of my childhood and a smile in my memories

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spread by clonally growth (LSCG), root depth (RD), starting flowering period (SFP) and
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ABBREVIATIONS AND ACRONYMS

ANPP	above ground net primary productivity
ARP22	cumulative rainfall in a 22-day period
LDMC	leaf dry matter content
LFS	length of the period from flowering to seed shed
LLS	leaf life span
LS	leaf size
LSCG	lateral spread by clonal growth
PH	plant height at onset of flowering
RD	root depth
SFP	start of flowering period
SLA	specific leaf area

CHAPTER 1

GENERAL INTRODUCTION

1.1 RATIONALE FOR THE RESEARCH

Conversion of natural forest ecosystems to pasture is one of the major causes of change in the biotic composition of ecosystems globally (Rockström *et al.* 2009), that often results in significant change in bio-geochemical cycling (Hooper *et al.* 2005; Garnier *et al.* 2007). In the humid and sub-humid neotropics, the most widespread type of pasture is that derived from forests to support livestock farming (Fisher *et al.* 1994; Maass 1995). These formations characterize a silvopastoral landscape consisting of a matrix of grasslands with some tree cover (Harvey and Haber 1999). They account for about 90% of the area dedicated to livestock production in Nicaragua and they are the most important forage source for the dual-purpose cattle system which represents 78% of the livestock inventory of Nicaragua (Argel 2006).

Grasslands in the study area are mainly unsown wild herbaceous communities, that I refer to as semi-natural grasslands, because their plant communities are natural, with a predominance of various prostrate grasses of the genus *Paspalum* and are maintained by grazing and the removal of woody vegetation (Ospina 2005).

A constraint of livestock production in Central America is the scarcity of forage, especially during the dry season. Farmers generally cope with seasonal forage constraints by using silage, hay, crop residues and cut-and-carry grasses during the dry season (Dávila *et al.* 2005). However, to utilize these options, producers need capital and

adequate returns for their production, which are often important limitations (Dagang and Nair 2003). Furthermore, the use of introduced grasses implies considerable investments and their management requires large inputs (fertilizers and weed control): in some regions, this only mitigates a lack of forage during the early dry season (Bishop *et al.* 1993).

In Central America, as in other areas with sub-humid and seasonally dry climates, savannas and semi-natural grasslands are often replaced by sown pastures with the aim of increasing the amount and quality of the feed offered to cattle (Dias-Filho 2007). In the area of the research reported in this thesis, this conversion involves replacement of the vegetation cover without changes in resource supply (water and nutrients) for the establishment and management of sown pastures. Despite being the basal resource for livestock production in the region, and in contrast to grasslands and pastures in temperate and subtropical regions, basic knowledge about semi-natural grasslands in the neotropics is fragmented and limited because of the paucity of information about their ecological characteristics (Scurlock *et al.* 2002; Baruch 2005). Moreover, the majority of the existing studies have been focused on the negative aspects of the conversion of rain forest to semi-natural grasslands and the introduction of sown grasses (Serna-Isaza *et al.* 2001).

Ecological theory predicts that changes in the composition and diversity of communities may have significant impacts on ecosystem function in terms of process rates, biomass and element pool sizes and variability, measured by the extent of their fluctuation in space and time (Hooper *et al.* 2005). The effects of community composition and diversity are often linked to particular attributes of the dominant species and to how

these relate to rates of resource acquisition and conservation (Grime 1998; Garnier *et al.* 2004), in interplay with levels of resource supply and disturbance. At the same time, higher productivity can be expected with higher diversity because diverse communities are likely to include combinations of species that are functionally complementary (Hooper *et al.* 2005). It has been found that co-existing species in savannas and grasslands in the neotropics reach peak biomass at different times of the season (Sarmiento 1984; 1992). In contrast, monocultures of sown pastures could be expected to be more productive over short periods of time and at small spatial scales, but probably not during a complete year or at larger spatial scales (Simpson and Langford 1996; Pieters and Baruch 1997; Rivas and Holmann 2005) because in monocultures, productivity is essentially channelled through systems with minimal functional and genetic diversity, with the process of domestication and selection acting on a subset of attributes, narrowing the genetic pool (Dias-Filho 2005). Moreover, to make full use of potentially productive monoculture in seasonally dry environments, the enhancement of the resource supply through inputs such as fertilizer and or irrigation is often required.

Before the dynamics of seasonal patterns of productivity in semi-natural grasslands and sown pastures can be adequately understood, the temporal and spatial variation of the productivity of those systems must be clarified. There have been few studies of semi-natural grassland productivity in the neotropics (Rippstein *et al.* 2001; Rao *et al.* 2001), with earlier studies having focused only on sown pastures, using standing biomass as a surrogate for above-ground primary productivity (Rusch *et al.* 2010a). Within this context, I focus the present research on the temporal variability of the above-ground net primary productivity (ANPP) within a range of semi-natural grasslands and sown

pastures. It is essential to understand the consequences of transforming grasslands into sown pastures considering insights into the amount, and temporal pattern of primary productivity of spontaneous and introduced vegetation common in the region, and their relationships with rainfall seasonality; this potentially provides indications about the consequences of such transformations in view of expected shifts in rainfall patterns anticipated as a result of global warming. Another consideration was that a massive replacement of semi-natural grasslands in the region would be ecologically and economically a high-risk enterprise (Rusch *et al.* 2010a); important because the functional and genetic diversity, which may confer a buffer mechanism to spatial and temporal shifts in the biophysical environment (Loreau *et al.* 2002), would be reduced.

1.2 WHY DRY SEASONAL SEMI-NATURAL GRASSLANDS?

The natural vegetation in Central Nicaragua corresponds to a transitional tropical sub-humid forest (Holdridge 2000), with semi-deciduous vegetation, and is referred to as seasonally dry tropical forest (Bullock *et al.* 1995). Semi-natural grasslands have become the most widespread type of vegetation after the clearing of this forest vegetation; therefore it is axiomatic that these systems experience pronounced rainfall seasonality.

That particular feature provides an interesting setting for the study of patterns of trait differentiation and of plant strategies along major environmental gradients, especially those related to temporal patterns of nutrient and water supply. Since water supply is a primary determinant of seasonal plant growth (Bonnet *et al.* 2010), and the amount of water available is directly linked to the capacity of the plant to capture nutrients (Weltzin *et al.* 2003), the temporal pattern of rainfall represents, locally, the most important resource supply gradient in these types of pasturelands.

Other relevant aspects of these grasslands are that they are relatively rich plant communities that are composed primarily of species native to Central America (ca 75 % of the plant cover). A total of 158 herbaceous species have been recorded in the study area of the present research, with 36 species in the grass family (Poaceae), which comprise 51 % of the total number of species (Ospina 2005). Another evident feature is that soils are spatially variable in these areas (Chapter 4), adding complexity to the understanding of temporal gradients of resource supply, and the need for special attention and effort on methodologies that allow and control for the various sources of variability.

1.3 PLANT FUNCTIONAL ATTRIBUTES: LINKING BIODIVERSITY AND ECOSYSTEM FUNCTION

A plant functional trait is any trait that impacts on plant fitness because it affects vegetative and reproductive output and plant survival (*sensu* Violle *et al* (2007)). These attributes of plants can be interpreted as specializations that mediate their physiological and ecological responses to the environment (Semenova and van der Maarel 2000; Garnier *et al.* 2001) and consequently they provide a most promising avenue for a mechanistic understanding of vegetation dynamics and for predicting responses to various ecological conditions in changing landscapes (Weiher and Keddy 1995).

Since ecosystems are experiencing unprecedented land use and climate change (Rockström *et al.* 2009), the knowledge of physiological and morpho-phenological characteristics of plants has become increasingly relevant in a global context (Lavorel *et al.* 2007; Suding *et al.* 2008). Recent research findings indicate that knowledge of vegetation based on the traits of the component species is a valuable framework for up-

scaling plant responses to environmental change at the community level, and for predicting how changes in plant composition will affect ecosystem functioning (Lavorel *et al.* 2007; Suding *et al.* 2008).

At local scales, knowledge of these characteristics offers interesting perspectives for tracing mutual feedback between global scenarios, locally dominant plant characteristics, ecosystem properties and ecosystem goods and services (Chapin 2003; Díaz *et al.* 2007; Díaz *et al.* 2007a). For example, in the French Alps, where community farming is based on sheep and cattle production, Quétier *et al.* (2007) showed how plant traits and classifications based on these traits were effective predictors of grassland services, such as forage and land stewardship.

1.4 AXES OF TRAIT DIFFERENTIATION AND PLANT STRATEGIES

Plant ecological strategy schemes (Grime 1977; Westoby *et al.* 2002) classify plants according to morpho-physiological axes of plant specialization or axes of ecological differentiation. These axes capture a large portion of the plant trait variation into “plant strategies” that encompass trade-offs in the allocation of resources to different tissues and plant organs (Grime 1977; Westoby 1998). Among the several axes of trait differentiation that have been identified (e.g., Westoby *et al.* 2002); the resource economy axis seems to be the most striking. It distinguishes between species that have high rates of resource acquisition, circulation and growth, low resource use efficiency, and that are successful at high levels of resource supply; and species that have a strategy of conservative resource use, low demand, low growth rates, that perform well in chronically poor environments (Grime *et al.* 1997; Díaz *et al.* 2004; Wright *et al.* 2004).

A series of foliar traits are widely recognized as powerful indicators of this functional trade-off, but there are also indications of a covariance with other sets of traits strengthening the concept of plant strategies of resource use (Sammul *et al.* 2003; Rusch *et al.* 2010b). The understanding of these fundamental trade-offs in plants can help to predict the effects of environmental change on species composition, community properties and ecosystem processes and services (Suding *et al.* 2008). This approach also allows an improved understanding of species responses to local sources of stress and disturbance (Díaz *et al.* 2004; Lavorel *et al.* 2007).

Plant functional traits not only represent plant strategies for growth and survival, they also influence important ecosystem processes (Chapin *et al.* 2000). The functional properties of individuals and populations, represented by plant traits can be scaled up to communities and ecosystems (Díaz *et al.* 2004; Violle *et al.* 2007) through the biomass ratio hypothesis (Grime 1998), according to which the trait values of the most abundant species at any given time will capture the magnitude of the ecosystem properties (Garnier *et al.* 2004).

The functional trait composition of an assemblage, calculated as the community weighted mean of a trait (henceforth, CWM) or the community aggregated trait, is a synthetic metric for up-scaling species functional characteristics to communities representing the dominant traits values in a community (Violle *et al.* 2007). The CWM has been promoted and used for the evaluation of how changes in community structure and composition affect ecosystem processes (Garnier *et al.* 2004; Garnier *et al.* 2007; Díaz *et al.* 2007). Generally, species differentiation is large compared to within species differences, justifying the calculation of CWM based on trait values averaged within

species. However, when within-species trait variability can be large, and therefore in these cases an approach that incorporates within species variation, i.e. through population or case-specific averages should be more appropriate (Garnier *et al.* 2004; Garnier *et al.* 2007; Reiss *et al.* 2010).

The aim of this thesis is to determine the main patterns of morpho-phenological trait differentiation among species in seasonally-dry tropical grasslands in Central Nicaragua as possible determinants of seasonal changes in primary productivity, of plant responses to environmental factors. In this way, I expect to improve the current understanding of the diversity of species strategies and community functional parameters of these communities.

Additionally, and beyond the role of main patterns of species trait differentiation, I expect to provide the first insights about the amount and the temporal pattern of the ANPP of the spontaneous and introduced vegetation common to these regions and their relationships with rainfall seasonality.

1.5 STUDY SITE

The study was conducted in the Río Grande de Matagalpa watershed in Central Nicaragua (12°31'-13°20'N; 84°45'-86°15'W). The area is located in the municipality of Muy Muy, at sites within an altitudinal range of 280 to 380 m. The predominant land-use is livestock farming with relatively homogeneous livestock management. Livestock production is generally extensive, with an average-stocking rate of 1 livestock unit ha⁻¹ and pasture management consisting of rotational grazing, fence maintenance and weeding, either manually by chopping down weeds or recently by the use of herbicides. During the dry season, grazing frequency is irregular.

The natural vegetation of the region corresponds to a transitional tropical sub-humid forest (Holdridge 2000) with semi-deciduous vegetation, and is referred to as seasonally dry tropical forest (Bullock *et al.* 1995). The vegetation in the study is an assemblage of native and naturalized species including grasses, herbs and woody plants. The term semi-natural grassland is used herein to refer to a pastureland area covered by spontaneous vegetation that grows naturally after forest clearing or on fallow land and which is maintained by grazing management, including fencing and weed control. Semi-natural grasslands often occur in association with scattered trees and consist mostly of grass species of the genus *Paspalum*.

Geologically, the area consists of tertiary volcanic tuff, a type of pyroclastic rock. The tuff presents a clear stratification, including softer and harder layers. It is probable that many of the landscape undulations are associated with differences in the hardness of the rocks and their variable resistance to erosion. Furthermore, tectonic movements are likely to have influenced the landscape, especially in the transition to the higher mountainous landscape of the Muy Muy municipality. Tuff in the area seems to be relatively impermeable, which explains the limited infiltration of rainwater observed during the early rainy season, especially in flat areas. Even on slopes, there are extensive areas where drainage is limited during part of the rainy season. In most flat areas, it is common to find soils with vertic properties: soils present wide cracks during the dry season and have prismatic structures, while on steeper slopes soils are more variable (Andreas Nieuwenhuyse, pers. comm. 2007).

The topography of the study area is undulating, with slopes of 5-40%, and the dominant soil type in the study sites is a greyish to black coloured vertisol with high organic

matter content in the upper horizons (5-8%), clayey subsoil, with a pH between 5.9 and 6.8 in the topsoil, and relatively poor in phosphorous (2-10 ppm). In most cases, the soil has limited drainage during the rainy season. Where the natural slope favours better drainage, browner colours are observed.

1.6 GENERAL OUTLINE OF THE THESIS

In this thesis, I aimed to understand of the main patterns of morpho-phenological trait differentiation and community functional properties that vary with seasonal changes according to rainfall, and that can affect the main patterns of primary productivity. In Chapter 2, I examine the amount and pattern of productivity of semi-natural grasslands and sown pastures, and their relationships with rainfall seasonality. Based on this knowledge, patterns of rainfall during a year (directly) and seasonal primary production (indirectly) are incorporated in later chapters with the hypothesis that the high species diversity in grasslands would encompass diverse vegetative attributes, reproductive phenologies and strategies for resource use. In Chapter 3, I explore the presence of axes of species trait differentiation with respect to resource use, and the co-variation across life histories and life forms of a set of primary foliar traits, specific leaf area, leaf dry matter content, leaf lifespan and the concentrations of leaf nitrogen and phosphorus with other traits with less well-documented evidence about their correspondence with the resource use trade-off. These traits were plant height, leaf size, leaf calcium, potassium, and magnesium concentration, lateral spread by vegetative growth, root depth, start of flowering period, and length of the period from flowering to seed shed. Furthermore, the notion of the resource economy axis as a primary specialization of species for resource use is discussed. In Chapter 4, I determine the community aggregated composition of

traits that correspond with the species strategies for resource use and how these community trait averages shift with time and according to seasonal changes in rainfall. This is done by establishing a community weighted mean of each trait. Here, I discuss the results in the light of concepts about differentiation and specialization of species through shifts in dominance along a seasonal sequence of resource availability change. In Chapter 5, I discuss and summarize the results that were obtained in the previous chapters. I also discuss the possible influence of omitted sources of variation in species traits and the community properties that were not considered in the different chapters. I conclude by identifying some questions not answered in this study and point to needs for further research.

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CHAPTER 2

BIODIVERSITY INCREASES SEASONAL STABILITY OF PRODUCTIVITY IN A SEASONALLY DRY CLIMATE¹

ABSTRACT

This article examines how above-ground net primary productivity (ANPP) in semi-natural grasslands and in sown pastures in Central America responds to the rainfall regime by: (i) assessing the relationships between ANPP and accumulated rainfall and indices of rainfall distribution, (ii) evaluating the variability of ANPP between and within the seasons, and (iii) estimating the temporal stability of ANPP in both vegetation types. We conducted sequential biomass harvests during 12 periods of 22 days for semi-natural grasslands and sown pastures and related them to rainfall. There were significant relationships between ANPP and cumulative rainfall in 22-day periods: a model including a linear and quadratic term showed the best fit. There was also a significant correspondence between ANPP and the number of rainfall events. Annual ANPP was similar in semi-natural grasslands and sown pastures. Sown pastures had higher ANPP increments per unit rainfall and higher ANPP at the peak of the rainy season. In contrast, semi-natural grasslands showed higher ANPP early in the dry season. The temporal stability of ANPP was higher in semi-natural grasslands than in the sown pastures in the dry season ($p=0.0402$), and in the annual cycle ($p=0.0127$). We conclude that contrary to

¹ A modified version of this chapter will be submitted for publication as: Ospina, S.D., Rusch, G.M., Pezo, D., Casanoves, F., Sinclair F. Biodiversity increases seasonal stability of productivity in a seasonally dry climate.

conventional thinking amongst pasture scientists, there appears to be little justification for replacing semi-natural grasslands with sown pastures in seasonally dry climates if the aim is to increase ANPP.

2.1 INTRODUCTION

Conversion of natural forest ecosystems to pasture is one of the major causes of change in the biotic composition of ecosystems globally (Rockström *et al.* 2009), that often results in significant change in bio-geochemical cycling (Garnier *et al.* 2007; Hooper *et al.* 2005). In the humid and sub-humid Neotropics, the most widespread type of pasture is that derived from forests to support livestock farming (Sarmiento 1984; Fisher *et al.* 1994; Maass 1995).

Typically, a silvopastoral landscape results, comprising a matrix of grasslands with some tree cover (Harvey and Haber 1999; Cajas-Giron and Sinclair 2001). The grasslands are mainly unsown wild herbaceous communities that we refer to as semi-natural grasslands, because their plant communities are natural, with a predominance of various prostrate grasses of the genus *Paspalum* and are maintained by grazing and the removal of some woody vegetation. Their large extent implies that they have a significant role in the global carbon cycle but their contribution is not well understood because of the paucity of high quality data on primary productivity and limited information about their ecological characteristics (Scurlock *et al.* 2002; Baruch 2005). In much of tropical America these pastureland types occur in areas where the natural vegetation has been classified as seasonally dry forest, characterized by a strongly seasonal growth pattern determined by the distribution of rainfall (Sarmiento and Pinillos, 1999).

Primary productivity and total rainfall are known to correspond closely in sub-humid and arid regions (Knaap *et al.* 2006; Baeza *et al.* 2009) and long-term data show a significant linear relationship between those variables for many sites around the world (Lauenroth and Sala 1992; O'Connor *et al.* 2001; Knapp *et al.* 2006). An understanding of the response of above-ground net primary productivity (ANPP) to rainfall becomes especially important in view of global warming. For Central America, scenarios for the dry and wet seasons predict a relative decrease in rainfall of 10 and 20%, respectively, for the period 2090-2099, relative to 1980-1999 (IPCC 2007).

In seasonally dry climates, primary productivity is affected not only by the total annual or seasonal rainfall, but also by rainfall distribution (Camberlin *et al.* 2009; Snyman 2009; Swemmer *et al.* 2007). For semi-arid systems it has been suggested that the majority of the primary productivity occurs in the form of short-duration pulses following rainfall events (Noy-Meir 1973) and in a mesic tall-grass prairie, Knapp *et al.* (2002) found that when the total rainfall for the season was kept constant, extending the dry interval between rainfall events reduced ANPP by about 10%; however, a correspondence between primary productivity and distribution of rainfall has not been well established for tropical sub-humid regions. Specifically in Central America, there is little published information about inter-seasonal variations of rainfall components (total amount, number of rain events, rain event size, length of dry intervals), but some evidence from climatically similar regions shows that seasonal rainfall distribution is more closely correlated to primary productivity than the overall amount of seasonal rainfall (Marengo *et al.* 2001). In five tropical regions including the Brazilian Northeast, inter-annual variations in the number of rainy days within a season were more highly

correlated with ANPP than fluctuations in the total seasonal rainfall (Moron *et al.* 2007), but it remains unclear whether different rainfall components have varying effects depending on the kind of vegetation cover.

At local levels, factors other than climate, including the composition of vegetation, become important determinants of productivity (Hector *et al.* 1999), but whether different rainfall components have varying effects depending on the kind of vegetation cover remains unknown. This knowledge is important, particularly because human interventions often change the vegetation cover. Both community composition and ground vegetation cover may alter the relationship between productivity and annual rainfall (O' Connor *et al.* 2001; Chidumayo 2003), therefore site-specific models are required for effective prediction of production in response to climate (O' Connor *et al.* 2001). In Central America, as in other areas with sub-humid and seasonally dry climates, savannas and grasslands are often replaced by sown pastures with the aim of increasing the amount and quality of feed offered to cattle (Dias-Filho 2007). In the area of the research reported here, this conversion involves replacement of the vegetation cover without changes in resource supply (water and nutrients). Ecological theory predicts that changes in the composition and diversity of communities may have significant impacts on ecosystem function in terms of process rates, biomass and element pool sizes and variation, measured by the extent of their fluctuation (Hooper *et al.* 2005). The effects of community composition are often linked to particular attributes of the dominant species and to how these relate to resource acquisition rates (Grime 1998; Garnier *et al.* 2004) and their response to disturbance (Garnier *et al.* 2007).

Higher productivity can be expected with higher diversity because diverse communities are likely to include combinations of species that are functionally complementary (Hooper *et al.* 2005). Co-existing species in savannas and grasslands in the Neotropics reach peak biomass at different times of the season (Sarmiento 1984). These patterns could be explained by specific differences in traits that determine plant responses to between- and within-seasonal variations in rainfall. In contrast, monocultures of cultivated varieties could be expected to be more productive over short periods of time and at small spatial scales, but probably not during a complete year or at larger spatial scales (Simpson and Langford 1996; Pieters and Baruch 1997; Rivas and Holmann 2005) because in monocultures, productivity is essentially channelled through a single life form and the process of domestication and selection acts on a subset of attributes, narrowing the genetic pool (Dias-Filho 2005). Often in seasonally dry environments inputs, such as fertilizer and/or irrigation, are required for monocultures to be productive. Whereas annual ANPP is a measure of the annual ecosystem function (Fahey and Knapp 2007), the variability of ANPP through the season provides an insight into how an ecosystem responds to fluctuations in rainfall within a year (Swemmer *et al.* 2007). Variability in productivity has been strongly associated with grassland stability (Elton 1958), and through the concept of community temporal -stability, linked to species diversity (Tilman 1999).

The research reported here focuses on the temporal variability of ANPP within a range of semi-natural grasslands (hereinafter, grasslands) and sown pastures. An insight into the amount and pattern of primary productivity of the spontaneous and introduced vegetation common to the sub-humid regions of Central America, and their relationships

with rainfall seasonality, is essential for understanding the consequences of transforming grasslands into sown pastures; and the consequences of such transformations in view of expected shifts in rainfall patterns anticipated as a result of global warming.

We hypothesize that sown pastures will have higher ANPP in the peak of the rainy season, but grasslands will start growth earlier in the season and continue longer into the dry season, when rainfall events are more erratic. We specifically aimed to find out: (i) whether ANPP in sown pastures and grasslands was related to rainfall and its distribution over short time periods; (ii) which rainfall parameters best explained the variation in ANPP; (iii) whether the ANPP of sown pastures was higher than for grasslands, when rainfall parameters in the dry and the rainy seasons were controlled for; (iv) whether ANPP was more stable through time for grasslands than sown pastures; and (v) what was the annual ANPP for grasslands and sown pastures.

2.2 METHODS

2.2.1 Site description

The study was conducted in the Río Grande de Matagalpa watershed in Central Nicaragua (12°31-13°20'N; 84°45-86°15'W). The area is located in the municipality of Muy Muy, in sites within an altitudinal range of 200 to 400 m. (Figure 2.1). The predominant land-use is livestock farming with relatively homogeneous livestock management. The natural vegetation of the region corresponds to a transitional tropical sub-humid forest (Holdridge 2000) with semi-deciduous vegetation, and is referred to as seasonally dry tropical forest (Bullock *et al.* 1995). The vegetation in the study is an assemblage of native and naturalized species including grasses, herbs and woody plants.

Here we use the term semi-natural grassland to refer to a pasture covered by spontaneous vegetation that grows naturally after forest clearing, or on fallow land, and which is maintained by grazing management, including fencing and weed control.

There is a clear contrast between rainy and dry periods seasonally. Rainfall recorded between November/December and April/May is usually less than 10% of the normal annual rainfall (1971-2000) for Muy Muy (1547.1 ± 125 mm) and the annual mean air temperature is 24.3°C <http://www.ineter.gob.ni/Direcciones/meteorologia/clima%20nic/caracteristicasdelclima.html>. Topography is undulating, with slopes between 5-45%. The bedrock consists of Tertiary volcanic tuff, a type of pyroclastic rock. Tuff in the area seems to be impermeable, which explains the limited infiltration often observed in flat areas during the rainy season. In most flat areas, it is common to find soils with Vertic properties, while on steeper slopes soils are more variable. The dominant soil type in the studied sites was a greyish to black Vertisol with high organic matter content in the upper horizons (8%). A clayey subsoil, pH between 5.8 and 6.8 in the topsoil, high in Ca, Mg, and K contents, but relatively low in phosphorous, Olsen-P < 10 ppm (Nieuwenhuys *et al.* unpublished data).



Figure 2.1 Map showing the location of the Muy Muy municipality in Central Nicaragua, and the country limits on a map of Central America (Figure modified from Google Earth).

2.2.2 Study design

The study layout was a fully randomized design with two types of ground vegetation cover: semi-natural grasslands, consisting of various species which share dominance, mostly prostrate grasses of the genus *Paspalum* and sown pastures dominated by *Brachiaria brizantha* cvs. Marandu and Toledo (cultivars of an African species used to improve pasture productivity in tropical America). Five plots with grasslands and four with pastures were sampled. Grassland and pasture plots were at least 10 and 3 years old, respectively, and were managed under grazing, hand weeded at least once a year, and had no fertilizers applied. Each of the five grassland plots was fenced with an area of at least 3200 m²; the four sown pasture plots were paddocks of 5000 to 6000 m².

The location of the selected plots in the study layout was not related to the distance between plots in the study layout, but based on soil similarities and topography. Given the management of weeding in the area at the beginning of the 2007 rainy season (early June) and in order to control the woody vegetation and non palatable species in the grasslands and pastures, all plots were clipped to ground level and woody species taller than 20 cm were cut at ground level, and the harvested biomass was removed from the plots.

Each of the nine plots was divided into four quadrants, each corresponding to a quarter of the plot, where each quadrant corresponded to a cardinal point (N, S, E, and W). At each sampling period one biomass sample (1 m x 1 m) was taken from each of the four quadrants (1 m x 1 m). Sampling units were assigned randomly with degrees (°) from randomly central datum point of the plot and with the distance from the datum point randomly generated. This procedure was repeated at each sampling period. If a subsequent randomly generated sampling unit resulted in a sampling unit falling on part of a previous sampling unit, it was discarded and a new sampling unit was randomly generated. This removed the problem of overlapping sampling units.

Four weeks after the initial clipping, all plots were grazed by cattle. The occupation period for each plot ranged between 1 and 2 days with the purpose of producing a uniform height of the above-ground biomass with the grazing before starting the new period of measurement. Once the occupation period ended, the residual biomass was clipped and used to estimate the biomass at the start of the ANPP estimation period (T₀). Grazing was then prevented for the following 21 days (or growth period). On day 22 (T₁), the above-ground biomass was clipped again to estimate the biomass increment in

the growth period (T0-T1). Sequential biomass harvests after 22-day periods of grazing exclusion were conducted approximately monthly for each plot, until June 30th, 2008.

2.2.3 Above-ground biomass sampling and estimation of above-ground net primary productivity

Standing biomass was clipped at 0.02 m and 0.12 m above the soil surface level for the grasslands and sown pastures, respectively. The clipped biomass was sorted by hand into green and standing dead components directly in the field, and the total green biomass was weighed immediately with a mechanical balance (± 0.1 g; Ohaus; triple beam) to avoid mass losses as a result of plant respiration. Once the standing biomass was sorted, and the total green biomass was weighed, the litter on the soil surface (detached dead matter) was collected by careful hand-picking in the area of each sampling unit without any sort of species separation. The litter samples were cleaned in the laboratory to discard soil particles and any live components of vegetation. The dry matter content of all above-ground biomass components was estimated on a subsample of approximately 250 g fresh weight which was oven dried at 65 °C, until constant weight. In addition, a composite sample of 100 g of litter (fresh weight) taken from each of the four samples was washed by soaking in water to eliminate possible soil contamination that could affect mass. ANPP was estimated as the sum of the positive differences in the three biomass components collected at the start (T0) and end (T1) of each sampling period of 22 days, applying a correction for senescence and for the transference of standing dead mass to litter (Sala and Austin 2000; Scurlock *et al.* 2002).

Annual ANPP was calculated by adding the ANPP estimated for each sampling period of 22 days during the 12-month growing cycle (July – June). Given that the ANPP

measurements were taken after 22 days of re-growth and that the interval between measurements periods was close to 30 days, there was at least one ANPP measurement per month per replicate during the whole study period. However, due to unforeseen difficulties in sampling, there were only 11 biomass measurements for the computation of the annual ANPP. One site with less than 11 ANPP observations in the one-year period was dropped from the analysis. In total, eight sites were included for the analysis of annual ANPP; five for grasslands and three for sown pastures. We used the community temporal stability (S) (Tilman 1999) to study the relationship between types of ground vegetation cover and temporal variability of biomass production over the dry season (December–May) and a 12 month growing cycle (July-June).

2.2.4 Rainfall data collection

Four rain gauges were located in four villages within the study area, and rainfall data were recorded daily. Also, data from a weather station of the Nicaraguan Institute of Land Studies (INETER) located within 5.7 km distance from the study sites were used. For six study sites, we used the rainfall data from the rain gauges and for three sites data from the weather station (Figure 2.2, Table 2.1).

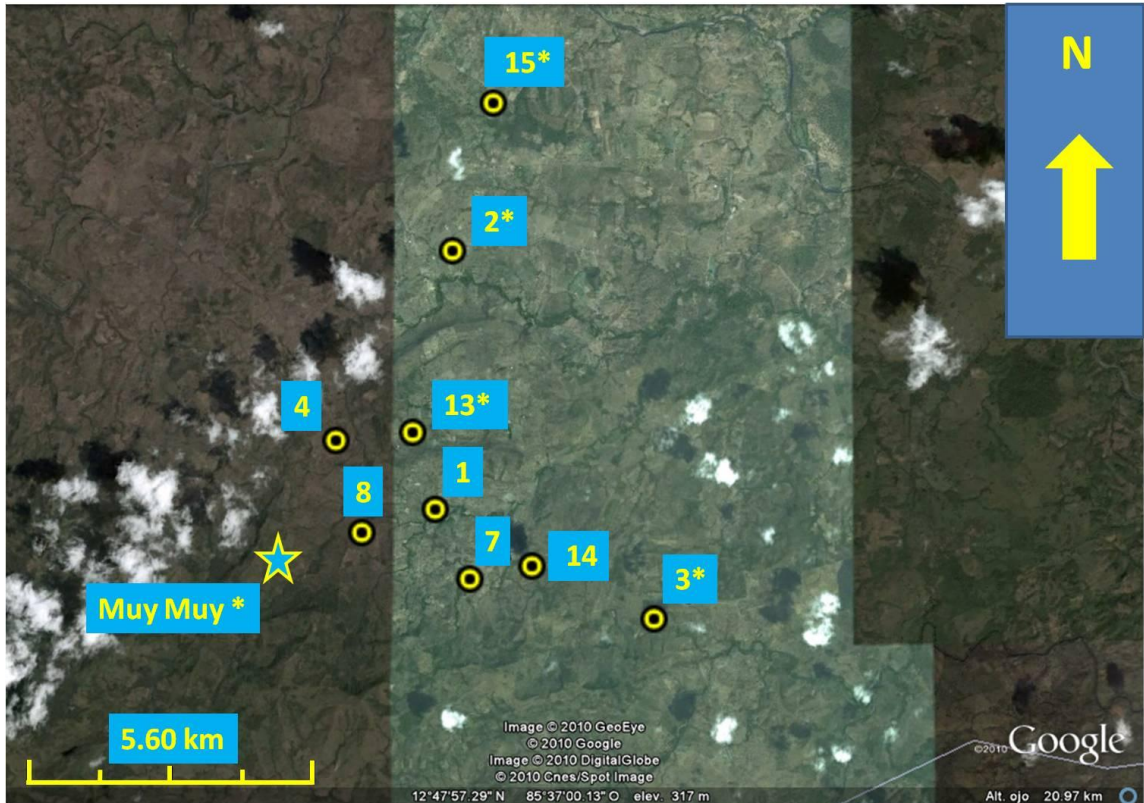


Figure 2.2 Study plot locations and associated rain gauges stations in Muy Muy, Matagalpa, Nicaragua: 1-El Marandu, 2-El Guanacaste, 3-La Laguna, 4- Los Técnicos, 13-El Mango, 14-El Genízaro, 15-El Mono, 7-El Plan, 8-El Llano. 1 – 4 are sown pasture plots; 7, 8, 13, 14 and 15 are semi-natural grassland plots. An asterisk denotes the location of a rain gauge or a weather station (Figure modified from Google Earth).

Between July 1 2007 and June 30 2008 the four rain gauges daily rainfall records were accumulated for each sampling period of 22 days and for each study site, the variable resulting from this is hereafter referred to as cumulative rainfall in each sampling period of 22 days (ARP22). Also the daily rainfall data in the same period were classified into rain events. Daily rainfall records that were greater or equal to 2 mm were considered as a rainfall event. In some cases a rain event coincided with one day. However, when rainfall was measured over consecutive days, these were collectively considered as one rain event. In addition, three metrics of rainfall distribution were calculated for each biomass sampling period: the number of rainfall events (NRE), the size of the rainfall

event (SRE) and the interval between rainfall events (IRE). To prevent overestimation of the size of the rainfall event and underestimation of the number of rainfall events, we followed Heisler-White *et al.* (2008), and divided cases with ≥ 3 days of consecutive measured rainfall into two events.

Table 2. 1 Names and characteristics of villages and rain gauge stations where study plots were located in Muy Muy, Matagalpa, Nicaragua

Village	Rain gauge station	Elevation (m)	Site (plot label)	Distance to nearest rain gauge station (km)
El Coyolar	El Coyol, Farm	300	La Laguna	0.9
			El Genízaro	2.3
			El Marandú	3.7
El Guanacaste	La Cruz, Farm	314	El Guanacaste	0.8
El Corozo	San Felipe, Farm	378	El Mango	2.1
Maizama Adentro	La Lucha, Farm	280	El Mono	1.2
Muy Muy, town	Weather Station Muy Muy 055027 (INETER)	320	Los Técnicos	2.1
			El Llano	4.5
			El Plan	5.7

2.2.5 Statistical analysis

Multiple linear regressions with polynomials and classification variables (treatment: grassland and sown pasture) using dummy variables, were used to fit the relationships between ANPP and ARP22 to test the hypotheses about differences between vegetation type response to cumulative rainfall. The relative contribution of cumulative rainfall and

rainfall distribution variables on ANPP was tested in the linear portion of the regression between ANPP and rainfall, when the relationship was significant, using the linear portion of the curve (below 300 mm of cumulative rainfall). Because of the presence of heteroscedasticity of residuals, we performed a mixed model analysis to test the hypothesis about differences amongst vegetation types for the regression parameters (intercept and slope) taking into account the lack of homoscedasticity. In order to examine the effects on ANPP of season and of the two types of vegetation, we used a two-way analysis of covariance with season (rainy season 2007, early dry season 2007, late dry season 2007 and rainy season 2008) and type of vegetation as main factors, and the interaction terms. ARP22 and ARP22 squared were used as co-variables. Due to variance heteroscedasticity between seasons, a mixed model was used to carry out the analysis of variance. Model selection was based on the Likelihood Ratio Test and the Akaike Criterion. To perform mean comparisons we used the DGC test (Di Rienzo *et al.* 2002).

Annual ANPP for the two types of vegetation cover was computed as an addition of the estimates of monthly ANPP for the period July 1st 2007 to June 30th 2008 and the F statistics were calculated according to the model : $Y_{ijk} = \mu + T_i + M_j + T_iM_j + \delta_{ijk}$, where, Y_{ijk} = ANPP ($\text{g m}^{-2} \text{ month}^{-1}$) of the treatment i , the month j and the replicate k . T_i and M_j are the two main effects (the treatment i and month j in which the measurement period occurred), and T_iM_j and δ_{ijk} , the first-order interaction and error terms, respectively.

The community temporal stability was measured as the mean biomass production of each site, divided by the standard deviation that results from the temporal variation in that biomass production. If there were no variation, community temporal stability would

be maximal (infinite). When the variation relative to the mean is large, community temporal stability is small (near 0) (Lehman and Tilman 2000). The community temporal stability was computed based on the mean biomass production of each site through the dry season (December 2007-May 2008) and a year (July 2007-June 2008). In order to test for differences attributed to type of vegetation we used ANOVA and Fisher' s LSD was used to perform mean comparisons. All statistical analyses were conducted with the InfoStat package (Di Rienzo *et al.* 2009) and R language.

2.3 RESULTS

2.3.1 Summary of current rainfall trends

The annual rainfalls recorded in 2007 and 2008 were 1638 and 1858 mm, respectively. In 2007, the rainy season, (6th June-30th November) accounted for 83% of the annual rainfall. In 2008, the rainy season lasted from May to October comprising 1506 mm, of which 71% was concentrated in the first trimester (28th May-28th August). The start of the rainy season in 2008 was at the end of May. The cumulative rainfall for the month was 95 mm, but 75% of it occurred in the last four days of the month.

2.3.2 Above-ground net primary productivity, rainfall and its distribution

Overall, the frequency of rain events was lower in the 2007 than in the 2008 rainy season (Table 2.2). No significant differences in the amount of rainfall ($p= 0.56$) or in the number of rainfall events ($p= 0.19$) were detected between grasslands and sown pastures sites.

The relationships between ANPP and ARP22 show significant linear and quadratic trends for pastures ($p < 0.0001$, in both cases) and grasslands ($p < 0.0001$, in both cases).

There were no differences between treatments in the intercepts ($p= 0.0770$), and the quadratic coefficients ($p= 0.5564$), but there were differences in the linear coefficients ($p= 0.0228$) (Figure 2.3). Under 300 mm of ARP22, the increase in ANPP with rainfall was larger in sown pastures than in grasslands but there was no difference between inflection points, indicating a similar rate of decline of ANPP in both treatments with higher ARP22.

We tested the contribution of the different rainfall components (cumulative rainfall and rainfall distribution metrics) in explaining ANPP variance in the linear portion of the ANPP vs. ARP22 regression curve. The relationship between ARP22 and NRE, and ANPP was significant (adjusted $R^2 = 0.67$; $p < 0.0001$). ANPP in sown pastures increased with cumulative rainfall (ARP22) at higher rates in the linear portion, than that in grasslands ($p= 0.0008$). Overall ANPP was also significantly related to NRE ($p=0.0045$) but there were no differences in this relationship between the treatments ($p= 0.1914$). The other rainfall distribution variables tested (SRE and IRE) were not significantly related to ANPP.

Table 2.2 Mean cumulative rainfall and distribution statistics in grassland and sown pasture sites during the rainy season 2007, dry season 2007/2008 and rainy season 2008. Mean cumulative rainfall over the period of measurement of above-ground net primary productivity (ARP22) and rainfall distribution metrics (n= the number of sampled rain gauges contributing to the mean). S.D= standard deviation

Seasons/Types of ground vegetation cover	ARP22 (mm)	Mean number of rainfall events	Mean size of rainfall events (mm)	Mean interval between rainfall events (days)
Rainy Season 2007: Grasslands (n= 4)	175	10	18	2
S.D.	48	2	5	1
Rainy Season 2007: Pastures (n= 3)	196	11	19	2
S.D.	49	2	7	1
Dry Season 2007/2008: Grasslands (n= 4)	32	4	9	7
S.D.	16	2	9	5
Dry Season 2007/2008: Pastures (n= 3)	28	4	8	8
S.D.	15	2	6	5
Rainy Season 2008: Grasslands (n= 4)	261	11	22	2
S.D.	130	3	7	1
Rainy Season 2008: Pastures (n= 3)	294	12	24	3
S.D.	155	3	9	2

2.3.3 Seasonality of above-ground net primary productivity

After adjusting ANPP by ARP22 and ARP22 squared there was a significant vegetation type x season interaction ($F= 10.47$, $p= <0.0001$), as well as significant main effects (vegetation type: $F= 4.04$, $p= 0.0338$, and season: $F= 113.10$, $p < 0.0001$). The smallest variation corresponded to the late dry season 2007 and the highest to the rainy season 2008. Sown pastures had higher ANPP in the rainy season 2008, while grasslands had higher ANPP in the early dry season 2007 (Figures 2.4 and 2.5). The temporal stability of ANPP was higher in grasslands than in sown pastures for the dry season ($F_{6.3131}$; $n= 8$; $p= 0.0402$) and the annual cycle ($F_{11.0474}$; $n= 8$; $p= 0.0127$) (Figure 2.6). The annual ANPP was 898 and $955 \text{ g m}^{-2} \text{ yr}^{-1}$ for grasslands and pastures, respectively.

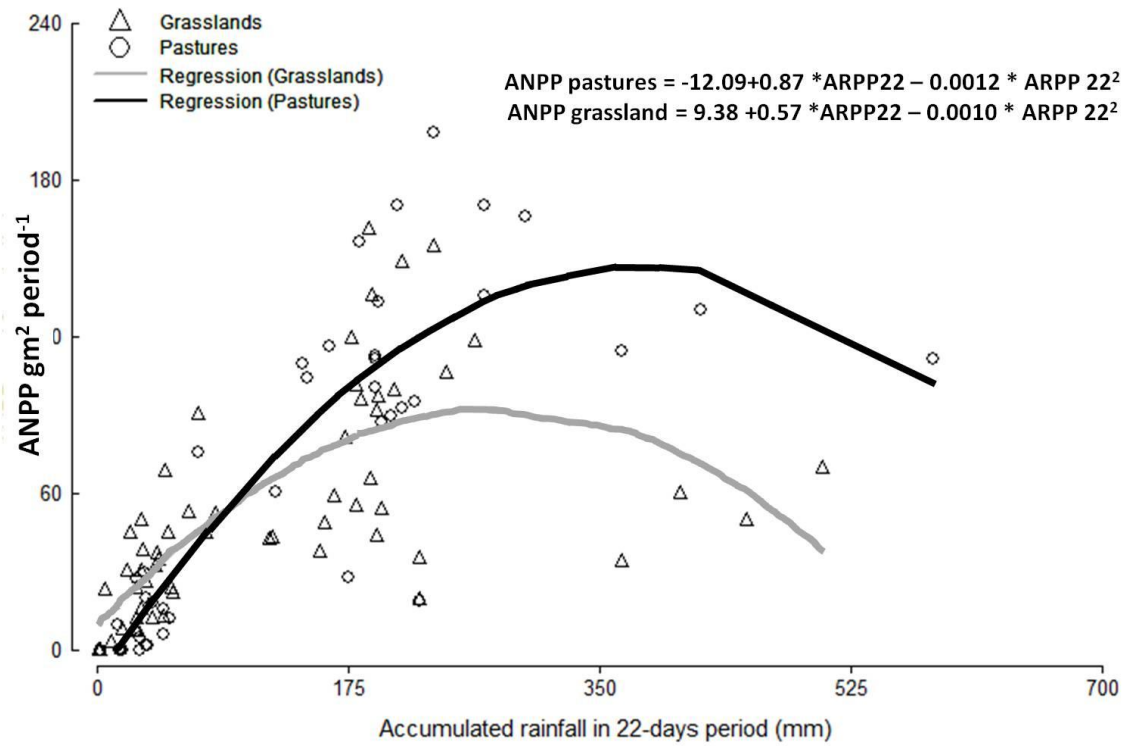


Figure 2.3 Relationships between above-ground net primary productivity (ANPP) and cumulative rainfall in 22 day periods (ARP22) for sown pastures and grasslands. Sown pastures: n= 44, adjusted r²= 0.74; grasslands: n= 60, adjusted r²= 0.47

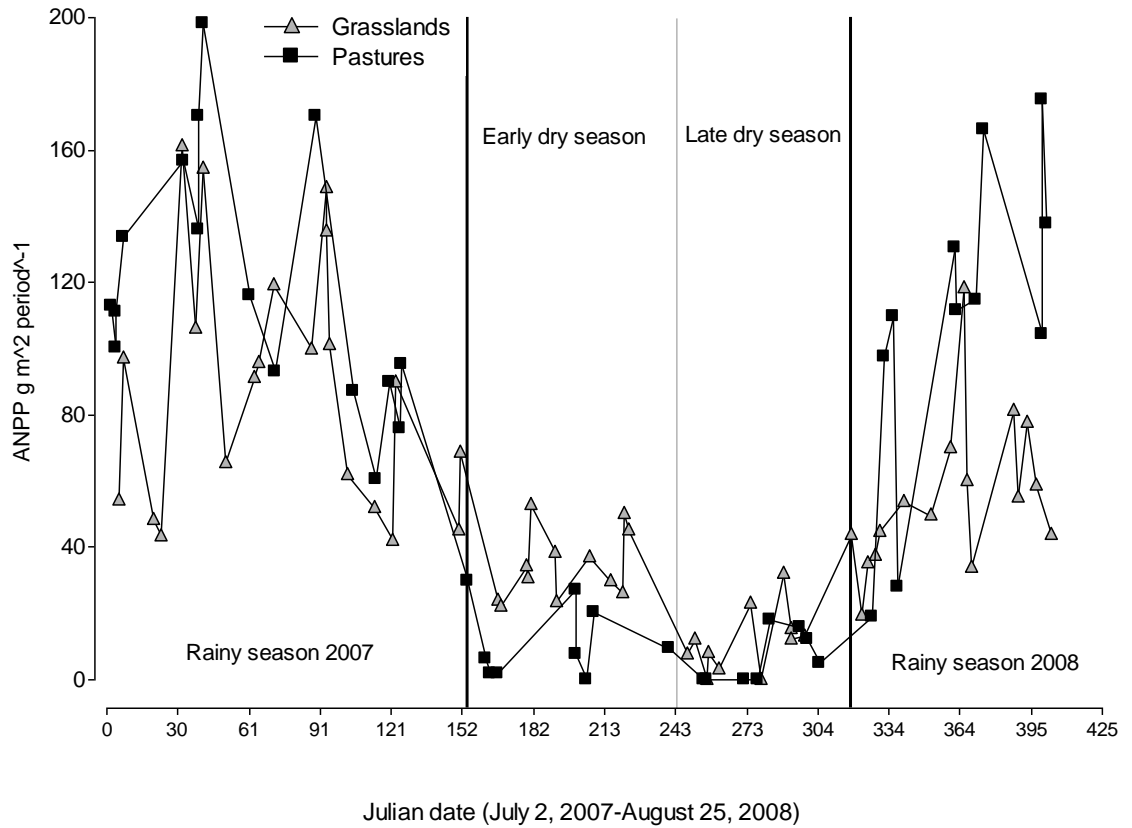


Figure 2.4 Variation of above-ground net primary productivity (ANPP in g m² 22 day period⁻¹) through time for grasslands and sown pastures in Muy Muy, Nicaragua. Black vertical lines separate seasons. The dry season was divided into early and late phases denoted by the grey vertical line

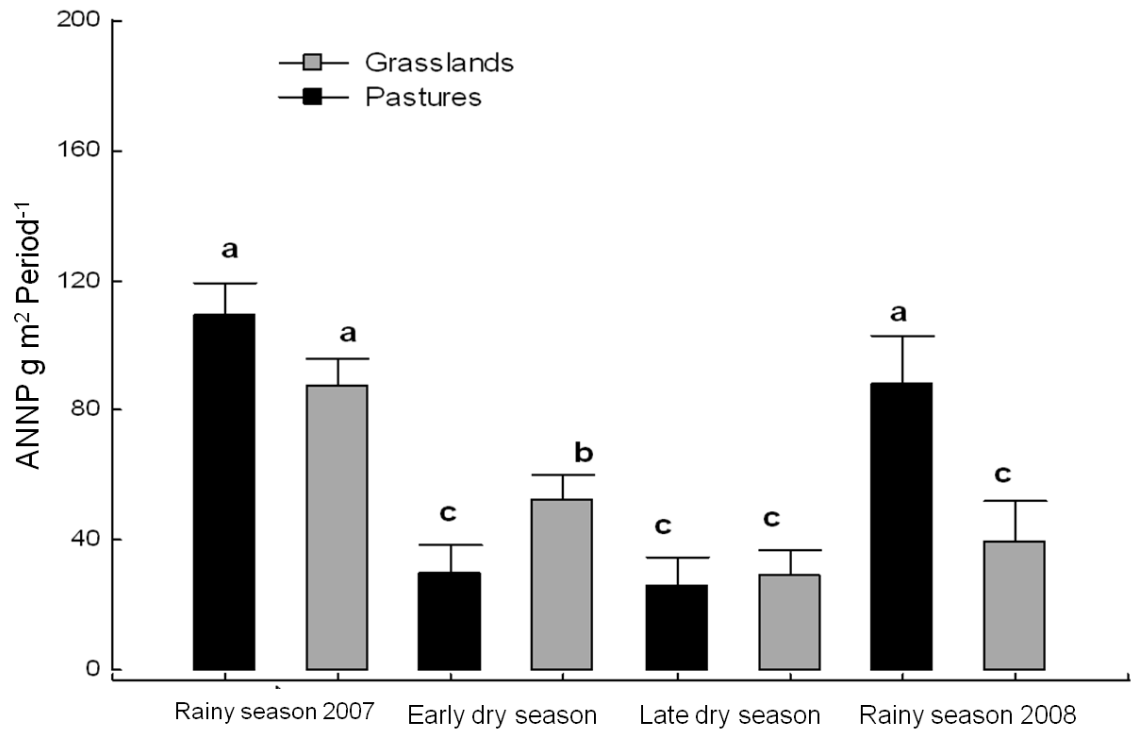


Figure 2.5 Mean above-ground net primary productivity (ANPP) for the different vegetation types in different seasons \pm 1 SE, based on LSD test. Significant differences ($p < 0.05$) between treatments are indicated by different letters

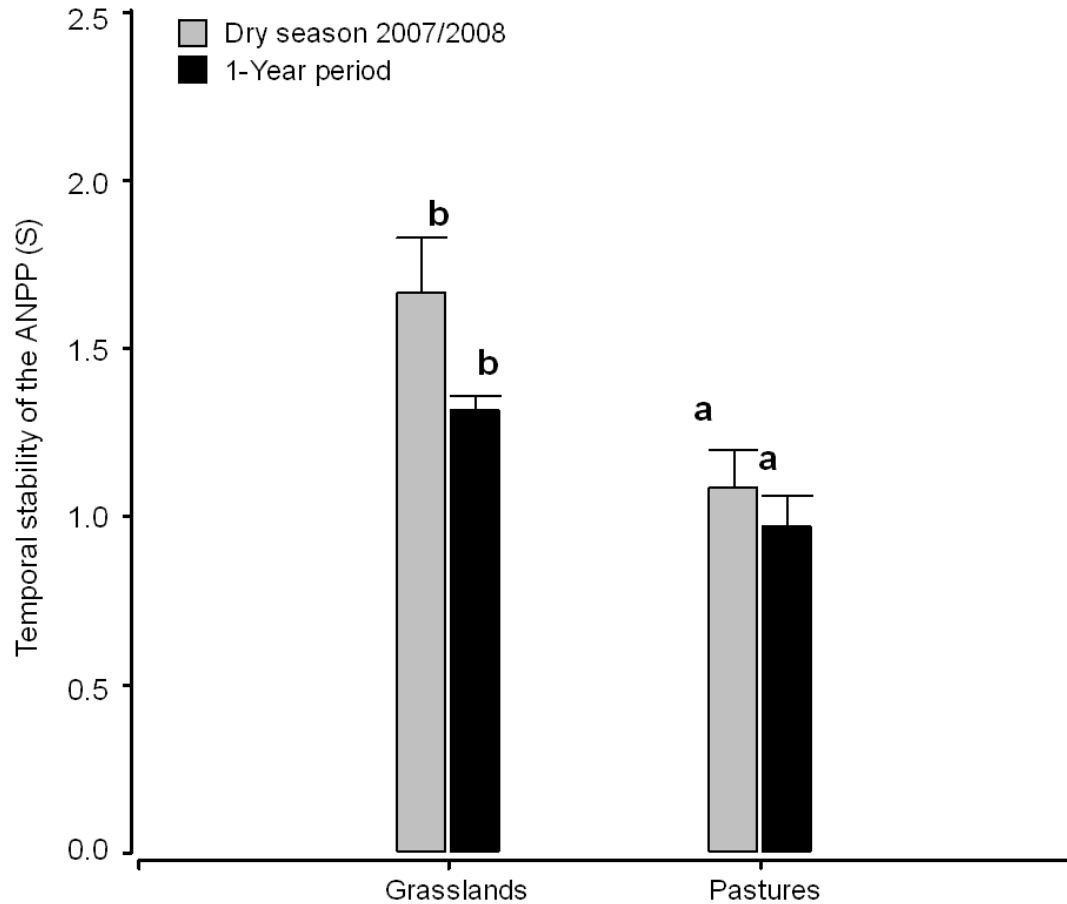


Figure 2.6 Yearly (July 2007-June 2008) and dry season (December 2007 – May 2008) temporal stability of ANPP, estimated in four plots with grasslands and at three plots with sown pastures in a sub-humid region of Central Nicaragua. Bars denote ± 1 SE, based on LSD test. Different letters in the same time period indicate significant differences ($p < 0.05$) between grasslands and sown pastures.

2.4 DISCUSSION

2.4.1 Relationships between ANPP, rainfall and its distribution

ANPP was positively associated with cumulative rainfall over 22 day-periods (ARP22).

A positive relationship between ANPP and rainfall is consistent with empirical evidence from most grasslands and savannas of the world showing a correspondence of ANPP with the variability between- (Lauenroth and Sala 1992; Sarmiento and Pinillos 1999; Knapp *et al.* 2006) and within- years (Swemmer *et al.* 2007; Heisler-White 2008) in the

amount of rainfall. In arid climates ANPP and rainfall correspond linearly, indicating that under these conditions water availability is the key limiting factor for ANPP. In our study, a quadratic model resulted in a better fit than a linear relationship. ANPP was less when the cumulative rainfall in a 22-days period (ARP22) was higher than ca. 300 mm. Apparently, the timing and intensity of the rainfall events were important in determining when ANPP increased with rainfall and at which point ANPP was lesser despite high cumulative rainfall.

The intensity of rainfall events is an important determinant of the amount of rainfall that infiltrates the soil (Ludwig *et al.* 2005) and in turn of productivity. Low retention of water and lower productivity per unit rainfall have been observed in grasslands of various conditions and composition (O' Connor *et al.* 2001) when large volumes of water fell within a month. In our study, the highest ARP22 occurred at the onset of the rainy season, when soils were dry, which are conditions conducive to high runoff. The vertic properties of the soils probably exacerbated this. The importance of the water holding conditions of the soil on the balance between the magnitude of rainfall runoff and infiltration may also be one reason for the large spread found in the responses between cumulative rainfall (ARP22) and ANPP in this study.

The number of rain events is a metric that corresponds with the temporal distribution of rainfall and is related to the length of the dry interval, given that fewer events will always result in longer dry intervals. Although the interval between rain events could be expected to be a more direct indicator of the effect of spacing between rain events (Swemmer *et al.* 2007), the number of rain events can also correspond with ANPP (Savadojo *et al.* 2007; Swemmer *et al.* 2007). Our results are in agreement with those

findings. We found that, in addition to the amount of rainfall, the number of rain events explained part of the variation in ANPP and indicated that the pattern of rainfall distribution is an important determinant of ANPP in this context. ANPP responded positively to more frequent and more evenly distributed rainfall events in both vegetation types. Also the response of ANPP to the number of rainfall events is likely to be in close correspondence with the properties of the soils in the study site. As indicated above, the soils of the area have strong vertic properties which cause contraction and cracking when drying. More frequent and evenly distributed rainfall will not only maintain more even levels of water supply for the vegetation, but may also have an effect in maintaining less stressful physical conditions for root growth. In contrast, the size of each event was less important; as we found no relationships between either the size of the rainfall event or the interval between rainfall events and ANPP. As discussed above, this lack of response could also be related to low infiltration rates and high runoff losses when large amounts of rain fall in a short time period (Loik *et al.* 2004). These findings evidence the importance of understanding rainfall distribution patterns as drivers of ecological processes in seasonally dry ecosystems.

2.4.2 Evaluating effects of pastureland type on ANPP and seasonality

ANPP is a fundamental property of ecosystems because it determines the amount of energy that is made available to other trophic levels, which in pastoral systems include livestock. ANPP assessments in tropical seasonal grasslands are scarce (Sarmiento 1984; grey literature in Scurlock *et al.* 2002), and where attempted, proxies such as standing biomass have often been used. The annual ANPP values measured in this study are within the ranges recorded for similar grasslands, savannas and sown pastures in the

Neotropics (Table 2.3). Under the pasture management applied in the study sites, we found no differences in annual ANPP between the *Brachiaria brizantha* pastures and the grasslands.

The similarity of annual ANPP amongst vegetation types is probably a result of two counteracting properties of the vegetation: the attributes of the dominant species and the complementary attributes of other species in the plant assemblage. *Brachiaria* cultivars are expected to be more productive and to have higher resource uptake and evapotranspiration than semi-natural grasslands (Fisher *et al.* 1994; Guenni *et al.* 2004) commensurate with a growth form indicative of high growth rates and rapid resource acquisition strategies (Baruch and Gómez 1996; Poorter and Garnier 2007). Erect growth has been related to high growth rates and correspondingly high rates of resource use, whilst prostrate growth form, which is characteristic of dominant species in the grasslands, has been associated with lower productivity (Altesor 2005). *Brachiaria brizantha*, cvs. Marandu and Toledo are tolerant of mild water stress and such sown pastures have higher biomass availability and forage production than native grasses in the Neotropics (Guenni *et al.* 2004). These characteristics agree with our findings. As predicted, in the rainy season, the sown pastures dominated by *Brachiaria* seem to have utilized rainfall more rapidly (steeper ANPP increase at the start of the rainy season) and also more efficiently; initially producing more biomass than grasslands and reaching higher yields before ANPP starts to decline. Accordingly, the attributes of the dominant species in the vegetation appear to be an important determinant of wet season ANPP, although differences in management of sown pastures and grasslands may also contribute to their different responses. Although not quantified, the amount of biomass

that remains after grazing events, a property of the pasture directly related to plant growth and the capacity to recover from tissue loss appeared to be larger in sown pastures than in grasslands (pers. obs.).

Despite more rapid growth in the rainy season, sown pastures appear to stop growth more abruptly at the end of the rainy season and, during the dry season, low rainfall translated into significantly lower levels of ANPP than in grasslands. We speculate that this pattern may be attributed to the occurrence of species in the grassland assemblage with attributes that enable them to maintain growth when water supply becomes limiting. Both the sown pastures and the grasslands were very low in productivity in the latter part of the dry season which is consistent with the widespread practice of retaining trees in pastures in seasonally dry climates to provide some nutrition for livestock from tree leaves, fruits and pods in the dry season (Cajas-Giron and Sinclair 2001).

Table 2.3 Estimated annual above-ground net primary productivity (ANPP) for grasslands and savannas in the neotropics.

Grassland type and Pasture	Region/Country	Annual ANPP g m ⁻² yr ⁻¹	Mean annual rainfall (mm)	References
Seasonal and humid savanna: savanna vegetation	Calabozo, Venezuela	1121	1252	San Jose and Medina 1976 after Scurlock <i>et al.</i> 2002
Seasonal and humid savanna: savanna vegetation	Calabozo, Venezuela	700-1100	1839	Sarmiento 1984
Sub-humid ecosystem: pasture <i>Hyparrhenia rufa</i>	Cañas, Costa Rica	1378	1538	Daubenmire 1972, cited by Sarmiento 1984
Sub-humid ecosystem: pasture <i>Brachiaria brizantha</i>	Cañas, Costa Rica	1560	1500	Andrade <i>et al.</i> 2008
Seasonal dry ecosystem: pasture <i>Brachiaria brizantha</i>	Muy, Muy Nicaragua	955	1547	This study
Seasonal dry ecosystem: seminatural grassland	Muy, Muy Nicaragua	898	1547	This study

2.4.3 Temporal stability

The result of a more defined peak of production of the sown pastures in the rainy season compared with the grasslands is also reflected in higher values of temporal stability and lower within-season coefficients of variation of ANPP for the grasslands than for the pastures. These results coincide with those reported by Tilman (1996) and Caldeira *et al.* (2005) about higher temporal stability of above-ground biomass in grasslands than in sown pastures, but further demonstrate that grasslands stabilize function in time more than sown pastures. The higher stability in grasslands is consistent with the presence of a larger number of species and possibly of more functional diversity (de Bello *et al.* 2009). It could be expected that higher species diversity in grasslands will encompass a wider set of adaptive traits, such as diverse vegetative and reproductive phenologies and root depths, compared with sown pastures. Some further supporting evidence of the different functional stability of the two types of pastureland is their apparent difference in sensitivity to rainfall fluctuations. Although the interaction term vegetation type x number of rainfall events was not significant in the overall ANPP model, ANPP was more sensitive to the number of rainfall events in sown pastures (NRE $n=41$ $p=0.0045$) than in grasslands (NRE $n=56$; $p=0.1914$), but the large variability in the ANPP data preclude more definite conclusions.

2.5 IMPLICATIONS

Understanding the outcome of predicted rainfall distribution in climate change scenarios remains a significant challenge for predicting the amount and the seasonal variability of ANPP for grasslands but more so for sown pastures in relation to their higher sensitivity to rainfall amount and its distribution. The introduction of sown pastures in the area of

the study does not appear to be an effective means to increase ANPP, since there were no significant gains on an annual basis. Furthermore, ANPP of sown pastures was more variable within seasons than for grasslands, and there was an indication that they were more sensitive to variation in the distribution of rainfall. Sown pastures were slightly more productive in the rainy season than grasslands, and the surplus produced in this period could be used in the dry season through harvest and storage. If this practice is not implemented, less even seasonal production is likely to impose greater challenges in grazing management for sown pastures than grasslands. Overall, the gains of replacing grasslands with sown pastures are questionable, and need to be weighed against increased costs and the ecological and environmental risk of reducing the diversity of vegetation. These suggestions are based entirely on above ground biomass but there could also be differences in nutritive value between sown pastures and grasslands. Both sown pastures and grasslands were equally unproductive in the latter part of the dry season, vindicating the widespread local practice of retaining trees in pastures.

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CHAPTER 3

BEYOND THE ‘LEAF ECONOMICS SPECTRUM’: MULTIPLE DIMENSIONS OF SPECIALIZATION IN PLANTS IN A TROPICAL GRASSLAND[†]

ABSTRACT

Several axes of plant trait differentiation have been identified, among which the resource economy axis that distinguishes between species that have rapid resource acquisition and those with high conservation of resources seems to be the most conspicuous and is based mainly on leaf traits. Here, we explore whether the resource economy axis is detectable in a seasonally dry tropical grassland and whether relatively unstudied traits relate to this axis: the nature of these relationships is examined. We measured a set of five primary foliar traits and another set of nine morpho-phenological traits for 36 species that were chosen to describe differences in plant strategies in these areas with variable seasonal water supply, involving different life cycles and life forms common in these grasslands. Ordinations showed three axes of trait differentiation. The first axis indicated that high foliar concentrations of nitrogen, phosphorus, calcium and magnesium, high specific leaf area and short leaf lifespan formed the basis of an resource acquisitive strategy, while low concentration of these foliar nutrients and low specific leaf area and long leaf lifespan were interpreted as a resource conservative strategy. The second axis separated

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differences in life-history and life forms among species. Within perennials, species with high leaf dry mass and long leaf lifespan formed a consistent trend having wide lateral spread, deep roots and high leaf nitrogen concentration. The third axis separated variation in plant height and leaf size and its major interpretation was phylogenetic differences between species life-history and life forms.

3.1 INTRODUCTION

Traits are often interpreted as adaptations or specializations that mediate plant responses to disturbance, resources and climate (Semenova and van der Maarel 2000) and consequently they provide a most promising avenue for a mechanistic understanding of vegetation dynamics and for predicting responses to various ecological conditions including environmental change (Weiher and Keddy 1995).

Plant traits are components of integrated individuals. Over the evolutionary history of a species, some attributes (i.e. trait values) may have limited the possible range of other attributes that could evolve in conjunction and be successfully passed on to the progeny. Trait co-variation can be examined by identifying groups of traits that are correlated across species and its functional significance can be investigated by studying the relationship between axes of trait differentiation and species performance under specific ecological conditions (Easdale *et al.* 2007).

Much progress has been made towards understanding plant morpho-physiological differentiation in other ecological regions through the identification of axes of ecological differentiation. The main axes capture a large portion of the plant trait variation into 'plant strategies' encompassing fundamental trade-offs in the allocation of resources to different tissues and plant organs (Grime 1977; Westoby *et al.* 2002). Several axes of

trait differentiation are considered important (e.g., Westoby *et al.* 2002), among which the resource economy axis seems to be the most conspicuous. It distinguishes between species that have high rates of resource acquisition and circulation, low resource use efficiency, and that are successful at high levels of resource supply; and species that have a strategy of conservative resource use and low demand, and that perform well in chronically poor environments (Grime *et al.* 1997; Díaz *et al.* 2004; Wright *et al.* 2004). Most of the knowledge about the resource economy axis derives from a set of primary foliar traits, which are widely recognized as powerful indicators of this trade-off. Thus, for instance, rapid acquisition of resources is generally correlated with high specific leaf area (SLA) and high leaf nitrogen (N) and phosphorus (P) concentrations, while high leaf dry matter content (LDMC) and long leaf lifespan (LLS) reflect the resource conservation strategy (Wilson *et al.* 1999; Wright *et al.* 2005; Freschet *et al.* 2010).

Most research about trait differentiation and trait trade-offs has been bound to temperate and subtropical ecosystems across a world-wide variety of vegetation types. Other ecosystems remain unstudied in this regard. This is the case of neotropical grasslands, the most widespread type of vegetation derived from forest after clearing (Fisher *et al.* 1994; Maass 1995). In sub-humid areas, these communities occur in a system with temporal shifts in resource availability and an important characteristic of their function is that they possess strong seasonal growth patterns determined by rainfall (Ospina *et al.*, *submitted*). Seasonal climates determined by rainfall provide an interesting setting for the study of axes of trait differentiation and of plant strategies along environmental gradients, especially, those related to temporal patterns of nutrients and water supply.

The objective of this study is to summarize the diversity of plant morpho-functional attributes in relatively unstudied communities, to explore whether the resource economy axis is detectable in this seasonal climate, to test whether relatively unstudied traits relate to the resource economy axis, and to examine the nature of these relationships. This is a first step to understand eco-physiological determinants of seasonal changes in primary productivity, community dynamics, plant responses to environmental factors and mechanisms of species co-existence. To our knowledge, no previous study has examined the relationships between primary foliar traits, below-ground traits, reproductive phenological traits and clonal growth capacity. We explored the relationships and trade-offs among both some widely studied leaf traits associated with the resource economy strategies of plants and also other traits about which evidence is scarce but indicative of an association with strategies along this primary axis of specialization in plants (Grime *et al.* 1997; Díaz *et al.* 2004; Roscher *et al.* 2004; Wright *et al.* 2005).

Specifically, we explored axes of species strategies of resource use with a set of primary foliar traits: SLA, LDMC, LLS, and the concentration of N and P; and others with less well-documented evidence about their correspondence with the resource economy axis. Among those traits are: plant height at onset of flowering (PH), leaf size (LS), leaf calcium, potassium, and magnesium concentrations (Ca, K and Mg, respectively), lateral spread by vegetative growth (LSCG), root depth (RD), start of flowering period (SFP) and length of the period from flowering to seed shed (LFS).

For some of those traits, the evidence about the correspondence with the resource economy axis or primary foliar traits is weak, partly due to the difficulty of measuring below-ground traits (Freschet *et al.* 2010), and because the assessment of seasonal

phenological traits is comparatively time consuming. The examination of the variation of traits usually not associated with the resource economy axis, or with low correspondence with primary foliar traits, provides a wider scope to research patterns of trait differentiation that may be relevant for understanding species responses to local sources of stress (see Díaz *et al.* 2004; Lavorel *et al.* 2007). Some studies show that primary foliar traits co-occur with other morpho-physiological traits along resource supply gradients, strengthening the interpretation of integrated plant adaptive strategies of resource use. Other evidence indicates that various combinations of traits may confer similar adaptation under a particular environmental setting, highlighting the necessity of considering a wide range of traits in order to understand plant responses to the environment (Ryser and Eek 2000).

Small LS generally corresponds with stressful environments (dry, disturbed) (Wright *et al.* 2004; Niinemets *et al.* 2007) likely due to a lower evaporative demand of small leaves and to the high cost of the investment in veins to support big leaves (Niinemets *et al.* 2007). At the same time, LS can be linked to allometric factors (plant and twig size, anatomy and architecture) (Westoby *et al.* 2002) that could be related to life form (Cornelissen *et al.* 2003).

Potential plant height across species has been positively related to gradients of soil resources (Fonseca 2000; Cornelissen *et al.* 2003; Rusch *et al.* 2009), but height is affected by ontogeny change through the lifetime of individuals, which may confound responses to the environment (Niklas 2004; Westoby and Wright 2006). In brief, LS and PH have been found to be associated with the resource economy axis in some cases and to be independent in others.

With coarse roots, the relationship of RD with primary foliar traits is observational (Berendse 1981), but based on a limited number of studies, which mainly show life-form specific responses. For example, phenotypic variation in RD across six herbaceous species evidenced niche differentiation in nutrient use, grasses became shallower rooted with N and P additions, and legumes tended to be deeper rooted when N was added (Mamolos *et al.* 1995). Likewise, Nippert and Knapp (2007) found differentiation in water use between C3 forbs/shrubs and C4 grasses under natural climatic conditions in tall grass prairies; grasses used water in shallower surfaces, while C3 species shifted the source of water-used in response to water availability. Chemical root traits depend on phylogeny, reflecting the effect of life form and life cycle (Roumet *et al.* 2006), and respond also to properties of the soil (Hodge 2004). However, whether RD is affected by gradients of resources across and within life forms and species, and whether it fits the expected variation according to the resource-use strategy is still uncertain. Grime *et al.* (1997) found positive correlations of leaf K with N and P and concluded that leaf N, P, K, Ca, and Mg are associated with rapid growth under high nutrients supply. However, Wright *et al.* (2005) found that leaf K showed clearly weaker relationship with leaf mass per area, leaf lifespan and photosynthetic capacity per unit leaf mass and concluded it cannot be considered a core trait to the ‘leaf economics spectrum’ (Wright *et al.* 2004). There is some evidence of a correspondence between LSCG with the resource economy axis. Fertilization has been found to increase vegetative mobility and branching, and low rates of spread have been found to be associated with low fertility (Sammul *et al.* 2003; Craine *et al.* 2005; Rusch *et al.* 2010).

SFP appears to be related to the plant's resource use strategy and to correspond with primary foliar traits associated with the plant's resource use strategy. In general, because it has been associated with plant development and with the temporal differentiation of co-existent species in their resource use over seasonal periods (Roscher *et al.* 2004), the timing at which species reach phenological stages is related to species growth rate and the seasonal use of nutrients (Sosebe and Weibe 1973). In particular, early SFP has been related to low LDMC both under controlled conditions and in natural grasslands (Ansquer *et al.* 2009; Duru *et al.* 2009). These studies reported that early SFP was associated positively with LDMC at the species and at the community level but whether LFS responds to species differentiation in resource use is still unclear.

Focussing on primary foliar traits corresponding to the resource economy axis, and on a set of foliar and whole-plant traits that can potentially describe differences in plant strategies in areas with variable seasonal water supply we asked (i) what are the main axes of trait variation and which traits or set of traits underlie each of these axes?; (ii) do primary foliar traits and phenological traits correlate with the same axis of trait variation?; and (iii) are there significant correlations among primary foliar traits and PH, LS, Ca, K, Mg, LSCG, RD, SFP and LFS? The basis for this question is that primary foliar traits have been shown to have significant correlations among themselves; this type of correlation has been observed worldwide, and the expectation is that similar patterns of leaf variation will be found in neotropical semi-natural grasslands. Regarding specific trait relationships, we further predicted that (i) species with large LSCG would have high SLA and high leaf N and P; (ii) variation in PH and LS would be associated with the resource economy axis; and (iii) SFP would correlate positively with LDMC.

3.2 METHODS

3.1.1 Study site

The study was conducted in the Río Grande de Matagalpa watershed in Central Nicaragua (12°31'-13°20'N and 84°45'-86°15'W). The area is located in the municipality of Muy Muy, in sites within an altitudinal range of 260 to 380 m. At these elevations, there is a clear contrast between rainy and dry periods. The natural vegetation of the region corresponds to a transitional tropical sub-humid forest (Holdridge 2000) with semi-deciduous vegetation, and is referred to as seasonally dry tropical forest in Bullock *et al* (1995). The vegetation is an assemblage of native and naturalized species including grasses, herbs and woody plants. Here, we use the term semi-natural grassland to refer to a pastureland that grows naturally after forest clearing or on fallow land and which is maintained by fenced grazing and weed control. The mean annual rainfall (1971-2000) for Muy Muy was 1547.1±147 mm, and the mean annual air temperature is 24.3 °C (<http://www.ineter.gob.ni/direcciones/meteorologia/clima%20nic/caracteristicas%20delclima>). The topography is undulating, with slopes between 5 and 45%. The bedrock consists of Tertiary volcanic tuff, a type of pyroclastic rock. Tuff in the area seems to be impermeable, which explains the limited infiltration often observed in flat areas during the rainy season (Nieuwenhuyse *et al.* unpublished data). In the region, soil has high organic matter (5-7%), intermediate pH (6.1-6.6), a relative wide range of available P (2-9 ppm), and relatively high contents of clay (25-59 %), which is also associated with high levels of calcium (12-30 mg/kg) and potassium (10-35 mg/kg) (Chapter 4).

3.1.2 Species selection and trait measurements

The composition of these grasslands was assessed in two previous studies: the first was a floristic survey of 330 transects placed randomly in 8 paddocks between the dry and early rainy season of 2004 (Ospina 2005); the second was an inventory of species cover conducted in 20 paddocks between March and April 2007 (Ospina *et al.*, *submitted*). Based on these studies, thirty-two herbaceous and four woody species (three shrubs and a commonly occurring liana) in these grasslands were selected as the basis for this study (Table 3.1). The selected species encompass all dominant herbaceous species including seven common annual species (*Baltimora recta*, *Ageratum conyzoides*, *Caperonia palustris*, *Maranta arundinacea*, *Panicum laxum*, *Setaria parviflora* and *Indigofera* spp.), and two pteridophytes (*Ligodium venustum* and *Selaginella sertata*), the shrubs (*Mimosa albida*, *Mimosa pigra* and *Combretum fruticosum*), and a liana (*Serjania atrolineata*). All traits were measured following the procedures listed in Table 3.2. Details of the protocols and modifications followed for LLS, LSCG, RD, SFP and LFS, are given in Chapter 4.

3.1.3 Data analysis

To assess the significance of the relationships of primary foliar traits with plant nutrient status we applied Pearson correlation analysis between all combinations of SLA, LDMC, LLS and leaf N and P concentrations. These correlations were assessed for the entire set of species and for herbaceous species only. The distribution of mean species' values of all of these traits had skewness ≤ 0.77 ; therefore, trait values were suited for analysis without transformation. In order to discriminate the main axes of trait differentiation, a PCA based on a matrix of mean values of 14 traits for 32 herbaceous species was carried

out. Axes that explained a significant amount of variation were identified with the broken-stick model distribution. Since each eigenvalues of a PCA represents a measure of each axis's variance, an axis was retained if its associated eigenvalue was larger than expected from the broken-stick distribution (Legendre and Legendre 1998). The interpretable traits explaining each axis were identified with the broken-stick distribution, which in this case was applied to the squared loading of traits across axes in a vector matrix (Peres-Neto *et al.* 2003). Species mean foliar (LS, Ca, K and Mg), whole plant (PH and RD) and phenological (SFP and LFS) traits with skewness ≥ 1 were transformed with log functions and LFS with rank transformation, in order to reduce the effect of asymmetry before conducting the PCA analysis. Pearson correlations were used to determine specific correlations between pairs of traits and between traits and the PCA axes that exceeded the broken stick model criteria identified previously. Statistical analyses were conducted with the InfoStat package (Di Rienzo *et al.* 2009).

Table 3.1 Species used for trait analysis by family, life cycle and life form. Three or four-letter codes are used for species identities in figures.

Family	Species	Code	Life cycle	Life form
Acanthaceae	<i>Blechum pyramidatum</i> (Lamb.) Urb	Bl p	Perennial	Forb
	<i>Dyschoriste quadrangularis</i> (Oerst) Kuntze	Dy q	Perennial	Forb
Asteraceae	<i>Baltimora recta</i> L	Ba r	Annual	Forb
	<i>Ageratum conyzoides</i> L	Ag c	Annual	Forb
Combretaceae	<i>Combretum fruticosum</i> (Loefl. Stuntz)	Co f	Perennial	Shrub
Convolvulaceae	<i>Ipomoea hederifolia</i> L	Ip h	Perennial	Forb
Cyperaceae	<i>Cyperus rotundus</i> L	Cyz	Perennial	Sedge
	<i>Dichromena ciliata</i> Vahl.	Dy c	Perennial	Sedge
	<i>Cyperus articulatus</i> L	Cy a	Perennial	Sedge
	<i>Scleria melaleuca</i> Rchb. Ex Schitdl&Cham	Sc m	Perennial	Sedge
Euphorbiaceae	<i>Caperonia palustris</i> (L.) St. Hill	Ca p	Annual	Forb
Fabaceae	<i>Calopogonium muconoides</i> Desv	Ca m	Perennial	Legume
	<i>Desmodium distortum</i> (Aubl.) J.F Macbr	De d	Perennial	Legume
	<i>Centrocema pubescens</i> Benth	Ce p	Perennial	Legume
	<i>Desmodium procumbens</i> (Mill.) Hitche	De p	Perennial	Legume
	<i>Rhynchosia minima</i> (L) DC	Rh m	Perennial	Legume
	<i>Indigofera</i> sp.	Ind	Annual	Legume
Hydrophyllaceae	<i>Hydrolea</i> sp.	Hyd	Perennial	Forb
Malvaceae	<i>Sida acuta</i> Burm. F	Si a	Perennial	Forb
	<i>Sida jussieana</i> DC	Si j	Perennial	Forb
Marantaceae	<i>Maranta arundinacea</i> L	Ma a	Annual	Forb
Mimosaceae	<i>Mimosa albida</i> L	Mi a	Perennial	Shrub
	<i>Mimosa pigra</i> L	Mi pi	Perennial	Shrub
	<i>Mimosa pudica</i> L	Mi p	Perennial	Legume
Poaceae	<i>Dichanthium aristatum</i> (Poir.) C.E.Hubb.	Di a	Perennial	Grass
	<i>Paspalum centrale</i> Chase	Pa ce	Perennial	Grass
	<i>Paspalum conjugatum</i> Bergius	Pa c	Perennial	Grass
	<i>Hyparrhenia rufa</i> (Nees) Stapf in Prain	Hy r	Perennial	Grass
	<i>Paspalum notatum</i> Fluggé	Pa n	Perennial	Grass
	<i>Oplismenus burmanii</i> (Retz.) P. Beauv	Op b	Perennial	Grass
	<i>Panicum laxum</i> Sw	Paz	Annual	Grass
	<i>Setaria parviflora</i> (Poir.) Kerguelen	Se p	Annual	Grass
	<i>Paspalum virgatum</i> L	Pa v	Perennial	Grass
Sapindaceae	<i>Serjania atrolineata</i> C. Wright	Se a	Perennial	Liana
Schizaeaceae	<i>Ligodium venustum</i> Sw	Li v	Perennial	Pteridophyte
Selaginellaceae	<i>Selaginella sertata</i> Spring	Se s	Perennial	Pteridophyte

Table 3.2 Species traits with measurement unit, type of statistical variable, number of replicates, brief procedure for measurement, and references.

Trait type	Trait	Measurement unit	Statistical type	Number of replicates	Mode of measurement	Reference
Leaf	Leaf size (LS)	mm ²	Interval	9 to 30	On scanned leaves, average size of simple and compound leaves, including petioles	<i>Cornelissen et al. 2003</i>
	Specific leaf area (SLA)	mm ² mg ⁻¹	Ratio	9 to 30	Fresh lamina area/dry lamina mass including petioles	<i>Cornelissen et al. 2003</i>
	Leaf dry matter content (LDMC)	mg g ⁻¹	Ratio	9 to 30	Oven-dry mass/ Fresh mass, including petioles	<i>Cornelissen et al. 2003</i>
	Leaf lifespan (LLS)	weeks	Interval	10 to 14	One-week interval counts of leaves of each species on about 900-1000 leaves tagged from leaf emergence to leaf fall into eight different isolated modules set up for this study	<i>Ryser and Urbas 2000; Cornelissen et al. 2003</i>
	Leaf nitrogen concentration (N)	mg g ⁻¹	Ratio	1 compound sample ≥100 individuals	With an auto-analyzer (ThermoFinnigan, Flash EA 1112) by the total combustion method. Total amount of N / Leaf dry mass	<i>Cornelissen et al. 2003</i>
	Leaf phosphorus concentration (P)	mg g ⁻¹	Ratio	1 compound sample ≥100 individuals	Colorimetry using the molybdate and stannous chloride and read by spectrophotometer UV/V method (absorbance at 660 nm). Total amount of P / Leaf dry mass	<i>Cornelissen et al. 2003</i>
	Leaf calcium concentration (Ca)	mg g ⁻¹	Ratio	1 compound sample ≥100 individuals	With spectrophotometer of atomic absorption (Equipment Analysis 100, Perkin Elmer). Total amount of Ca / Leaf dry mass	
	Leaf potassium concentration (K)	mg g ⁻¹	Ratio	1 compound sample ≥100 individuals	With flame emission spectrometry (absorbance at 768 nm). Total amount of K / Leaf dry mass	
	Leaf magnesium concentration (Mg)	mg g ⁻¹	Ratio	1 compound sample ≥100 individuals	With spectrophotometer of atomic absorption (Equipment Analysis 100, Perkin Elmer). Total amount of Mg / Leaf dry mass	
Whole plant	Plant height at onset of flowering (PH)	cm	Interval	30	At the beginning of flowering the shortest distance between the highest photosynthetic tissue and the ground level	<i>Cornelissen et al. 2003</i>
	Lateral spread by clonal growth (LSCG)	cm	Interval	10 to 14	Measuring of the horizontal yearly growth of ramets by following those over time	<i>Knevel et al. 2005 with modification</i>
	Root depth (RD)	cm	Interval	10 to 14	Digging of trenches to determine depth of the root systems for each species, taking samples from three walls of each trench	<i>Schuster 1964 with modification</i>
Phenological	Start of flowering period (SFP)	week of the year (WOY)	Interval	8 per week	Continuous weekly surveys by observing the phenological status of individuals present along a 50 m long transect	-
	Length of the period from flowering to seed shed (LFS)	weeks	Interval	8 per week		-

3.3 RESULTS

3.3.1 Axes of trait differentiation and related traits

The first three axes of the PCA ordination based on 32 herbaceous species explained together 63% of the between species trait variation. Axes four and five represented in general new combinations of some of the traits integrated by axes 1 to 3 and none of the traits loaded significantly on them. Most traits loaded significantly on one principal component axis (1-3), except for LLS that loaded on both PCA axis 1 and 2, and for RD which loaded significantly on PCA axes 2 and 3 (Figure 3.1; Table 3.3).

PCA axis 1 (28% of the variation) was identified as an axis of resource capture based on primary foliar traits such as LLS and SLA, concentrations of N and P and of other foliar nutrients such as Ca and Mg. All these traits, except for LLS, had high scores on axis 1 (Figure 3.1; Table 3.3). The consistency and significance of the positive correlations between the foliar nutrients (Ca, Mg, N and P), as well as the significant associations of leaf Ca and Mg with SLA further supported the strength of this axis as the one representing the resource economy spectrum. Two major plant groups discriminate on axis namely grasses and sedges with low scores, and legumes and other forbs at high scores (Figure 3.1 b). In addition, Species with high leaf nutrient concentrations tended to have high SLA, and short LLS, but SLA and LLS, had no correspondence with leaf P concentration (Table 3.4).

PCA axis 2 (21% of the variation) integrates two primary foliar traits associated with resource conservation in plants, LDMC and LLS, and other traits such as Leaf K, LSCG, RD, SFP and LFS. High LDMC, long LLS, high LSCG, deep roots, late SFP, long LFS

and low leaf K concentration were associated with high values on PCA axis 2 (Figure 3.1; Table 3.3).

The strongest correlations corresponded to the positive relations of LDMC with LLS, and of LSCG with RD, but also the negative associations of leaf K with LDMC and LSCG (Table 3.4). PCA axis 2 reflects the contrast between annual species (low scores) and perennials (high scores) (Figure 3.1 b).

Table 3.3 Axes eigenvalues, expected variance values under a broken-stick model, loadings of plant traits on the first three PCA axes and percentage of variance explained by each axis all obtained from a matrix of 14 traits x 32 species. Traits were subsequently sorted according to the absolute value of their loadings in PC1, PC2 and PC3. Bold numbers represent significant loadings according to the broken-stick method.

Traits	PC 1	PC 2	PC 3
Explained variance	28.00%	21.00%	14.0%
Eigenvalue	3.82	2.82	1.97
Broken-stick eigenvalue	3.61	2.76	1.63
Expected broken-stick percentage	27	25	23
Leaf calcium concentration (Ca)	0.46	-0.06	0.07
Leaf magnesium concentration (Mg)	0.41	-0.09	0.12
Leaf nitrogen concentration (N)	0.39	0.11	0.03
Leaf phosphorus concentration (P)	0.30	-0.14	0.19
Specific leaf area (SLA)	0.27	-0.19	-0.12
Leaf dry matter content (LDMC)	-0.19	0.42	-0.22
Leaf potassium concentration (K)	-0.18	-0.42	0.12
Leaf lifespan (LLS)	-0.28	0.38	0.18
Lateral spread by clonal growth (LSCG)	0.22	0.37	0.07
Start of flowering period (SFP)	0.14	0.36	0.07
Length of the period from flowering to seed shed (LFS)	-0.04	0.28	0.17
Plant height at onset of flowering (PH)	-0.21	-0.12	0.54
Leaf size (LS)	-0.14	-0.12	0.53
Root depth (RD)	0.15	0.30	0.46

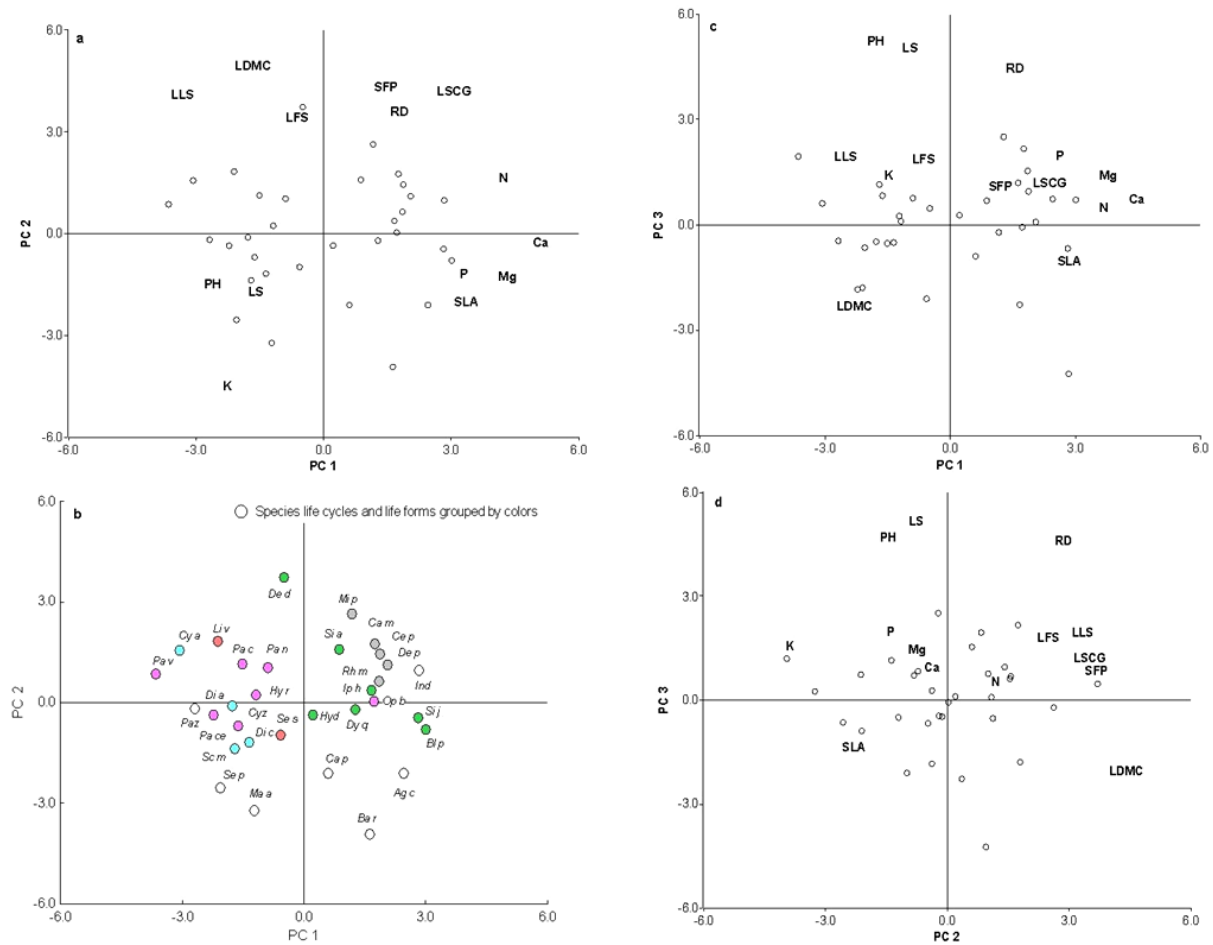


Figure 3.1 Arrangement of plant traits along the first 3 axes of a 32 species by 14 traits PCA of semi-natural grasslands in Central Nicaragua. Loading for all traits along PCA axes 1-3 are in Table 3.3. Table 3.2 gives the full names of the plant traits shown here in 3.1 a, 3.1 c and 3.1 d, as abbreviations. In Figure 3.1 b species life cycles and life forms are grouped by colors, *open circles* annual species, *color-filled circles* perennial species: pink grasses, blue sedges, grey legumes, green forbs and red pteridophytes.

Perennial legumes, grasses and a pteridophyte (*Ligodium venustum*) were associated with high values of LDMC, long LLS, deep roots, long LSCG and long LFS. The factor that corresponded strongest and positively with annual species was leaf K concentration (Table 3.3, Figure 3.1b).

The pairwise correlations generally support the associations identified in the PCA (Table 3.4). Species having high leaf K concentration had low LDMC and species with high LDMC had long LLS. Leaf K, LSCG and RD co-varied, i.e. species with the capacity for lateral spread had consistently deep roots and low leaf K concentration, although low leaf K was not always associated with deep roots (Table 3.4). Although SFP and LFS had significant loads on axis 2, some inconsistency of these traits with this axis was evident because their correlations with LDMC and leaf K were not significant. Late flowering was associated with wide lateral spread and deep roots, and species having long leaf longevity had also longer period from flowering to seed shed.

PCA axis 3 accounted for 14% of the total variance and appears to be size-related, given the strong association of PH with LS and RD with this axis. Tall species, with big leaves, and deep roots were found at high values of axis 3, while, short species with small leaves, and shallow roots occurred at low values (Figure 3.1, Table 3.3). The tight association of PH and LS with axis 3 is supported by a highly significant and positive correlation of PH with LS; however, the Pearson correlations of PH with RD, and of LS with RD were not significant, indicating a weaker structure in this size-related axis (Table 3.4).

Table 3.4 Pearson correlation coefficients between pair traits of 32 herbaceous species, grouped by each of the first three PCAs axes: (a) foliar traits representing the acquisitive type, (b) foliar traits, lateral spread and phenological traits representing the conservative type, (c) leaf size and whole plant traits representing the architectural variation, and (d) the first three PCAs axes. See table 3.2 for definitions and abbreviations. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

		a					b					c			
		Ca	Mg	N	P	SLA	LDMC	K	LLS	LSCG	SFP	LFS	PH	LS	RD
a	Ca														
	Mg	0.81***													
	N	0.59***	0.38*												
	P	0.65***	0.47**	0.39*											
	SLA	0.45**	0.42*	0.34*	0.09										
b	LDMC	-0.40*	0.56***	-0.01	-0.34*	-0.28									
	K	-0.29	-0.13	-0.49***	-0.02*	-0.08	-0.53***								
	LLS	-0.42*	-0.36*	-0.39*	-0.29	-0.43***	0.50***	-0.21							
	LSCG	0.25	0.22	0.37*	0.01	0.15	0.17	-0.50***	-0.02						
	SFP	0.20	0.18	0.44*	0.13	-0.12	0.32	-0.32	0.32	0.36*					
c	LFS	0.07	0.07	-0.26	-0.17	-0.22	0.12	-0.19	0.57***	0.29	0.07				
	PH	-0.21	-0.21	-0.24	0.07	-0.22	-0.11	0.32	-0.33	-0.33	-0.02	0.08			
	LS	-0.19	-0.19	-0.04	-0.05	0.01	-0.18	0.18	0.17	-0.09	-0.22	-0.12	0.72***		
d	RD	0.19	0.28	0.38*	0.14	-0.17	-0.05	-0.27	0.21	0.60***	0.38*	0.32	0.17	0.28	
	PC 1	0.89***	0.81***	0.75***	0.59***	0.53***	-0.37*	-0.35*	0.55***	0.45**	0.27	-0.08	-0.41*	-0.27	0.31
	PC 2	0.10	-0.15	0.19	-0.24	-0.32	0.70***	-0.70***	0.61***	0.58***	0.59***	0.47**	-0.21	-0.19	0.54***
	PC 3	0.10	0.17	0.05	0.26	-0.17	-0.32	0.17	0.25	0.10	0.09	0.23	0.76***	0.75***	0.65***

3.3.2 Correlations between primary foliar traits and other traits

For the whole set of 36 species we found the expected patterns of correlation among primary foliar traits highlighting two strong relationships: positive correspondence between LDMC and LLS, and negative between LLS and SLA (Table 3.5, Figure 3.2). In addition, leaf N correlated positively with leaf P and SLA, and negatively with LLS. However, leaf N was unrelated to LDMC, and SLA correlated only with leaf N but not with leaf P. Thus, high LDMC is not necessarily associated with low leaf N, and there was not a positive correlation between SLA and leaf P (Table 3.5).

The correlations between pairs of primary foliar traits for the herbaceous species were slightly different from those calculated on the set that included woody species; in particular, the lack of significant correlations in the herbaceous set between LLS and leaf P, and between LDMC and SLA. There was a significant negative correlation of LLS with leaf N and there was a significant though weak correspondence of LDMC with leaf P when the analysis comprised the herbaceous species only (Table 3.5).

Species with higher leaf N had a later start of flowering. Also, and independently of the trait associations along PCA axes, leaf N concentration was positively associated with SFP, the relationship being positive among herbaceous species ($n=32$, $r^2= 0.44$, $p= 0.0136$, Table 3.4), all perennials ($n=29$, $r^2= 0.48$, $p= 0.0186$) and within the annuals ($n=7$, $r^2= 0.74$, $p= 0.0447$) (Appendix 1 in Chapter 3, Figure. 3.3).

Root depth and leaf N concentration were also positively correlated, both for the entire data set, ($n= 32$, $r^2= 0.38$, $p= 0.0298$, Table 3.4) and when only perennial species were analyzed ($n= 29$, $r^2= 0.54$, $p<0.01$), but, there was no correspondence when the analysis

was restricted to annual species (Appendix 1 in Chapter 3). Species with greater lateral spread tended to have higher leaf N concentration ($n= 32$, $r^2= 0.37$, $p= 0.0341$, Table 3.4) for the herbaceous species, and across perennial species ($n= 29$, $r^2= 0.42$, $p= 0.0261$, Appendix 1). Also, species with long-lived leaves have slower reproductive development. Leaf lifespan and LFS were positively correlated among the herbaceous species ($n=32$, $r^2= 0.57$, $p<0.01$, Table 4), across perennials ($n= 29$, $r^2= 0.45$, $p= 0.0257$), and annuals ($n= 7$, $r^2= 0.78$, $p= 0.0290$) (Appendix 1 in Chapter 3, Figure 3.3). Leaf N, P, Ca and Mg concentrations were positively correlated. Independently of their loadings on the PCA, correlations between these traits revealed tight pair-wise correlations with a consistent array amongst leaf nutrients such as N, P, Ca and Mg ($n= 32$, $r^2= 0.39$ to 0.81 , $p= 0.0296$ to <0.01 , Table 3.4). Leaf K had negative correlations with all other foliar nutrients but these were only significant with leaf N ($n= 32$, $r^2= - 0.49$, $p<0.01$) (Table 3.4).

Table 3.5 Pearson correlation coefficients of primary foliar traits for whole set of 36 species (32 herbaceous plus 4 woody species, below the diagonal) and for 32 herbaceous species (above the diagonal)

	LDMC	SLA	LLS	N	P
LDMC	1.00	-0.28	0.50***	-0.01	-0.34*
SLA	-0.33*	1.00	-0.43**	0.34*	0.09
LLS	0.66***	-0.45**	1.00	-0.39*	-0.29
N	0.03	0.33*	-0.26	1.00	0.39*
P	-0.38*	0.13	-0.34*	0.38*	1.00

See Table 3.2 for definitions of abbreviations. * $p<0.05$; ** $p<0.01$; *** $p <0.001$.

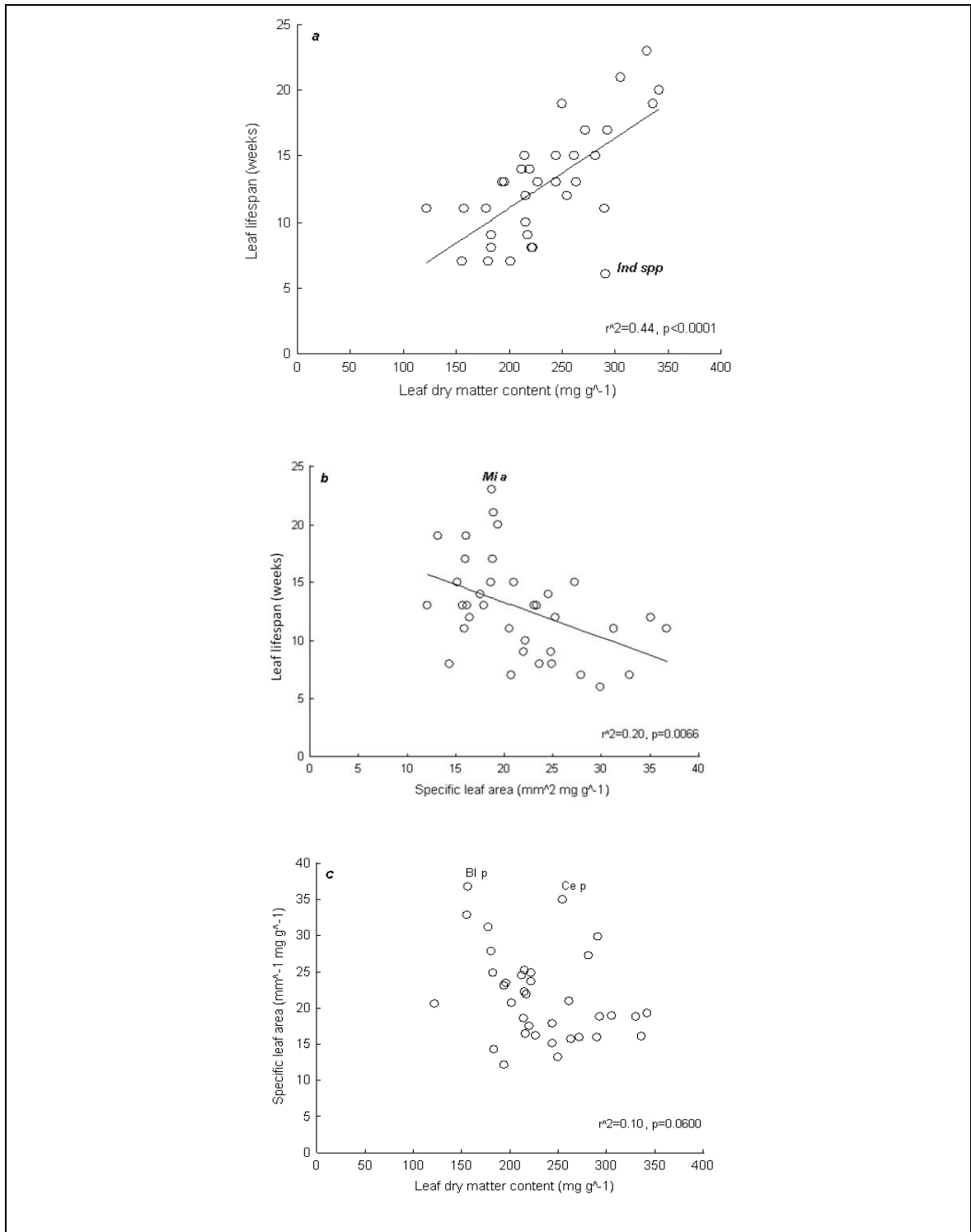


Figure 3.2 Relationships in the 36 herbaceous and shrubby species for some highly significant correlations between (a) leaf lifespan (weeks) and leaf dry matter content (mg g^{-1}); (b) leaf lifespan (weeks) and specific leaf area ($\text{mm}^2 \text{mg}^{-1}$) and (c) specific leaf area ($\text{mm}^2 \text{mg}^{-1}$) and leaf dry matter content (mg g^{-1}). Lines indicate significant regressions $p<0.05$.

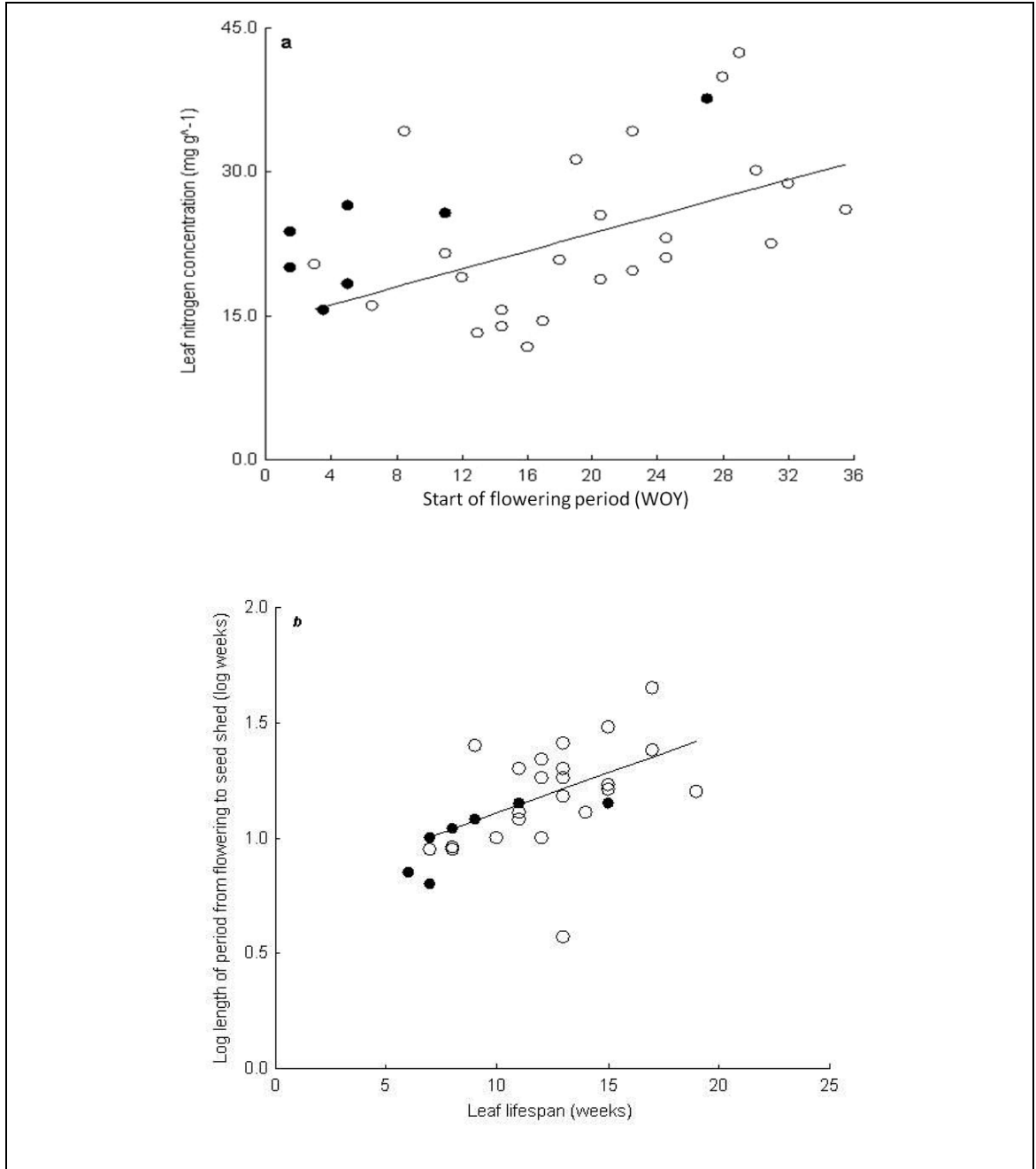


Figure 3.3 Two primary foliar traits of the 32 studied herbaceous species plotted against two phenological traits. (a) Relationship between leaf nitrogen concentration (mg g^{-1}) and the start of flowering period estimated as week of the year (WOY) from July 2, 2007 to June 30 2008. (b) Relationship between the log length of the period from flowering to seed shed and leaf lifespan (weeks). *Open symbols* perennial species; *closed symbols* annual species. Lines indicate significant regressions $p < 0.05$.

3.4 DISCUSSION

3.4.1 Main dimensions of morphological trait differentiation and correlated traits

We used a set of primary foliar traits with well-established correspondence with resource use strategies in plants (Grime *et al.* 1997; Reich *et al.* 1997; Díaz *et al.* 2004; Wright *et al.* 2004). While linkages between primary foliar traits and the plant's resource economy strategy have been established, the correspondence with other important traits for plant persistence and reproduction (PH, LS, RD, and leaf K, Ca and Mg concentrations, LSCG, SFP and LFS) has not yet been tested due to their complex variability, and to time-consuming measurements.

All morpho-physiological traits measured and analyzed in this study were significantly associated with the first three axes of trait differentiation, and in some cases, such as LLS and RD, variation was linked to more than one dimension of trait differentiation. The main axis of trait differentiation was centered on leaf nutrient concentration (Ca, Mg, N and P) and other leaf traits that are related with resource acquisition strategies (SLA and LLS). These results are in agreement with findings reported in the literature from other regions and vegetation types, further supporting the notion of the importance of the resource economy axis encompassing fundamental trade-offs in plants which is reflected in a primary axis of specialization in resource use strategies.

This first axis discriminates primarily perennial and annual dicotyledoneous species, and one grass species, *Oplismenus burmanii*, characterized by high foliar nutrient concentrations (Ca, Mg, N, and P), high SLA and low LLS., from perennial and annual grass species (of the genus *Paspalum*, *Hyparrhenia* and *Dichanthium*), Cyperaceae,

pteridophyte, and one perennial dicotyledoneous species. Beyond the relevance and consistency of this primary axis of differentiation, correlations of leaf P concentration with both LLS and SLA were not significant across the herbaceous species. The lack of correspondence could be attributable to the generally low leaf P concentrations found in this set of species, in a range between 1.31 and 2.96 mg g⁻¹, and reflecting the extremely low soil P concentration in the area of the study. Further, some annual species had low leaf P concentration and short LLS, but also other species with high SLA had relatively low leaf P concentrations. Low leaf P concentration in the annuals might be explained by their short time window to acquire nutrients and the strong soil P limitation in the study area (Chapter 4). The perennials instead have the potential to recycle nutrients through re-sorption from senescing leaves (Aerts 1996). These results indicate that species can differ in resource acquisition strategies despite the strong limitation of a particular nutrient. Similar to other studies in areas with low soil P concentration, leaf P concentration was correlated with primary foliar traits such as leaf N concentration and LDMC, although in general more weakly in our study (Reich *et al.* 1991; Wright *et al.* 2004; Wright *et al.* 2005; Orwin *et al.* 2010).

The second dimension was underlined by primary foliar traits (LDMC and LLS), that are frequently associated with resistance to physical damage by means of structural investments in leaf protection (physical defence) that make more likely the conservation of internal nutrients longer (Wright and Cannon 2001; Cornelissen *et al.* 2003). Other traits that underlined the variation explained by this axis such as leaf K concentration, LSCG, RD, SFP and LFS, reflected a distinction between life cycles and life forms among the species. High LDMC, low leaf K concentration and long LLS, the three most

important traits in this axis' underlined the species with higher investment in foliar structure and separated at the highest end perennial grasses and sedges, from species with high leaf K concentration and lower investment in leaf structural compounds; with mainly the annuals in the extreme and perennial forbs and legumes in intermediate ranges.

Regarding leaf K concentration, no previous studies have reported a negative relationship with leaf dry mass or longer leaf longevity. Potassium has higher mobility in soils and plants than Ca and Mg, and the highest concentrations in the soil are found in the top soil, indicating the importance of organic matter mineralization as the main source of K supply for the plants (Salisbury and Ross 1992; Jobaggy and Jackson 2004). Accordingly, it could be expected that leaf K concentration would be higher in plants with litter that decomposes fast, i.e. with low LDMC and low concentrations of leaf structural carbohydrates (Cornwell *et al.* 2008; Fortunel *et al.* 2009).

The results in this study show that grasses and sedges were the plant groups with lowest leaf K concentration, supporting the evidence from other studies where soil K limited more the growth of forbs and legumes than that of grasses and sedges (Thurston 1969 cited in Grubb 1977).

SLA and LDMC are generally considered to scale inversely along the resource acquisition axis and to correspond in an opposite way with leaf N concentration (Cornelissen *et al.* 2003; Gross *et al.* 2007). However, this study showed that SLA and LDMC are primarily associated with different and independent axes of trait differentiation. The second axis of differentiation appears to correspond to a strategy of

resistance through physical defence and to be rather unrelated to primary foliar nutrients concentrations. Lower LDMC was a common feature in annual species, and the lower investment in leaf structure is likely an advantage for these species compared with the benefits of stronger leaf structure of the perennial species (Fitter 1997).

It has been found that species with high LDMC exhibit low leaf N concentration (Duru *et al.* 2005; Gross *et al.* 2007) and low LDMC is generally related to a strategy of resource acquisition and use (Vile *et al.* 2005; Pontes *et al.* 2007). However, in our study, LDMC and leaf N concentration were uncorrelated across the herbaceous species, though LDMC was negatively correlated with the other axis - linked foliar nutrients concentration – it indicated an inconsistent relationship between LDMC and other traits related to the resource use axis. This complex relationship could be related to strategies of both resource use and resistance to biomass loss. In grasses, the presence of structural compounds in leaves is a main defence mechanism to deter herbivores, in contrast to chemical defence which is uncommon in this plant group. In this study, also legumes had high LDMC and high leaf N concentration. In these cases, high LDMC could confer protection against herbivory in plants with otherwise highly nutritious tissues. Another possibility is that the high leaf N concentration in the legumes is part of a strategy for water conservation in low rainfall habitats such as found in the studies of Wright *et al.* (2004) with diverse sclerophyllous species in eastern Australia.

Interestingly, axis 2 is also associated with LSCG, RD, SFP and LFS. The association of these traits implied that the second axis summarized other dimensions of converging specialization that include clonal growth, root depth, flowering, and seeding phenology.

Most of the variation in these traits is comprised first by the distinction of life cycles and second, by differences within life forms. The functional traits that differentiate perennials were the presence of clonal organs, late start of flowering, deep roots and long period from flowering to seed shed. Within perennial species, differences in LSCG, RD, and LFS were to some extent explained by the inherent differences between sedges, grasses, forbs and legumes. Thus, legumes had a generally greater capacity for lateral spread and longer duration of flowering than grasses, also legumes showed the deepest roots, followed by other forbs, grasses and sedges. As mentioned the strong positive correlation of RD with LSCG applied to perennials, corresponding with the findings of Roumet *et al* (2006) where chemical root traits were first affected by life cycles and life form differences among herbaceous species.

Positive correlations in perennial species of LSCG and RD with leaf N concentration are indicative that both the lateral spread by clonal growth and the depth of the root could be related to the resource acquisition strategy. This is in agreement with earlier findings showing that high rates of lateral spread and branching are common in fertile soil conditions and *vice versa* (Sammul *et al* 2003; Rusch *et al.* 2010) and with studies that evidenced life-form differentiation in nutrients and water use strategy between grasses and legumes (Mamolos *et al.* 1995; Nippert and Knapp 2007).

Additionally, we identified a third dimension reflecting size differences among the various herbaceous life forms. PH and LS underlined the variation in this axis, which coincided with findings from a number of studies (Chazdon 1991; Westoby 1998; Westoby *et al.* 2002) where this type of association has been interpreted as an axis of

allometric or architectural trait variation. RD was also associated with this axis, although not consistently correlated with PH and LS. We found that forbs, the tallest plants with largest leaves were differentiated from pteridophytes, grasses and sedges, and shorter species. However, annual forbs consistently had shallow roots. Legumes achieved a diverse range of plant heights and showed consistent correspondence between PH and LS, and a stronger relationship of PH and LS with RD than in the other life forms. For these reasons, RD has to be interpreted with caution when analyzed for allometric differences across herbaceous life forms. Our findings are partially supported by Schenk and Jackson (2002) showing that the stature of plants was a relevant and consistent predictor of RD. They found that across life forms taller species tend to have deeper roots and that within life forms, species with larger aboveground biomass tended to have deeper roots. Our results do not contradict the fact that some variation in PH and LS, not detected here, may hold across life forms and can be linked to the seasonal resource supply, however, the major pattern of variation for PH and LS has a basis on phylogenetically derived life forms and life cycles.

3.4.2 Linkages of phenological traits with resource use of the species

Based on earlier evidence (Ansquer *et al.* 2009; Duru *et al.* 2009) we hypothesized that SFP would correlate positively with LDMC. The expected positive correlation of SFP with LDMC was non-linear; and the result was similar for the correlation between SFP and LLS, although a relevant aspect of these correlations is that there was evidence of the expected positive direction across the herbaceous species (SFP vs. LDMC, $n= 32$, $r^2=0.32$, $p= 0.0865$; SFP vs. LLS, $n= 32$, $r^2=0.32$ $p= 0.0675$), which could be indicative

that later flowering tended to be present in species having higher investment in leaf structure and longer leaf longevity. In a corresponding way to LSCG and RD, the variation in reproductive phenological traits depended on differences in life cycles and life forms. All annuals and some sedges and forbs had the onset of flowering early in the rainy season while most perennials reached the onset of flowering late in the rainy season or during the dry season.

Across all herbaceous species and within annuals and perennials there was also a positive correlation of SFP with leaf N concentration. The explanation again has a basis mainly in the intrinsic differences in the phenological development of the different life forms, varying in lifespan and in the time of flowering onset, and having inherently different foliar concentrations of nutrients (particularly of leaf N), e.g. legumes with highest values of foliar N could reach flowering later, while annual species (with average high values of leaf N) had the earliest onset of flowering.

The length of the period from flowering to seed shed showed a strong positive relationship to LLS. A tendency of an association between long leaf lifespan and long period from flowering to seed shed has not been reported explicitly earlier. The longer LFS matching a longer leaf lifespan across all species, and within species groups with different life cycles, appear as another indication that duration of phenological stages (flowering/seeding) such as SFP has an important basis in the intrinsic differences in the phenological development of the different life forms.

3.5 CONCLUSIONS

Herbaceous species in tropical semi-natural grasslands have clear patterns of differentiation in their traits. Our data support the expectation that with high foliar concentrations of N, P, Ca and Mg, high SLA and short LLS form the basis of an acquisitive strategy where species having low leaf thickness per unit area, have high foliar nutrient content. There is strong evidence reported in the literature that these attributes correspond to a strategy in plants of high rates of resource acquisition and turnover, and growth. Conversely, low foliar concentration of N, P, Ca and Mg, low SLA and long LLS correspond to a resource conservative strategy. Relationships of leaf P concentration with LLS, and SLA are weak, but in the direction expected. The second axis of species traits differentiation is governed by differences in life-history and life form among species. A trend is evident of leaf K concentration with grasses and sedges having the lowest concentrations, perennial forbs and legumes, intermediate, and annual species the highest values. Similarly, across perennials, LSCG, RD, SFP and LFS were to some extent explained by the inherent differences between grasses, forbs and legumes. Both axis 1 and axis 2 point out a differentiation in the species strategies for resource use; the first one differentiating species with low foliar nutrients, long LLS indicating slow growth rate, and the second, those with high LDMC and longer LLS. Also associated with the second axis, there is a consistent trend among perennials to have higher capacity for lateral spread and deep roots, associated with high leaf N concentration. These plant features are associated with the acquisitive strategy and correspond also to the capacity to restore tissue, being therefore associated with tolerance to grazing (Rusch *et al.* 2009). In addition, the non-negative association of leaf

dry matter content and nitrogen concentration could be related to strategies of perennial life forms to cope with herbivory. High dry matter content that result from higher concentrations of structural carbohydrates (cellulose, lignin) confer resistance to physical damage and reduce attractiveness to herbivores and digestibility. High LDMC could confer a defence mechanism against herbivory in species that would otherwise be attractive due to high leaf nutrient contents. A third axis of trait differentiation includes the variation in PH and LS and its major explanation are phylogenetic differences between species life cycles and life forms. The size-related trait RD underlines the differentiation within this axis although it does not always correlate with PH and LS. Phenological traits appear to be indicative of different life-form strategies for resource use as they tend to be positively correlated with LDMC and LLS. Particularly, species with lower LDMC tend to have earlier SFP than species with higher LDMC, and longer LLS, and species with longer LLS have a longer period from flowering to seed shed.

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APPENDIX 1 - CHAPTER 3

Pearson correlation coefficients for pair trait correlations, calculated for particular groups of species: annuals (below the diagonal), perennials (above the diagonal).

	N	LLS	LSCG	RD	SFP	LFS
N	1.00	-0.40*	0.42*	0.54**	0.48*	-0.23
LLS	-0.51*	1.00	-0.32	-0.11	0.19	0.45*
LSCG	0.49	-0.18	1.00	0.55**	0.03	0.17
RD	0.23	0.47	-0.10	1.00	0.23	0.20
SFP	0.74*	-0.26	0.92***	0.04	1.00	-0.16
LFS	-0.41	0.78*	-0.29	0.35	-0.28	1.00

See Table 3.2 for definitions of abbreviations. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

CHAPTER 4

SEASONAL RESOURCE GRADIENTS INTERACT WITH SITE HETEROGENEITY IN DETERMINING COMMUNITY FUNCTIONAL PARAMETERS IN SEMI- NATURAL GRASSLANDS OF CENTRAL NICARAGUA *

ABSTRACT

Assessing the seasonal shift in the aggregated composition of traits that correspond with resource use strategies in plants is a functional approach to improve our understanding of the shifts in the composition of plant strategies in environments with temporal variation in resource supply. Fourteen morpho-phenological traits across 32 herbaceous and four woody species were measured: leaf size, specific leaf area, leaf dry matter content, leaf lifespan, foliar concentrations of P, N, Ca, K, and Mg, plant height, lateral spread by clonal growth, root depth, start of flowering period and the length of the period from flowering to seed shed. The community weighted means for five different paddocks were calculated for eleven periods (between July 2nd 2007 and May 26th 2008) that were divided into four seasonal periods according to the rainfall seasonality; early rainy season, late rainy season, early dry season and late dry season, with trait values weighted by species cover. The dominance of plants with high foliar concentrations of P,

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N, Ca, K and Mg, high specific leaf area, wide lateral spread, low leaf dry mass and short leaf longevity increased in the rainy season and declined towards the dry season, suggesting the relevance of rainfall seasonality to community functional properties in these grasslands. All aggregated traits analyzed, except plant height, were significantly affected by paddock heterogeneity, and soil variation was a relevant explanatory factor. The interaction between the seasonal period and paddock heterogeneity was another source of variation; however these interactions explained less variation than the main factors.

4.1 INTRODUCTION

Theory predicts a fundamental axis of specialization in plants, namely a resource economy axis, which entails contrasting strategies of resource use, from species with high rates of resource acquisition and circulation, low resource use efficiency, and that are successful at high levels of resource supply; to species that have a strategy of conservative resource use and low demand, and that perform well in chronically poor environments (Grime *et al.* 1997; Díaz *et al.* 2004; Wright *et al.* 2004).

Evidence about the distribution of plant traits on resource availability gradients (nutrients, water) strongly supports the functional interpretation of species strategies (Grime 1977; Díaz *et al.* 1998; Wright *et al.* 2004). These studies have been conducted along spatial gradients at various scales but analysis within plant assemblages indicate that trait variability can be as large or larger within sites than between sites with contrasting environment (Westoby *et al.* 2002), which supports the notion of niche differentiation among coexisting species (Silvertown 2004).

In environments with temporally fluctuating resource supply, a shift in species composition and plant strategies in time can be expected. Seasonally dry environments provide an opportunity to investigate shifts in dominance of plant traits with temporal changes in resource supply, which to our knowledge has not been reported in the published literature. Savannas and grasslands in many areas of the neotropics experience pronounced rainfall seasonality, with a pattern of increased irregularity in the number and intensity of rainfall events at the onset and end of the rainy season. Since water supply is a primary determinant of seasonal plant growth (Bonnet *et al.* 2010), and the amount of water available is directly linked to the capacity of the plant to capture nutrients (Weltzin *et al.* 2003), the temporal pattern of rainfall represents the most important resource supply gradient in these systems.

In the area of this study, the pattern of growth of the herbaceous vegetation closely reflects seasonal changes in water supply (Ospina *et al.*, *submitted*, Chapter 2). Above-ground productivity increases with rainfall and declines towards the end of the rainy season, however, there are indications of a sequence of species attaining dominance at different times of the growing period (Ospina *et al.*, *submitted*).

One hypothesis in this study was that in these grasslands, a set of species that have an acquisitive resource use strategy would attain dominance when resources are in high supply in the mid-wet season, and others that have traits enabling them to make use of more unreliable and scarce resources would sustain growth at the end of the rainy season and early in the dry season. Based on trait correlations among some foliar and phenological traits, and lateral spread in the dominant species, an acquisitive-

conservative spectrum was identified as the main axis of trait differentiation among the dominant species of the grasslands in this study (Chapter 3), which also suggests that temporal differentiation in plant strategies may exist. These observations are supported by findings in neotropical savannas, where co-existing species often reach peak biomass at different times of the rainy season (Sarmiento 1984; Sarmiento 1992).

The functional properties of individuals and populations, represented by plant traits can be scaled up to communities and ecosystems (Díaz *et al.* 2004; Violle *et al.* 2007) through the biomass ratio hypothesis (Grime 1998), according to which the trait values of the most abundant species at any given time will capture the magnitude of the ecosystem properties (Garnier *et al.* 2004). The functional trait composition of an assemblage, calculated as the community weighted mean of a trait (henceforth, CWM) or the community aggregated trait, is a synthetic metric for up-scaling species functional characteristics to communities representing the dominant trait values in a community (Violle *et al.* 2007). The CWM has been promoted and used for the evaluation of how changes in community structure and composition affect ecosystem processes (Garnier *et al.* 2004; Garnier *et al.* 2007; Díaz *et al.* 2007).

Following the notion of the biomass ratio hypothesis (Grime 1998) it was hypothesized that temporal shifts in community averages of plant traits would reflect seasonal changes in the composition of plant strategies. The study aimed at an assessment of whether there are seasonal changes in the average composition of traits that have a documented correspondence with the resource use strategies in plants and also of other traits with functional importance for which empirical evidence exists, but is limited. The general

expectation was that species with different strategies of resource use would shift dominance along a seasonal sequence.

Specifically, we hypothesized that the CWM of a series of foliar traits that are known to be sensitive to shifts in the soil conditions, namely specific leaf area (SLA), leaf dry matter content (LDMC), leaf lifespan (LLS), and leaf nitrogen and phosphorus concentrations (N and P, respectively) (Ryser and Urbas 2000; Cornelissen *et al.* 2003; Garnier *et al.* 2004), would vary amongst seasonal periods. We predicted that in the rainy season, CWM would be higher in the case of traits positively related to high resource acquisition and circulation (SLA, N and P), and in the dry season, the dominant species would have higher values of traits associated with a conservative strategy (LDMC and LLS).

Firstly, for the leaf concentration of calcium, potassium and magnesium (Ca, K, Mg), Grime *et al.* (1997) found positive correlations of leaf K with N and P and concluded that leaf N, P, K, Ca, and Mg are associated with rapid growth under favourable growth opportunities; however, Wright *et al.* (2005) found that leaf K showed clearly weaker relationships with leaf mass per area, leaf lifespan and photosynthetic capacity per unit leaf mass and concluded it cannot be considered a core trait to the 'leaf economics spectrum' (Wright *et al.* 2004). In the grasslands in this study, high leaf Ca and Mg concentrations are some of the foliar traits that indicate the acquisitive species strategy, and annuals, which mainly occur at the start or in the middle of the rainy season, have particularly high leaf K concentration compared with perennials (Chapter 3). According

to this evidence, we hypothesized that the CWM of leaf Ca, K and Mg concentrations would be higher in the rainy season than in the dry season.

Secondly, although root traits reflect an allometric pattern of variation (e.g., between annuals and perennials), and respond plastically to the properties of the soil (Hodge 2004; Roumet *et al.* 2006), there is some evidence of a relationship of this trait with strategies of resource use. Differences in root depth (RD) between C4 grasses and C3 (forbs) evidence differentiation in water use strategy. Grasses, consistently use water in the shallow soils layers, while C3 species increase their use of soil water from greater depths when the upper horizons become dry (Nippert and Knapp 2007). With N and P additions grass species became shallower rooted, but legumes had deeper roots with N addition (Mamolos *et al.* 1995). We therefore expected CWM of RD to be shallower in the rainy season than in the dry season.

Thirdly, the capacity of vegetative spread correlates with nutrient supply. Fertilization increased vegetative mobility and branching (Sammul *et al.* 2003) and lateral spread by vegetative growth (LSCG) correlated positively with leaf N concentration, but also with root depth (Chapter 3). Also low rates of lateral spread have been found at low soil fertility (Rusch *et al.* 2010). Therefore, we hypothesized that aggregated LSCG should be longer in the rainy season than in the dry season.

Fourthly, start of flowering period (SFP) and length of the period from flowering to seed shed (LFS) appear to be related with the plant's resource use strategy because those traits are associated with plant development and a temporal differentiation of resource use over seasonal periods (Roscher *et al.* 2004, chapter 3). The timing at which species

reach phenological stages is related to species growth rate and the seasonal use of nutrients (Sosebe and Weibe 1973); hence, we expected the CWM of SFP to follow the compositional change from annual- to perennial-dominated stages, but whether LFS would respond to the differentiation of resource use is unclear. However, we expect that the CWM of LFS will present a trend of seasonal variation similar to CWM of LLS because according to findings in the early analysis (Chapter 3), species with shorter LLS have shorter LFS, and *vice versa*.

Fifthly, for plant height (PH) and leaf size (LS) patterns of variation along resources gradients are regularly linked to allometric factors (plant size, anatomy and architecture) (Westoby 1998; Westoby *et al.* 2002), and this sort of variation appears to be an important distinction amongst life forms (Cornelissen *et al.* 2003). However, it is well known from general observation that species occurring at low fertility and availability of water or rainfall tend to be lower in height and to have a smaller leaves. Thus, small LS generally corresponds with stressful environments (dry, disturbed) (Wright *et al.* 2004; Niinemets *et al.* 2007) likely due to a greater evaporative demand of large leaves and to the high cost of the investment in veins to support big leaves (Niinemets *et al.* 2007). Potential plant height across species has been positively related to gradients of soil resources (Fonseca 2000; Cornelissen *et al.* 2003; Rusch *et al.* 2009), but height is affected by ontogenetic change through the lifetime of individuals, which may confound responses to the environment (Niklas 2004; Westoby and Wright, 2006). In the semi-natural grasslands studied, across 32 herbaceous species, PH and LS underlined a gradient of species variation in size represented in life forms differences (Chapter 3). Taking this evidence together and considering that the target species include diverse life

forms, aggregated at community level, we hypothesize that these traits would be less sensitive to shifts in the amount of resources between the rainy and dry seasons.

4.2 METHODS

4.2.1 Study site

The study was conducted in the Río Grande de Matagalpa watershed in Central Nicaragua (12°31'-13°20'N; 84°45'-86°15'W). The area is located in the municipality of Muy Muy, in sites within an altitudinal range of 280 to 380 m. In the study site there is a clear contrast between rainy and dry periods, and the predominant land use is livestock farming with relatively homogeneous livestock management. The natural vegetation of the region corresponds to a transitional tropical sub-humid forest (Holdridge 2000) with semi-deciduous vegetation, and is referred to as seasonally dry tropical forest in Bullock *et al* (1995). The vegetation in the study is an assemblage of native and naturalized species including grasses, herbs and woody plants. Here we use the term semi-natural grassland to refer to a pastureland area covered by spontaneous vegetation that grows naturally after forest clearing or on fallow land and which is maintained by grazing management, including fencing and weed control. Rainfall recorded between November/December and April/May during this study is less than 10% of the rainfall normal (1971-2000) for Muy Muy (1547.1±147 mm); the annual mean air temperature is 24.3°C (<http://www.ineter.gob.ni/direcciones/meteorologia/clima%20nic/caracteristicasdelclima>). The topography is undulating, with slopes between 5 and 40%. The bedrock consists of Tertiary volcanic tuff, a type of pyroclastic rock. Tuff in the area seems to be impermeable, which explains the limited infiltration often observed in

flat areas during the rainy season. In most flat areas, it is common to find soils with Vertic properties (Nieuwenhuysen *et al.* unpublished data).

4.2.2 Species selection and trait measurements

Based on earlier studies that assessed the composition of the grasslands (Ospina 2005; Ospina *et al.*, *submitted*, Chapter 2), thirty-two herbaceous and four common woody species were selected for a trait study. Our target species group comprised various life forms of annual and perennial plants including two pteridophytes, representing various vascular plant families with a wide range of functional traits (Appendix 1 in Chapter 4).

The traits were studied in plants occurring in eight paddocks with a size ranging between 2.5 and 5 ha. The paddocks were at least 12 years old, and were managed under grazing, with hand weeding at least once a year, and no fertilizer applied. At the beginning of the rainy season 2007 (early June), the paddocks were mown to ground level to homogenize the height of the sward at the start of the trait study. Also, woody species taller than 20 cm were cut at ground level and the harvested biomass was removed from the paddocks. Soon after clipping, one permanent sampling plot (henceforth, PSP) of 100 m x 100 m was randomly delimited in each paddock.

Fourteen traits were measured: nine foliar traits: leaf size (LS), specific leaf area (SLA), leaf dry matter content (LDMC), leaf lifespan (LLS), and foliar concentrations of P, N, Ca, K, and Mg; three whole plant traits: plant height (PH), lateral spread by clonal growth (LSCG), and root depth (RD); and two phenological traits, start of flowering period (SFP), and the length of the period from flowering to seed shed (LFS). The procedures for trait measurement are listed in Table 3.2 in the Chapter 3.

LLS was observed in the PSP, and the rest of the traits in the whole paddock. Plants were chosen randomly for trait measurement (except in the case of LSCG and LLS, where plants were subjectively chosen) with a minimum distance of 2 m between them. LS, SLA, LDMC, foliar N, P, Ca, K, and Mg concentrations were measured all along the rainy season of 2007, RD during the rainy season of 2008; while PH was measured at the beginning of the flowering period of each species. LLS, LSCG, SFP, and LFS were observed during one year, between July 2007 and June 2008. Thirty replicates (individuals) were taken for PH, LS, SLA and LDMC except for *Ligodium venustum* and *Selaginella sertata* for which nine replicates were measured for LS, SLA and LDMC. Foliar concentrations of N, P, Ca, K and Mg were assessed on a compound sample per species. For measurements of LLS, RD and LSCG, at least 10 to 14 replicates were used. SFP and LFS were determined weekly on at least eight replicates per species.

PH was assessed by measuring the shortest distance between the highest photosynthetic tissue and the ground level during the week when at least five individuals (of a total of eight random individual observed were starting the flowering period in at least five of the paddocks. For species with a rosette growth habit (e.g. *Blechnum* spp.), PH was the height of the rosettes leaves. In the case of *Cyperus articulatus* that at flowering time has photosynthetic shoots but not leaves, the average height of central shoots was considered to be PH.

For all foliar traits, except for LLS, measurements were conducted on fully developed, healthy-looking and undamaged mature leaves. LS, SLA and LDMC were determined following the protocol in Cornelissen *et al.* (2003). For species with pinnately-

compound leaves (*Mimosa pudica*, *Mimosa pigra*, *Ligodium venustum* and *Selaginella sertata*) the individual leaflets were counted, and the leaflets from one side were scanned without petiole and rachis. Mean leaf size was calculated by first multiplying the leaflet area by two and then, by multiplying the number of pinnae by two. Leaf samples for LS determination were transported to the lab in cooler boxes and processed within 8 hours after their collection. SLA and LDMC were determined on re-hydrated leaves, treated as those for LS determinations, rehydrated at 1 - 5° C for 6 - 10 hours, and processed within 24 hours after their collection. Leaves to determine foliar N, P, Ca, K and Mg concentrations were collected following the same procedure as for LS, SLA and LMDC, but in this case for each species a compound sample was collected from at least 100 individuals (1-12 leaves per individual), and rachis and petioles were removed.

LLS was assessed following the method of periodic records of tagged leaves (Cornelissen *et al.* 2003) and modified according to Ryser and Urbas (2000). Each grid, 1.6 x 0.6 m, was divided into 96 squares with thin white rope with 16 columns by 6 rows: each square had an area of 100 cm² (10 x 10 cm). LLS was assessed by identifying and marking with a white colour marker newly developed leaves in each 100 cm² square. 1.6 x 0.6 m grids were subjectively located on vegetation patches with relatively low single species dominance; each was covered with a metal cage to protect it from grazing. The grid was visited weekly and leaf status of grasses and forbs, alive or dead, was recorded per square. Leaves were considered to be dead when they were detached from the plant. If they were detached, but not brown it was recorded as a missing value. When the leaf was dead, a new leaf was tagged in the same square. Records in each grid started on 16th July 2007 and continued until 90% of leaves initially tagged were

recorded as dead. The grid was then relocated within the PSP. Grids were relocated successively, such that there was always a grid in a PSP. The weekly records were continued until July 31st 2008. LLS was calculated as the difference in time (weeks) between emergence and death of leaves.

LSCG in a year was assessed following the method described in Knevel *et al.* (2005) and modified according to the approach used by Pitelka *et al.* (1985). In each of the eight paddocks, three 1x1 m LSCG plots with between 4 and 10 species of the targeted group were selected. Within the LSCG plots, a section of 0.25 m² was marked and dug up by hand, looking for several well-developed plants with large and interconnected above-ground and below-ground structures. With this procedure the structure of the clonal growth organs was described by drawing and the information used to identify a method for tagging and measuring LSCG in each species. Then, at the border of the each of the 24 LSCG plots (8 paddocks x 3 LSCG plots 1x1m subplots), four new sections of 0.25 m² were delimited by their edges, and four species were chosen. For each species two distal parts of their clonal growth organs were measured, and tagged as the starting point (base line) for the next measurement to assess horizontal lateral spread. The first two observations were done with a one week interval and, afterwards, measurements were spaced to avoid excessive disturbance of the soil that during the first week of observations caused the mortality of at least one of the parts of the tagged clonal growth organs mainly in grasses and sedges. The measurements were done monthly until the end of the rainy seasons 2007, every two months in the dry season in 2008, and one last measurement was done at the start of the 2008 rainy season.

The assessment of RD was carried out using a trench profile method described by Schuster (1964), including some modifications. At the beginning of the rainy season 2008, in each of the eight paddocks, six RD random sub-plots (1 x 1 m) fulfilling the condition that at least four and less than ten of the target species occurred in the plots, a trench of 0.6m width x 0.6m length x 0.6 m depth was dug up. Using a sharp knife and a tape measure the species' root systems were carefully excavated and measured from three vertical walls of each trench. In general, measurements of both types of roots (fibrous/taproot) started following three separate branches of the roots (those branches chosen originated from a plant's shoot at the ground level). The average, minimum, and maximum depths of the root branches, penetration were estimated based on at least 10 replicates for each species and were completed combining the data from different trenches and different paddocks.

The SFP and the LFS were measured over continuous weekly surveys from July 2007 to June 2008 on each of the eight paddocks. The phenological stage of the species was assessed by observing the status of individuals present along a 50 m long transect located randomly in the paddock in north (first option) or south (alternative option) and using a random start point. A cross-shaped wooden tool (1 x 1 m) with a 3 mm hole in each of the four extremes was placed at intervals of 1.5 m on the transect length. All individuals intersecting the vertical projection of the holes were registered and their phenological status registered, totalling at least four records by point and 136 by transect (except in the presence of bare soil). That number of records was enough to have for each target species at least eight or more individuals per week when combining data from the eight paddocks. For *Lygodium venustum* and *Selaginella sertata*, we recorded

SFP and LFS when the spore receptacle in the margin of the back side of fronds turned turgid, friable and/or light brown and yellow respectively. The SFP was defined as the week when at least five individuals (of a total of eight random individual observed) were at this stage and was expressed in weeks of the year (WOY) from the 2nd July 2007. The end of the reproductive period was considered as the week when more than two individuals out of a total of eight individuals had dehisced fruits or seeds that had shed.

4.2.3 Species cover, soil and climate data

Species cover is one less time consuming measure than species biomass measurements and according to Fehmi (2010) and also the previous experience on methods for quantifying vegetation in semi-natural grasslands, species cover measurements may allow a reasonable comparability of species dominance between sites and within the time window when the phenological stages are similar. Species cover was studied in five different paddocks. A single plot area (cover plot) between 3200 and 6000 m² was fenced in each paddock, on soils of similar properties (organic matter ranges of 4.9-7.8%), pH_{water} (6.1- 6.6), relatively high levels of calcium (12-30 mg/kg) and with no deficit of magnesium (4.0-10.0 mg/kg) or potassium (0.30-1.10 mg/kg). However, in the course of the study, some differences in their physical properties, in the levels of phosphorus and of other nutrients were identified. All these five paddocks were previously managed under grazing; hand weeded at least one a year and had no fertilizer applied for at least 10 years prior to our study. More details about the soil chemical and physical properties of the five cover plots can be found in Appendix 2 of this Chapter (see also Study site in Chapter 1). Daily rainfall data from three rain gauges located in

three villages within the study area were collected. Also, we obtained data from a weather station of the Nicaraguan Institute of Land Studies (INETER) located at less than 5.7 km from the survey plots. For three cover plots, we used the rainfall data from the rain gauges and for two cover plots data from a weather station (Table 4.1); for the first 62 days (July 2nd – August 31st 2007) daily rainfall data for El Coyol, farm rain gauge was not available. We considered that the rainy season began when the daily average rainfall reached and remained above 11.5 mm, for seven consecutive days in at least two of the rain gauges, and ended when the daily average rainfall decreased and remained below 2.0 mm for the same period and rain gauge number. Accordingly, the 2007 rainy season started on June 21st and ended on November 30th, and the 2008 dry season started on December 1st, 2007 and ended on May 26th 2008.

For our study, daily rainfall records were accumulated between July 2nd 2007 and May 26th 2008, and both the rainy season 2007 and the dry season 2008 were also divided in two periods of similar number of days to characterize, in a better way, the rainfall variations within each season and the effect of a short duration of rainfall seasons on the variation of the aggregated traits. From July 2nd 2007, the four seasonal periods and their last date were: early rainy season 2007 (September 14th, 2007), late rainy season 2007 (November 30th, 2007), early dry season 2008 (February 27th, 2008), and late dry season 2008 (May 26th, 2008).

To assess plant cover change, four sampling areas were delimited in each of the five cover plot areas. Plant cover was estimated in four 1 x 1 m² sub-plots located in each of these areas during the period July 2nd 2007 - May 26th 2008. The interval between

consecutive observations was approximately one month and the surveys were started after 21 days of grazing exclusion following a grazing period of 1-2 days. In total, there were five cover plots, four sub-plots per cover plot two corresponding to the start and the end of each of eleven grazing exclusion periods, giving a total of 440 samples. We estimated species cover visually (Greig-Smith 1983) using a 1 x 1 m metallic frame subdivided into 5 x 5 cm segments (in total 400). The cover of each species was estimated as proportion of the projected area of the plant on the area of the segment, added over the 1 x 1 m² sub-plots, and independently from the cover of other species. With this methodology, total cover can exceed 100% per sub-plot due to the species aerial overlap (Appendix 3 in Chapter 4). We calculated the CWM for each trait and each survey cover plot combining the species cover data of the eight sub-plots for each of the eleven seasonal periods, weighting the trait values by species cover data using f-Diversity software (Di Rienzo *et al.* 2008).

4.2.4 Data analysis

Since the five cover plots were relatively close to each other (the longest distance between the cover plots was about 8.0 km), it was presumed that they experienced similar rainfall conditions. This was tested by using a correlation analysis to ascertain whether cover plots had similar patterns of accumulated rainfall. In order to test whether trait CWMs varied according to seasonal changes in resource availability in particular: i) between four periods according to rainfall: early rainy season 2007, late rainy season 2007, early dry season 2008, and late dry season 2008, from July 2nd, 2007-May 26th, 2008; ii) differences among cover plots and iii) their interactions, a two-way factor

analysis of variance of the CWMs were performed. We used a linear mixed models framework in order to account for the variance heterogeneity present in some traits. The analyses were done using an interface to R (R Developing Core Team 2009) implemented in InfoStat software (Di Rienzo *et al.* 2008). Mixed model estimations were based on the restricted maximum likelihood (REML). The residual error distribution was approximately normal and showed homoscedasticity of variances for CWMs of SLA, LDMC, P, K, LSCG, RD and SFP. For the other CWMs (LS, N, Ca, Mg, LLS, PH and LFS) the heteroscedasticity was modelled allowing different variances for the plot factor, and Fisher's LSD was performed using adjusted standard errors.

Table 4.1 Names and characteristics of villages and rain gauges where study plots for species cover determinations were located in Muy Muy, Matagalpa, Nicaragua

Village	Rain gauge	Elevation (m)	Site (plot number and label)	Distance to nearest rain gauge station (km)
El Coyolar	El Coyol, Farm	300	14-El Genízaro	2.3
El Corozo	San Felipe, Farm	378	13-El Mango	2.1
Maizama Adentro	La Lucha, Farm	280	15-El Mono	1.2
Muy Muy, town	Weather Station Muy Muy 055027 (INETER)	320	8-El Llano	4.5
			7-El Plan	5.7

4.3 RESULTS

The amount of accumulated rainfall at the three rain gauges and the weather station was correlated in all cover plots for the entire period of the study (between July 2nd, 2007 and May 26th, 2008) ($r^2 = 0.9880$; $p < 0.0001$), and also in: early rainy season 2007 ($r^2 = 0.9950$; $p < 0.0001$), late rainy season 2007 ($r^2 = 0.9643$; $p < 0.0001$), early dry season 2008 ($r^2 = 0.9602$; $p < 0.0001$) and late dry season 2008 ($r^2 = 0.9082$; $p < 0.0001$)

(Appendix 4 in Chapter 4). Two axes of soil variation were considered in the principal component analysis. The main axis (48% of the variation) was based on the relative proportions of sand and clay particles, and magnesium and calcium concentrations; and a second axis (25% of the variation) was related to levels of available P and pH (Tables 1 and 2, and Figure 1, in Appendix 2 of this chapter). Soils in plots 15, 13 and 8 showed highly significant increasingly higher proportions of sand, and lower concentrations of Mg and Ca, while, plots 14 and 7 had lower sand and higher Mg and Ca concentrations. Plots 7, 8 and 13 had a highly significant higher available P and lower soil pH, while plots 14 and 15 showed the opposite trend (Tables 1 and 2; and Figure 1, in Appendix 2 of this chapter).

4.3.1 Seasonal variation of the aggregated functional traits

Mean total plant cover varied little within the period studied. However, highly significant temporal differences of CWMs were observed for eight of the 14 traits. CWM of foliar traits such as SLA, LDMC and LLS, foliar nutrients (N, Ca, K and Mg) and LFS changed with high significant magnitudes among seasons, while, CWM of LS, foliar P, and LSCG changed also among season but the magnitude of those changes was less. There was also a significant interaction term of time and plot ($p < 0.05$) for six aggregated traits, SLA, LDMC, K, Mg, LSCG and LFS. Only in the case of LSCG this interaction term was highly significant (Table 4.2, Figure 4.1).

The dominance of plants with high foliar concentrations of P, N, Ca, K and Mg, high SLA, greater LSCG and LFS and lower LDMC and LLS increased in the rainy season, and declined in the dry season. There was a general trend of the weighted average

concentrations of the foliar nutrients to increase slightly through the rainy season and to decline steadily from the start of the dry season. Specifically, and as expected, there was a trend of higher concentrations of foliar P, N, Ca, K and Mg, higher SLA and longer LSCG late in the rainy season in comparison with the late dry season, and between the early and the late dry season the concentrations of these foliar nutrients, and of SLA and LSCG, decreased (Figure 4.1 d, e, f, g, h, b and j). The way in which CWM of SLA and LSCG varied over seasonal periods was similar to that of the CWM of LLS (Figure 4.1 b, j and i) and different from the CWM of foliar nutrients (Figure 4.1 d, e, f, g and h), having similar values of SLA and LSCG (Figure 4.1 b and j) over the rainy season and in the early dry season. Contrary to CWM of SLA, LSCG and LLS, the CWM of LDMC (Figure 4.1 c) responded early to the change between the rainy and the dry seasons, having higher values early in the dry season than in the late rainy season. The way in which CWM of LFS (Figure 4.1 k) varied over time was strongly linked to seasonality with dominance by plants with a longer reproductive period late in the rainy season than in the dry season and, contrary to our hypothesis, with an opposite trend to that of LLS (Figure 4.1 i) which showed shorter leaf longevity in the rainy season. The trend in CWM of LFS was, on the other hand, positively associated with CWM of leaf nutrient concentration (Figure 4.1 d, e, f, g and h). Finally, although the ANOVA showed a significant effect of seasonality on LS (ANOVA $F_{5,57} p= 0.0031$), the Fisher's LSD test did not clarify which specific periods differed (Figure 4.1 a).

4.3.2 Site heterogeneity and its effects on the variation of the aggregated functional traits

For all foliar traits, the two phenological traits, LSCG and RD, CWMs showed significant differences among plots (Table 4.2, Figure 4.2). In the case of aggregated LLS and LFS the seasonal variation was stronger than plot differences, while for the others (except for PH with no differences); plot differences were the greater source of variation (Table 4.2). Between-plot differences was the only significant source of variation for aggregated RD and SFP (Figure 4.2 k and l), and an important source for aggregated SLA and leaf K concentration (Figure 4.2 b and g). However, the way in which aggregated SLA, leaf K and RD varied did not correspond well with the main axes of soil differentiation among the plots, and it could be more related with other sources of between plots variations not considered in this study (Figure 4.2 b, g and k). In the cases of CWMs of SLA, LLS, LSCG and LFS (Figure 4.2 b, i, j and m), and leaf concentrations of K, N, Ca and Mg (Figure 4.2 g, e, f and h) an important part of the differences among the plots could be due to other edaphic factors not studied here, or specific patterns of floristic composition might also be relevant sources of variation. Specifically, for aggregated LS, foliar N, Ca and Mg concentrations, LLS, LSCG and LFS, plot differences appeared to be related to particular properties of plot 15 (Figure 4.2 a, e, f, h, i, j and m). CWMs of LDMC and, SFP (Figure 4.2 c and l), and part of the differences among the plots in foliar concentrations of N, P and Ca (Figure 4.2 e, d and f) were to some extent associated with textural composition of the soil, e.g. in plots with higher proportions of sand (plots 15 and 13), CWMs of leaf N and P were higher, and

the dominant species had early onset of flowering and lower LDMC (Figure 4.2 e, d, l and c).

The CWM of Ca across plots was low and affected by textural differences, with higher concentrations towards soils with lower clay content, and only the most clayish plot (plot 14, which also had the highest contents of soil Ca and Mg) showed the lowest CWM of leaf Ca concentration (Figure 4.2 f). Contrary to CWMs of LDMC, N, P and Ca, aggregated leaf Mg concentration was related to the second axis of soil differentiation, (Figure 4.2 h), e.g. plots with higher contents of soil available P, and more acidic properties showed a higher CWM of leaf Mg concentration (plots 7, 8 and 13). Contrary to our hypothesis aggregated RD, (Figure 4.2 k) was not affected by rainfall seasonality and the plots' differences in soil texture, acidity and P content explained only the variation in the range of plots with the lowest clay contents (plots 15, 13 and 8). It appears that in clayish soils there are other sources of edaphic variation that masked the effect of the textural gradient.

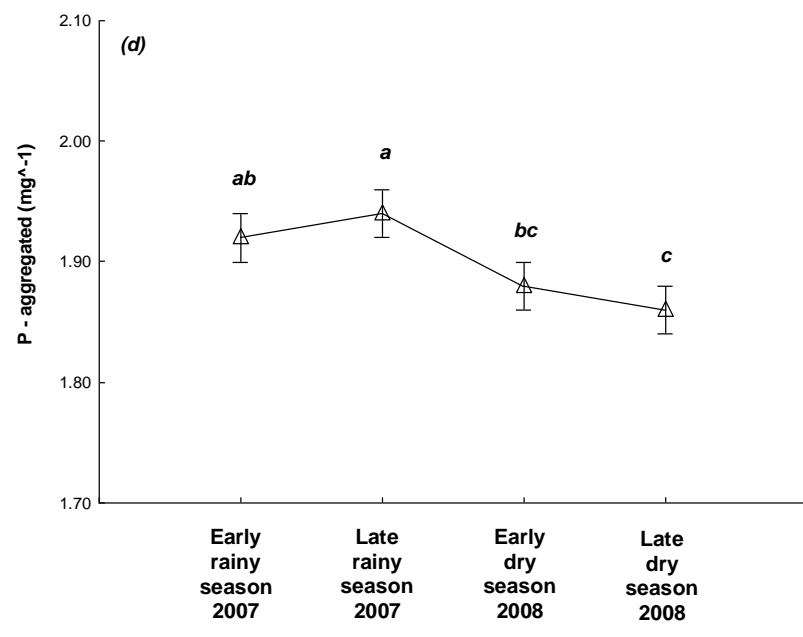
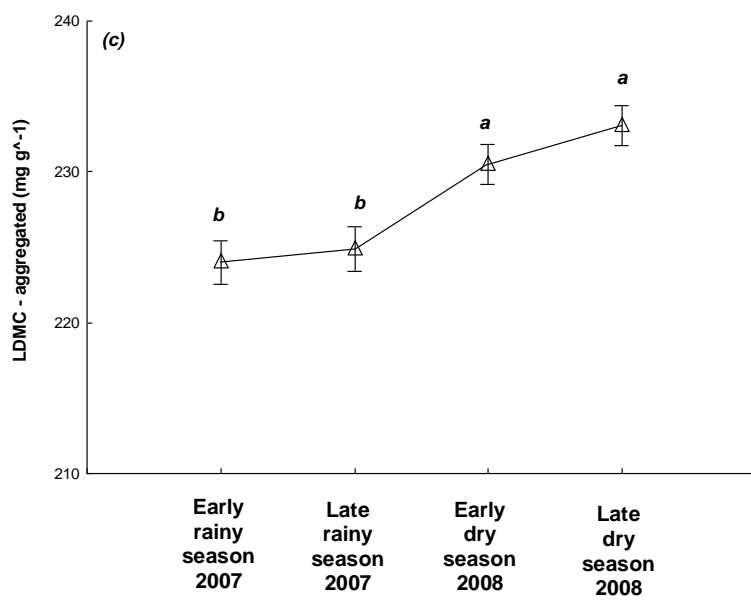
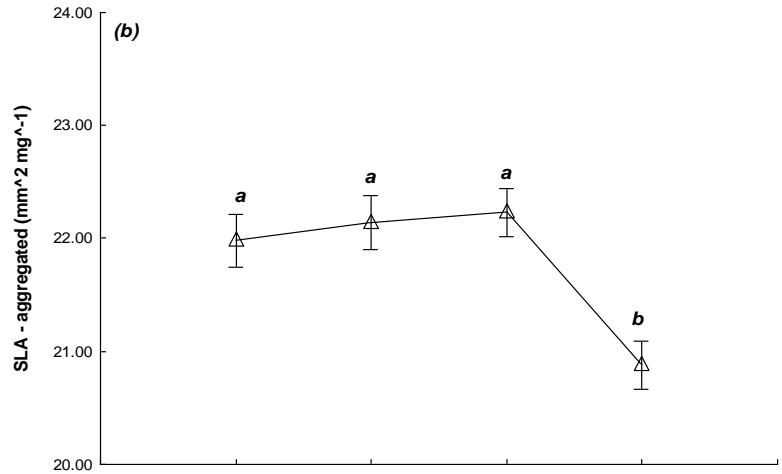
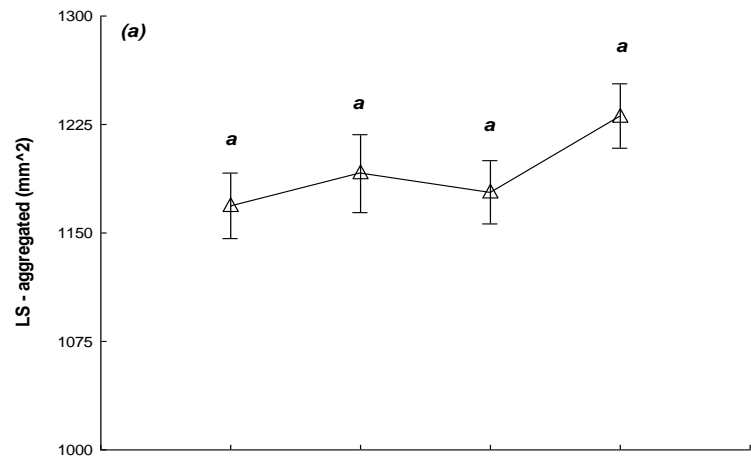
4.3.3 Seasonal and plot interactions of the aggregated functional traits

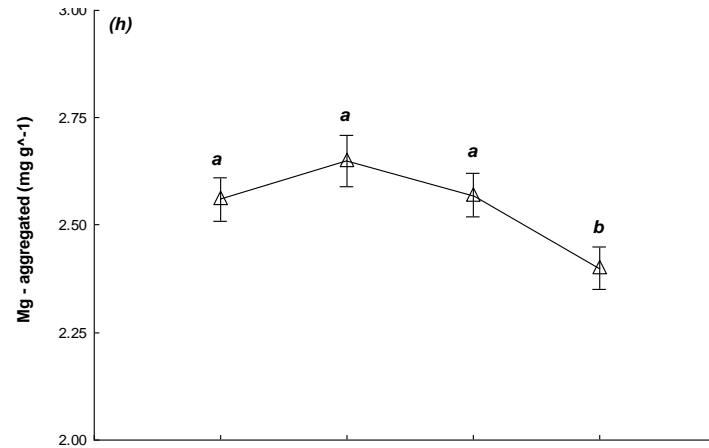
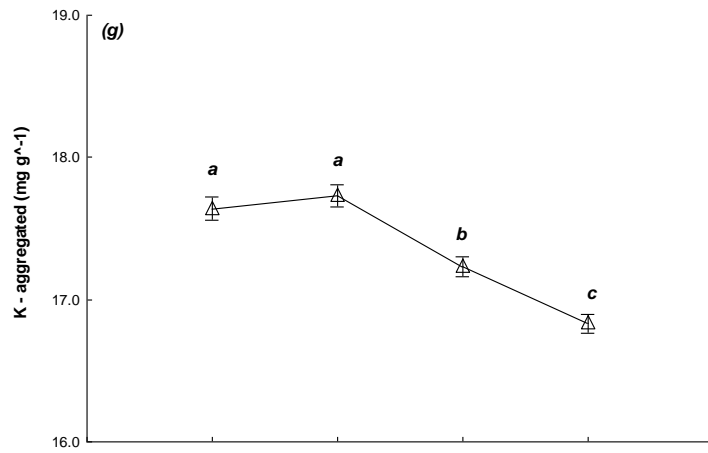
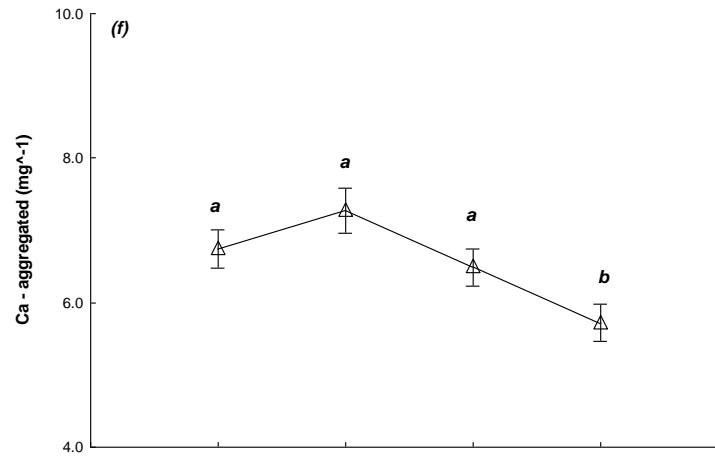
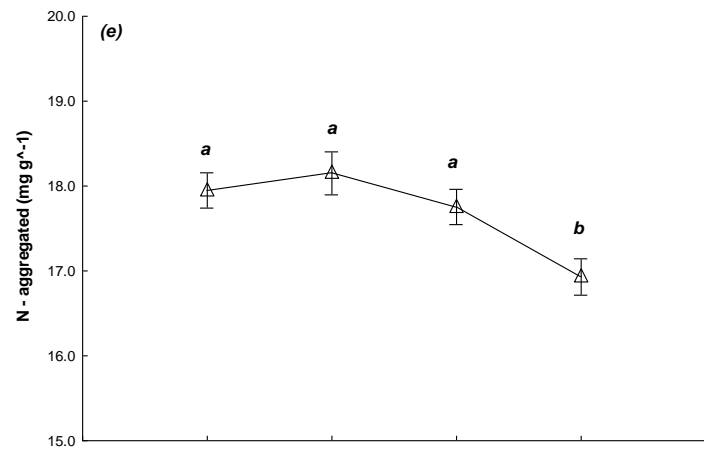
CWMs of SLA, LDMC, K, Mg, LSCG and LFS varied over seasonal periods, but are also affected by plot, and seasonal period and plot interacted significantly ($p < 0.05$, interaction terms). As expected, there was a trend of higher SLA in the late rainy season or at the onset of the dry season than in the late dry season, but there were important differences in this response among plots (ANOVA $_{\text{plot} \times \text{time}} F_{2,45} p = 0.0196$). The plot with the highest SLA early in the rainy season showed the stronger seasonal variability. CWM of LDMC fluctuated with time; were lower in the rainy season than in the dry

season and also there were important differences in this trend among plots (ANOVA_{plot x time} $F_{2.72}$ $p= 0.0104$). Plots with lowest LDMC had lower seasonality than those with highest values. Foliar K concentration differed significantly among the seasons. We found higher concentrations in the rainy season than in the dry season, with four plots that showed the highest values late in the rainy season and only one plot with little seasonal variation (ANOVA_{plot x time} $F_{2.87}$ $p= 0.0074$). Foliar Mg concentration also fluctuated with seasons; there were lower concentrations in the early dry season than in the rainy season, and all plots had higher or rather similar concentrations in the late dry season than early in the season (ANOVA_{plot x time} $F_{2.73}$ $p= 0.0103$). Most part of the plot variation was based on a plot with the highest values. The temporal trend of aggregated LSCG was not clear; the strong variation was attributed to plot differences (ANOVA_{plot x time} $F_{5.63}$ $p< 0.0001$). The way in which CWM of LFS varied over time was strongly linked to rainfall seasonality with longer LFS in the late rainy season than in the dry season (ANOVA_{plot x time} $F_{2.19}$ $p= 0.0353$).

Table 4.2 F-Statistics of variation in community weighted means of foliar, whole-plant and phenological traits with plot and four seasonal time periods in semi-natural grasslands of Central Nicaragua. One, two and three asterisks indicate significance levels at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. Leaf size (LS), specific leaf area (SLA), leaf dry matter content (LDMC), leaf phosphorus concentration (P), leaf nitrogen concentration (N), leaf calcium concentration (Ca), leaf potassium concentration (K), leaf magnesium concentration (Mg), leaf lifespan (LLS), plant height (PH), lateral spread by clonally growth (LSCG), root depth (RD), starting flowering period (SFP) and length of the period from flowering to seed shed (LFS)

Trait type	CWM traits	Source of variation		
		Seasonal period (<i>t</i>)	Plot (<i>p</i>)	<i>t x p</i>
Foliar traits	LS	5.57**	27.01***	1.72
	SLA	8.45***	29.50***	2.45*
	LDMC	10.43***	22.53***	2.72*
	P	5.11**	43.65***	1.86
	N	16.76***	46.61***	0.44
	Ca	9.32***	52.29***	0.98
	K	33.74***	73.80***	2.87**
	Mg	13.27***	26.71***	2.73*
	LLS	25.52***	10.85***	1.86
Whole-plant traits	PH	2.35	1.51	1.16
	LSCG	3.81*	138.86***	5.63***
	RD	0.44	71.53***	1.61
Phenological traits	SFP	1.91	90.48***	0.58
	LFS	32.24***	27.12***	2.19*





Early rainy season 2007 Late rainy season 2007 Early dry season 2008 Late dry season 2008

Early rainy season 2007 Late rainy season 2007 Early dry season 2008 Late dry season 2008

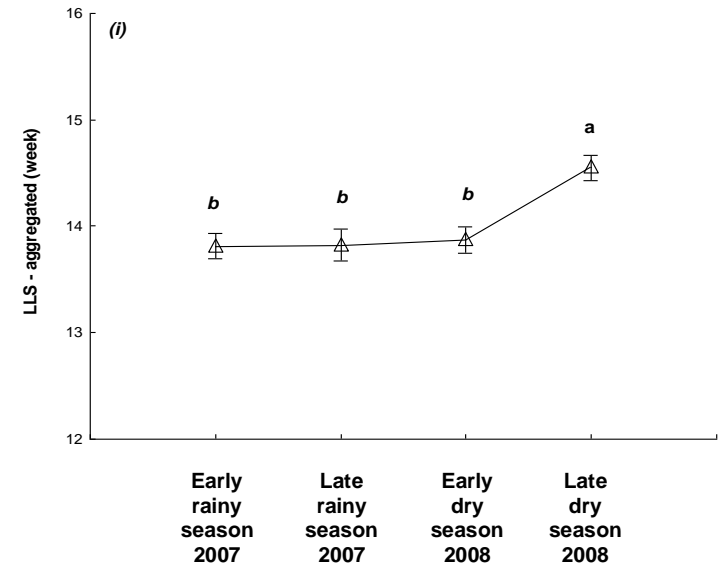
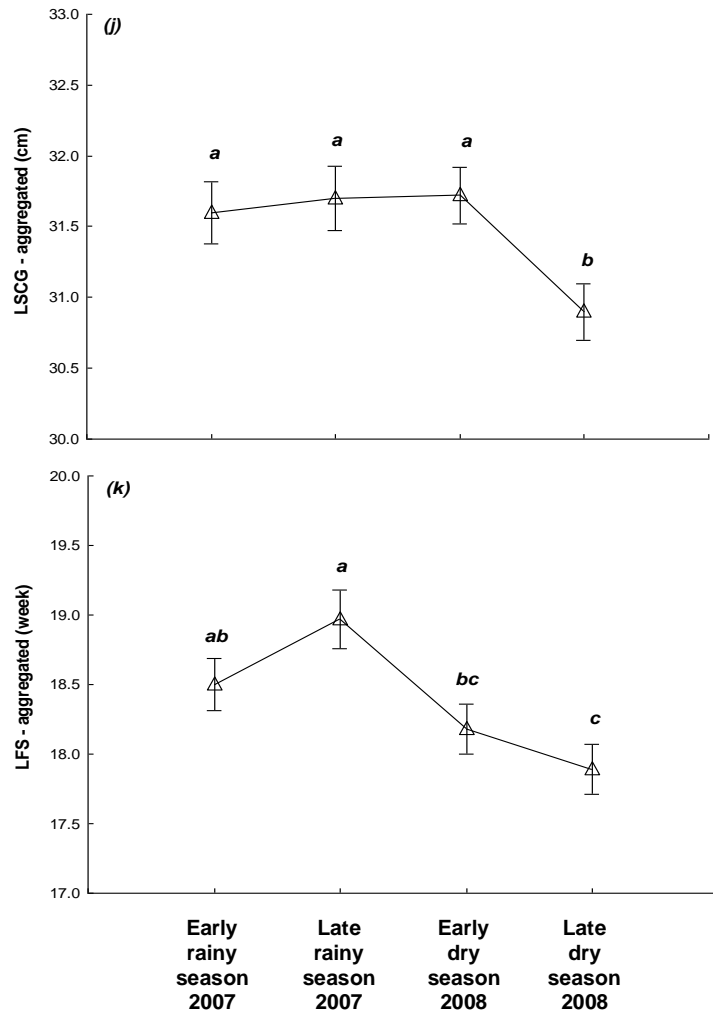
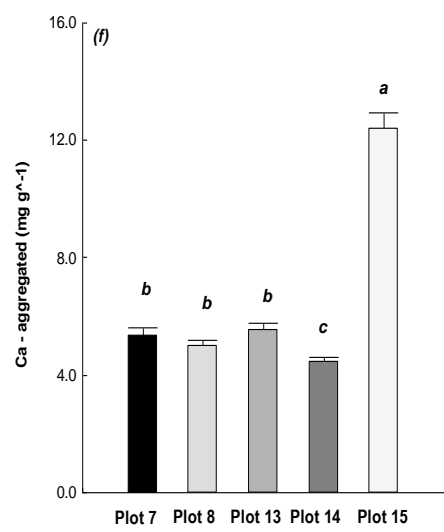
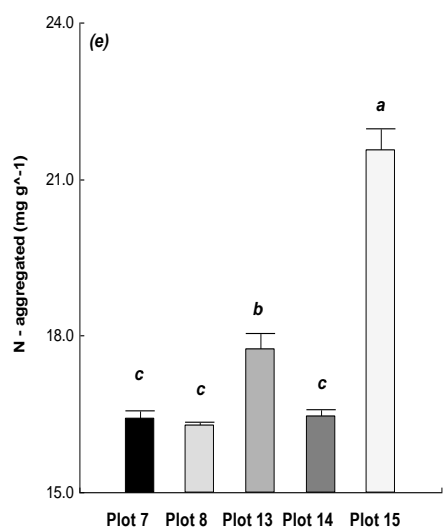
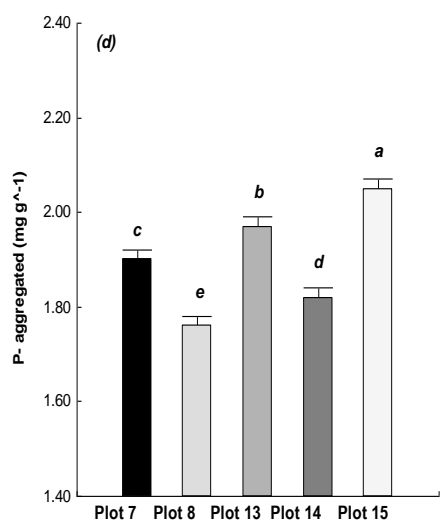
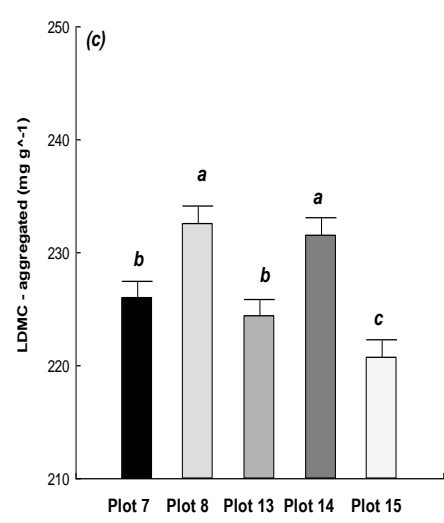
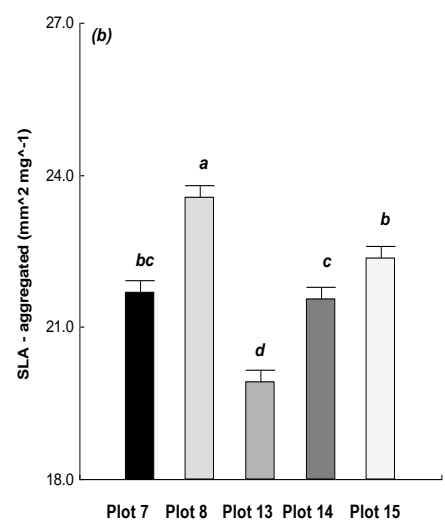
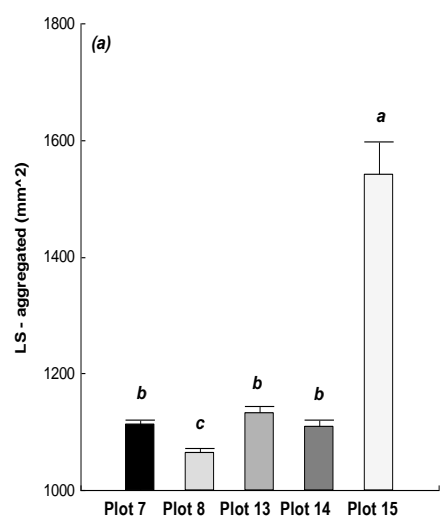
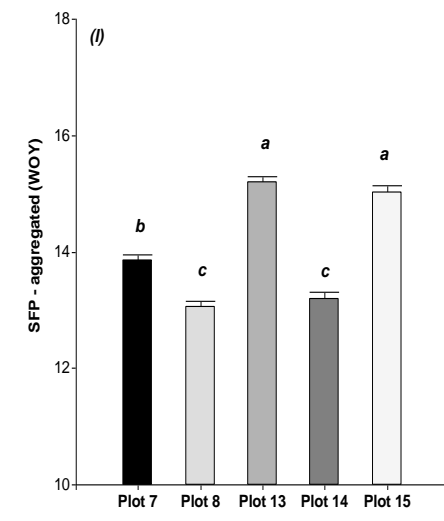
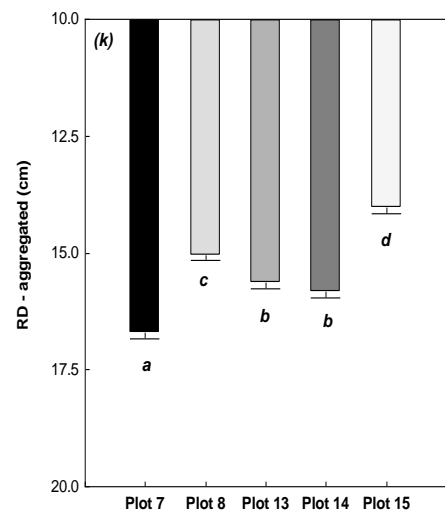
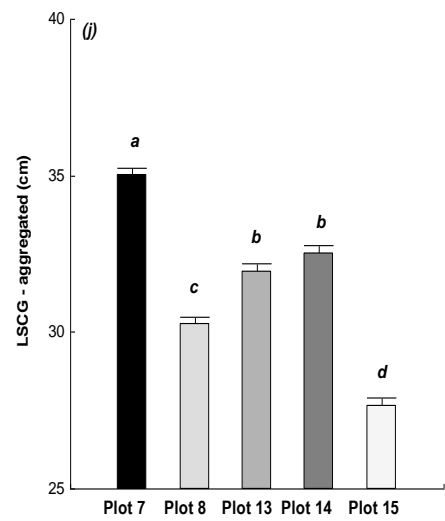
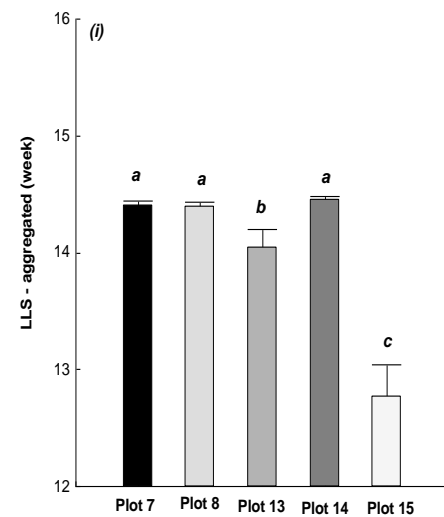
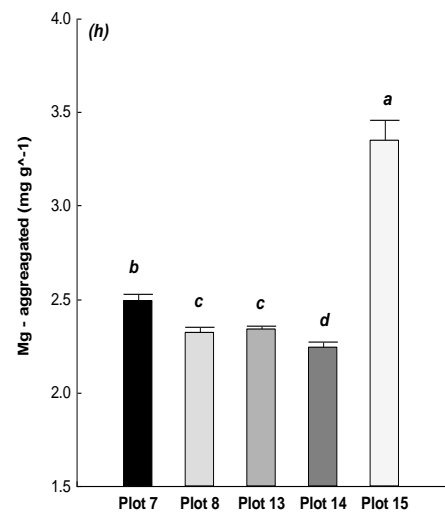
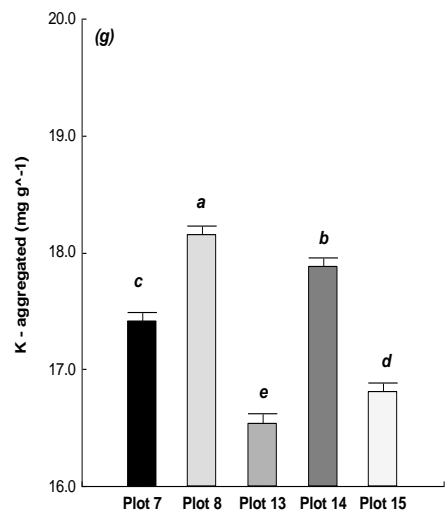


Figure 4.1 Mean and standard error of functional traits weighted by species cover during a continuous period (July 2nd, 2007 to May 26th, 2008) in five plots of semi-natural grasslands in Central Nicaragua. The letters on each point indicate statistical differences among the seasonal periods at $p < 0.05$ (Fisher's LSD). (a) LS, leaf size, (b) SLA, specific leaf area, (c) LDMC, leaf dry matter content, (d) P, leaf phosphorus concentration, (e) N, leaf nitrogen concentration, (f) Ca, leaf calcium concentration, (g) K, leaf potassium concentration, (h) Mg, leaf magnesium concentration, (i) LLS, leaf lifespan, (j) LSCG, lateral spread by clonal growth and (k) LFS, length of the period from flowering to seed shed.





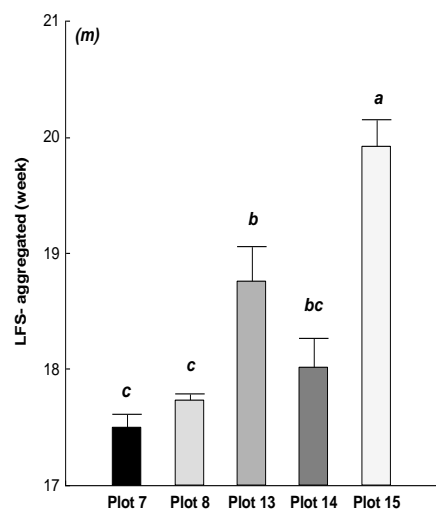


Figure 4.2 Mean and standard error of functional traits weighted by species cover in five plots of semi-natural grasslands in Central Nicaragua. The letters on each bar indicate statistical differences between cover plots at $p < 0.05$ (Fisher's LSD). (a) LS, leaf size, (b) SLA, specific leaf area, (c) LDMC, leaf dry matter content, (d) P, leaf phosphorus concentration, (e) N, leaf nitrogen concentration, (f) Ca, leaf calcium concentration, (g) K, leaf potassium concentration, (h) Mg, leaf magnesium concentration, (i) LLS, leaf lifespan, (j) LSCG, lateral spread by clonal growth, (k) RD, root depth, (l) SFP, starting of flowering period and (m) LFS, length of the period from flowering to seed shed.

4.4 DISCUSSION

The aim of this study was to investigate whether there are seasonal changes in the average composition of traits in a grassland community that have a documented correspondence with the resource use strategies in the plant species and also of other traits with functional importance for the primary productivity but for which empirical evidence is more limited. The general expectation was that along with a seasonal sequence there would be a turnover of species in these communities, and that species would have different strategies of resource use in the different seasons.

We found that community aggregated SLA, LDMC, LLS and leaf P, N, Ca, K and Mg concentrations, LFS, and to a lesser extent LSCG, responded to changes in the temporal patterns of species dominance defined over the rainfall seasons, and these findings supported predictions about the general species-level trade-off between resource acquisition and conservation. These findings could support the notion of differentiation and specialization of the co-existing species when resources change in time.

Generally, the results highlight the relevance of rainfall seasonality to community functional properties. However, the interaction between the temporal gradient and among-site heterogeneity was also an important source of variation for SLA, LDMC and foliar concentrations of K and Mg, LSCG and LFS; however these interactions explained less variation than the main factors.

All aggregated traits analyzed, except CWM of plant height, were affected by site heterogeneity. Differences in soil appear to be an important factor determining the temporal patterns of trait CWM; this was linked to differences in the composition of

major life form groups among sites. For example, in relation to the others, CWMs in plot 15 showed consistently bigger leaves, higher concentrations of foliar nutrients such as N, Ca and Mg, longer duration of phenological stages, reduced capacity for lateral spread by clonal growth and lesser leaf longevity. It is unlikely that those clearly different values of plot 15 resulted only from differences in the soil variables considered in this study and the different species composition of this plot in relation with the others must be other important explanation. Also, it is expected that CWM of LLS and leaf K concentration would be affected by the relative cover of perennial and annual species (Chapter 3), a difference which appears to be more related to the history of land use than to soil conditions (Ospina *et al.* 2009).

Another uncontrolled source of variation is that accounted by within-species differences in trait expression. Due to in the case of LS, SLA, LDMC, and foliar concentrations of N, P, Ca, K and Mg were measured during the 2007 rainy season. Similarly, RD was measured at the beginning of the 2008 rainy season. Garnier *et al.* (2007) indicated that when CWMs are calculated for traits that could differ in values according to treatment level, i.e. in this case traits differing in value according to the season, then differences in CWM can be caused either by within species trait variability, or by a change in species composition (Garnier *et al.* 2004; Garnier *et al.* 2007; Reiss *et al.* 2010).

Recent studies on grassland assemblages show that spatial gradients of soil resources interact with the temporal resource availability and with species composition in shaping the productivity of grasslands and species assemblages (Maestre *et al.* 2006; Maestre and Reynolds 2007). In a similar way, in the context of climate change scenarios Lavorel *et al.* (2007) have called for more studies that explore continuous and diverse

sets of plant traits in order to better capture responses to multiple combinations of natural gradients, such as climate variation, spatial gradients of resources and disturbance. Our results complement and support these studies because we found that site heterogeneity (spatial variation) interacted with resource supply changes defined over the rainfall seasons, and modified functional trait responses, probably through the differentiation of species by their resource use under the different portions of the rainfall gradient. The findings in this study support those of Ospina *et al.* (*submitted*, Chapter 2) where seasonal biomass stability in species diverse semi-natural grasslands was higher than in monoculture pastures that stopped growth earlier in the dry season. Those results were attributed to the presence of species with a wider set of strategies being responsible for continued growth beyond the peak rainy season due to the capacity to make use of more unreliable and scarce resources. The effect of fluctuations in the resource gradients through rainfall seasonality on the morpho-phenological traits of the 32 species in our study closely matched predictions based on published literature and in a previous study. We found that in the rainy season, the dominant grassland species had higher aggregated values of traits with well documented correspondence with high levels of resource supply, such as SLA, and leaf concentrations of N and P, while in the dry season, species with resource conservative traits, such as high LLS and LDMC, gained dominance (Ryser and Urbas 2000; Cornelissen *et al.* 2003; Garnier *et al.* 2004).

Likewise, the responses of foliar and whole-plant traits with less well-documented evidence about their correspondence with the resource economy axis confirmed our expectations. The dominant species in the rainy season had higher values of foliar Ca, K and Mg concentrations (Grime *et al.* 1997) and higher capacity for lateral spread than in

the dry season (Rusch *et al.* 2010). On the other hand, and contrary to our expectations, the length of the reproductive period was longer in species dominant in the rainy season than those dominant in the dry season. These results together indicate that high foliar concentrations of nutrients, higher capacity for clonal growth and longer duration of the reproductive phase correspond to the predominant strategies in these grasslands when water is in high supply during the rainy and the early dry seasons. In contrast, under reduced resource supply, particularly in the late dry season, dominant species tend to have low foliar Ca, K and Mg concentrations, lower capacity for clonal growth, and short duration of reproduction.

The trend found of higher capacity for clonal growth to be positively associated with resource acquisitive strategies matched the findings of previous studies at species and community levels indicating a high capacity for LSCG to correspond closely to strategies of rapid resource capture and circulation (Sammul *et al.* 2003; Rusch *et al.* 2010).

The fact that community averages of LFS were higher in the rainy season than in the dry season contradicts the finding of LFS being associated with a strategy of resource conservation, where LLS and LFS were positive correlated at the species level (Chapter 3). The lack of correspondence between CWMs of LFS and LLS could be due to the fact that, in these communities, the period in which there is active growth in the dry season is comparatively shorter than in the rainy season, so species that attained dominance in the dry season had a narrow time window for reproduction.

In a corresponding way, species with different life forms and life cycles flower at different times, and all annuals and some sedges and forbs had the onset of flowering

early in the rainy season. For some annuals, an investment in a longer period from flowering to seed shed could be an alternative strategy to that of vegetative growth. Towards the mid and late rainy season a large number of perennial species were flowering and it is likely that, for them, a longer LFS could also be related to other factors not considered here such as competition for pollinators during the peak growing season, or particular climatic factors (e.g. irradiance and air humidity) associated with population reproductive phenology (Michalski and Durka 2007; Sola and Ehrlén 2007).

The results for CWMs of RD do not fit the expectations: no significant differences in RD among the seasons could be detected. One reason might be the nature of the trait variation and the methodological approach. RD responds plastically to soil properties (Hodge 2004; Roumet *et al.* 2006) and Nippert and Knapp (2007) identified different water use strategies between C3 and C4 species when upper soil horizons become dry. In this study, RD measurements were made in the rainy season, when the soils in the area are friable and it is easy to carry out manual excavations of roots. Therefore, if the species' RD changed with growing conditions over the seasons, our method would not capture this variation, which would explain that CWM of RD appears non-responsive to the shifts in the dominance along the seasons.

4.5 CONCLUSIONS

In neotropical grassland assemblages in seasonally dry climates, we observe shifts in plant traits dominance, associated with changes in species composition probably due to temporal changes in resource supply given rainfall seasonality. In these communities, species seem to separate according to their strategies for resource use over the gradient of relative high resource supply in the rainy season, having high concentrations of P, N,

Ca, K and Mg, high capacity for clonal growth, long reproductive phenological development, short leaf longevity and low leaf dry mass. These acquisitive vegetative-phenological characteristics are characteristic of the dominant species till early in the dry season. However, leaf P and K concentrations, and LFS, are particularly sensitive to shifts in growing conditions, responding early to the change between the rainy and the dry season, and decreasing their concentrations from the late rainy season to the early in the dry season. In the case of dry mass leaf structure, of the dominant species, it increases from the late rainy season to the early dry season. In the late dry season, the grasslands show strategies for resource conservation of lower foliar concentrations of P, N, Ca, Mg and K, lower capacity for clonal growth and longer leaf longevity.

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APPENDIX 1 - CHAPTER 4

Species used for trait analysis by family and *a priori* taxonomic functional group (grass, sedge, forb or legume)

Family	Species	Code	Life cycle	Life form
Acanthaceae	<i>Blechnum pyramidatum</i> (Lamb.) Urb	Bl p	Perennial	Forb
	<i>Dyschoriste quadrangularis</i> (Oerst) Kuntze	Dy q	Perennial	Forb
Asteraceae	<i>Baltimora recta</i> L	Ba r	Annual	Forb
	<i>Ageratum conyzoides</i> L	Ag c	Annual	Forb
Combretaceae	<i>Combretum fruticosum</i> (Loefl. Stuntz)	Co f	Perennial	Shrub
Convulvulaceae	<i>Ipomoea hederifolia</i> L	Ip h	Perennial	Forb
Cyperaceae	<i>Cyperus rotundus</i> L	Cyz	Perennial	Sedge
	<i>Dichromena ciliata</i> Vahl.	Dy c	Perennial	Sedge
	<i>Cyperus articulatus</i> L	Cy a	Perennial	Sedge
	<i>Scleria melaleuca</i> Rchb. Ex Schitdl&Cham	Sc m	Perennial	Sedge
Euphorbiaceae	<i>Caperonia palustris</i> (L.) St. Hill	Ca p	Annual	Forb
Fabaceae	<i>Calopogonium muconoides</i> Desv	Ca m	Perennial	Legume
	<i>Desmodium distortum</i> (Aubl.) J.F Macbr	De d	Perennial	Legume
	<i>Centrocema pubescens</i> Benth	Ce p	Perennial	Legume
	<i>Desmodium procumbens</i> (Mill.) Hitchc	De p	Perennial	Legume
	<i>Rhynchosia minima</i> (L) DC	Rh m	Perennial	Legume
	<i>Indigofera</i> sp.	Ind	Annual	Legume
Hydrophyllaceae	<i>Hydrolea</i> sp.	Hyd	Perennial	Forb
Malvaceae	<i>Sida acuta</i> Burm. F	Si a	Perennial	Forb
	<i>Sida jussieana</i> DC	Si j	Perennial	Forb
Marantaceae	<i>Maranta arundinacea</i> L	Ma a	Annual	Forb
Mimosaceae	<i>Mimosa albida</i> L	Mi a	Perennial	Shrub
	<i>Mimosa pigra</i> L	Mi pi	Perennial	Shrub
	<i>Mimosa pudica</i> L	Mi p	Perennial	Legume
Poaceae	<i>Dichanthium aristatum</i> (Poir.) C.E.Hubb.	Di a	Perennial	Grass
	<i>Paspalum centrale</i> Chase	Pa ce	Perennial	Grass
	<i>Paspalum conjugatum</i> Bergius	Pa c	Perennial	Grass
	<i>Hyparrhenia rufa</i> (Nees) Stapf in Prain	Hy r	Perennial	Grass
	<i>Paspalum notatum</i> Fluggé	Pa n	Perennial	Grass
	<i>Oplismenus burmanii</i> (Retz.) P. Beauv	Op b	Perennial	Grass
	<i>Panicum laxum</i> Sw	Paz	Annual	Grass
	<i>Setaria parviflora</i> (Poir.) Kerguelen	Se p	Annual	Grass
<i>Paspalum virgatum</i> L	Pa v	Perennial	Grass	
Sapindaceae	<i>Serjania atrolineata</i> C. Wright	Se a	Perennial	Liana
Schizaeaceae	<i>Ligodium venustum</i> Sw	Li v	Perennial	Pteridophyte
Selaginellaceae	<i>Selaginella sertata</i> Spring	Se s	Perennial	Pteridophyte

APPENDIX 2 – CHAPTER 4

Soil properties of the five studied plots

Methodology

The soil type of each of the plots studied was described in a pit of 0.6 x 0.6 x 0.6 m, located in a representative area of each plot. Soil characteristics were described according to the FAO guidelines (FAO 1990). For chemical analysis, from each soil horizon composite samples from the soil pit walls were taken, air dried and analyzed at the CATIE laboratory in Costa Rica. The following variables were measured: pH in water, organic matter, through the combustion method in an elemental carbon auto-analyzer (ThermoFinnigan method); available phosphorus (P) and potassium (K) by a modified Olsen procedure at pH 8.5; and calcium (Ca) and magnesium (Mg) were determined with 1N potassium chloride. Soil texture was determined using the hydrometer with the Bouyucos method (Forsythe 1985).

Data analysis

To determine whether studied soil properties were different in the studied plots, a one-way factor analysis of variance was performed (Table 1). We used a linear mixed models framework in order to consider the heterogeneity of variances present in some soil variables for the plot factor. The analyses were done using an interface to R (R Developing Core Team 2009) implemented by InfoStat software (Di Rienzo *et al.* 2008). Mixed models used were based on the restricted maximum likelihood (REML) estimation method. The residual error distribution was approximately normal and showed homoscedasticity of variances for all soil variables. In a second approach, in

order to check the pre-supposed similarity of the plots based on physical properties of the soil profile and the soil nutrients in the A- horizon, a principal component analysis (PCA) was applied (Figure 1; Table 2).

Results

Significant soil differences were detected among the plots except for depth of the A-horizon (ADH) ($p= 0.3501$), and OM ($p= 0.0658$). The soils of the five plots were slightly acid, pH ranged from 6.1 to 6.6. Sand content in the sandiest plot (plot 15) was considerably higher (43.5%) than in more clayey paddocks (17.5 and 26.5%, plots 14 and 7 respectively). Effective soil depth (ESD) was similar in all soils. The gradient of variation of soil pH, and clay and sand content also explained the variation of Ca and Mg. The clayish soils had higher concentrations of these nutrients and the sandier paddocks (plots 15 and 13) displayed the lowest concentrations. In the case of soil P, the pattern of variation among the plots was not linked to other nutrients or physical properties. Soil K remained almost constant within each plot, though varied between them due to the high value in plot 7.

Multivariate patterns with soils properties across the plots

The first PCA axis on soil variables (48% of the variation) was an axis of textural variation based on clay/sand, Mg and Ca concentrations. Soil Mg and Ca are nutrients usually correlated with each other, and relatively high in clayish soils. The second PCA axis (29% of the variation) integrates fertility variables such as OM and available P with the depth of the A-horizon and soil pH. Soil K and ESD were not significantly associated with the variation of the first two PCA axes (Table 2). In plots with high

scores on axis 1 there was a predominance of soils with higher percentage of sand and lower concentrations of Mg and Ca (plots 15, 13 and 8), while low scores were associated with lower percentage of sand and higher concentrations of Mg and Ca (plot 14 and 7). In relation to the fertility gradient, plots with high scores on axis 2 were related to higher OM, available P, deeper A- horizon and more acidic soils (plots 7, 8 and 13), while low scores were related to lower OM, available P, shallow A- horizon and less acidic soils (plot 14 and 15) (Figure 1).

Taking into account all the results, the soils of the five studied plots showed two gradients of multivariate variation: texture and fertility, the latter was defined by OM, available P and pH, and also related to variation in the ADH (Table 2; Figure 1). However, according to the ANOVA, the variation of ADH and OM among the plots was not significant (Table 1); therefore the variation in the fertility gradient among the plots was mainly based on available P and pH.

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Table 1. Mean values and standard error of ten physical and chemical soil properties of five plots (of the species cover study) located in semi-natural grasslands in central Nicaragua. Except for effective soil depth, data shown represent the soil in the A-horizon and was tested with ANOVA and grouped with the Fisher's LSD test

Soil variables	Plot 7	Plot 8	Plot 13	Plot 14	Plot 15	P
Effective soil depth (ESD) [cm]	48.0±2.8a	35.0±2.8c	32.0±2.8c	42.0±2.8b	41.0±2.8b	0.0014
Average depth of the A-horizon (ADH) [cm]	14.0±1.2a	14.0±1.2a	16.0±1.2a	13.0±1.2a	12.0±1.2a	0.3502
Clay (Clay) [%]	42.0±3.3b	28.0±3.3c	29.0±3.3b	59.0±3.3a	25.0±3.3c	0.0036
Sand (Sand) [%]	26.0±1.2b	40.0±1.2b	39.0±1.2b	17.0±1.2c	43.0±1.2a	0.0001
Soil pH (pH)	6.3±0.06a	6.1±0.06c	6.2±0.06b	6.3±0.06a	6.6±0.06a	0.0199
Available calcium concentration (Ca) [mg/kg]	23.9±1.05	19.2±1.05	15.4±1.05	30.1±1.05	12.0±1.05	0.0004
Available magnesium concentration (Mg) [mg/kg]	7.3±0.29b	5.4±0.29c	4.0±0.29d	10.0±0.29	3.6±0.29d	0.0001
Available potassium concentration (K) [mg/kg]	1.10±0.05	0.25±0.09	0.30±0.09	0.40±0.09	0.55±0.09	0.0037
Available phosphorus concentration (P) [ppm]	9.1±0.8a	4.9±0.15b	3.7±0.15c	1.5±0.35e	2.6±0.15d	0.0002
Organic matter (OM) [%]	7.8±0.66a	7.5±0.66a	7.6±0.66a	5.4±0.66b	4.9±0.66c	0.0658

Different letters in rows indicate significance levels among plots at $p < 0.05$.

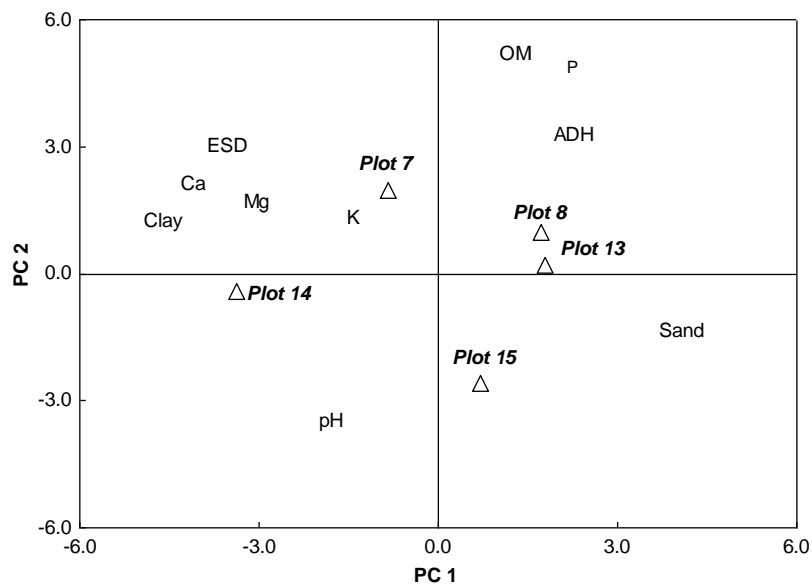


Figure 1. Arrangement of ten physical and chemical soil variables: effective soil depth (ESD), average depth of the A-horizon (ADH), percentage of clay (Clay), percentage of sand (Sand), soil pH (pH), available calcium concentration (Ca), available magnesium concentration (Mg), available potassium concentration (K), available phosphorus concentration (P) and organic matter (OM), along the first two axes of a five plots PCA ordination. Loadings for soil variables for PC1 and PC2 are in Table 2, below.

Table 2. Axis eigenvalues and loadings of ten soil variables on the first two PCA axes and percentage of variance explained by each axis and obtained from the matrix of the soil variables in five plots

Soil variables	Percentage of variation explained	
	PC 1 (48.0%)	PC 2 (29.0%)
Eigenvalue	4.70	2.95
Effective soil depth (ESD) [cm]	-0.30	0.15
Average depth A-horizon (ADH) [cm]	0.26	0.47
Clay (Clay) [%]	-0.44	0.13
Sand (Sand) [%]	0.44	-0.11
Soil pH (pH)	-0.16	-0.39
Available calcium concentration (Ca) [mg/kg]	-0.39	0.28
Available magnesium concentration (Mg) [mg/kg]	-0.42	0.21
Available potassium concentration (K) [mg/kg]	-0.16	0.10
Available phosphorus concentration (P) [ppm]	0.22	0.40
Organic matter (OM) [%]	0.16	0.54

Bold numbers represent meaningful loadings according to Pearson correlations coefficients $p < 0.05$.

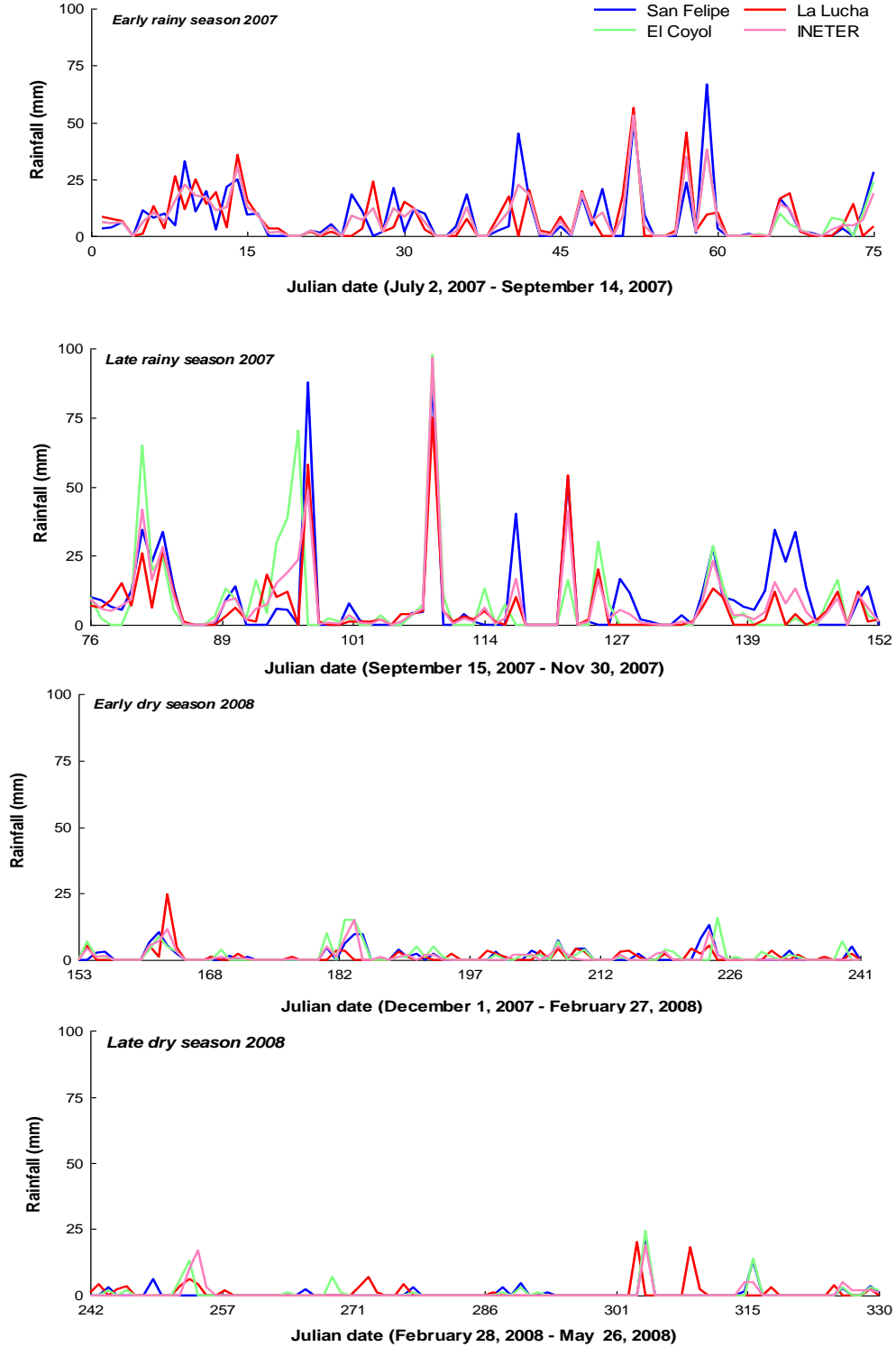
APPENDIX 3 – CHAPTER 4

Mean values of species cover of the target species group (thirty-two herbaceous and four common woody species), the open ground cover and the total cover of five plots in four seasonal periods that represent shifts in the resource availability gradients in semi-natural grasslands in Central Nicaragua

Seasonal periods	Plot number	Number of samples (sub-plots)	Species cover (target species group)	Number of species in the target species cover	Open ground cover (bare ground, stone)	Total plot cover
Early rainy season 2007 (July 2-September 14, 2007)	7	24	94.8	25	0.7	121
	8	16	86.8	16	2.3	110
	13	24	93.2	26	3.4	133
	14	16	86.1	23	1	116
	15	24	90.2	26	3.1	115
Late rainy season 2007 (September 15-November 30, 2007)	7	16	91	19	2.7	125
	8	24	92.2	18	3.2	109
	13	16	77.5	18	1.3	106
	14	24	89.7	14	3.2	119
	15	16	83.8	31	3.4	110
Early dry season 2008 (December 1, 2007-February 27, 2008)	7	24	93.2	18	0.9	128
	8	24	95.1	17	1.9	111
	13	24	84.9	19	2.2	104
	14	24	95.3	18	3.1	129
	15	24	83.2	28	0.7	110
Late dry season 2008 (February 28-May 26, 2008)	7	24	97.9	18	1.3	105
	8	24	99.1	17	2.8	115
	13	24	87.2	22	2.8	111
	14	24	98	17	4.1	111
	15	24	84.6	33	2.3	110

APPENDIX 4 – CHAPTER 4

Daily rainfall during four seasonal periods: early rainy season 2007, late rainy season 2007, early dry season 2008, and late dry season 2008. Data are from three rain gauges and a weather station of the Nicaraguan Institute of Land Studies (INETER).



CHAPTER 5

GENERAL DISCUSSION AND KEY FINDINGS

In Central America, as in other areas with sub-humid and seasonally dry climates, savannas and grasslands are often replaced by monocultures of sown pastures with the aim of increasing the amount and qualities of feed offered to cattle, and so, contributing to regional development (Dias-Filho 2007; Cruz *et al.* 2010). Sown pastures and other land uses that integrate these types of pastures can also contribute to mitigation of climate change because they improve forage digestibility reducing methane emissions (Ibrahim *et al.* 2007). In the area of the research reported in this thesis, this conversion involves replacement of the vegetation cover without changes in the resource supply (water and nutrients) for the establishment and management of sown pastures.

Hooper *et al.* (2005) suggested that changes in the composition and diversity of communities may have significant impacts on ecosystem function in terms of process rates, biomass and element pool sizes and variability. Additionally, Grime (1998) and Garnier *et al.* (2004) determined that the effects of community composition on ecosystem functions are linked to particular attributes of the dominant species and proposed that these attributes relate to rates of resource acquisition and conservation. In this chapter, I discuss these theories and at the same time, I summarize the findings presented in chapters 2, 3 and 4, regarding different aspects of the linkages among plant species (biodiversity), plant attributes, strategies of resource use and above-ground net primary productivity (ANPP) in semi-natural grasslands.

KEY FINDINGS

1. A quadratic model resulted in a better fit than a linear relationship between ANPP and the cumulative rainfall in a 22-days period (ARP22), because ANPP was low when ARP22 was higher than ca. 300 mm. Also, in both sown pastures and semi-natural grasslands, ANPP responded positively to more frequent and more evenly distributed rainfall events, although ANPP was more sensitive to the number of rainfall events (NRE) in sown pastures (NRE $n=41$; $p=0.0045$) than in grasslands (NRE $n=56$; $p=0.1914$).
2. The temporal stability of ANPP was higher in semi-natural grasslands than in sown pastures in the dry season ($p=0.0402$) and in the annual cycle ($p=0.0127$). Under the pasture management applied in the study sites, I found no differences in annual ANPP between the *Brachiaria brizantha* pastures and the grasslands (955 and 898 g m⁻² yr⁻¹ for the pastures and grasslands, respectively), and both sown pastures and grasslands had low productivity in the late dry season.
3. I found a main axis of plant morphological differentiation, distinguishing species according to foliar concentrations of nitrogen, phosphorus, calcium and magnesium (N, P, Ca and Mg), specific leaf area (SLA) and leaf lifespan (LLS). I interpreted this, as the basis determining resource acquisition strategies where species having short-lived leaves and with high SLA have high foliar nutrient content, traits that are associated with a resource acquisitive strategy. Conversely, species with the opposite attributes reflected a resource conservative strategy. This axis revealed an unexpected result; the correlations of leaf P concentration with LLS and SLA were not significant.

4. Also, I found a second axis of plant morpho-phenological differentiation underlined by LLS and leaf dry matter content (LDMC), traits frequently associated with resistance to physical damage by means of structural investments in leaf protection (physical defence). Other traits that linked to this axis such as leaf potassium concentration (K), lateral spread by clonal growth (LSCG), root depth (RD), start of flowering period (SFP) and length of the period from flowering to seed shed (LFS) were governed by differences in life-history and life forms among species. There was a trend among perennials to have greater LSCG and deeper roots, which was associated with high leaf N concentration, and indicative of a particular acquisitive strategy. An acquisitive strategy corresponds to high relative growth rate, however, in this case, can be in turn, related to the capacity to replace tissue after damage, a character which confers tolerance to defoliation in conditions of high resource supply (del Val and Crawley 2005; Rusch *et al.* 2009). Therefore, the differentiation along axis 2, could be associated with responses to tissue damage along a gradient from resistance to tolerance. The results failed to show the expected negative association of LDMC and leaf N concentration, which was the basis for indicating an inconsistent relationship between LDMC and other traits related to the resource use axis. In grasses, this relationship could be associated to strategies of both resource use and resistance to biomass loss through investment in structure to deter herbivores. Also, high LDMC and high leaf N concentration could confer protection against herbivores, or as found by Wright *et al.* (2004)

relative high leaf N concentration could be part of a strategy for water conservation in low rainfall habitats.

5. Plant height at onset of flowering (PH) and leaf size (LS) were independent of the resource acquisition-conservation axis and patterns of differentiation seemed to respond to differences in phylogeny, life cycles and life forms between species.
6. Averaged community functional traits provided insights about the shifts in plant traits associated with changes in species dominance following temporal changes in resource supply due to seasonality. Three specific findings about the sources of variation explaining the community functional parameters were: a) aggregated SLA, LDMC, LLS and leaf P, N, Ca, K and Mg concentrations, LFS, and to a lesser extent LSCG, responded to changes in the temporal patterns of rainfall seasons; b) site heterogeneity revealed clear textural differences in soils and less evident differences in soil phosphorus concentration and pH. At the same time, it affected significantly all the aggregated morpho-phenological traits, except PH. Part of the site heterogeneity could involve compositional differences among sites, which could be a result of land-use history; c) the interaction between the temporal patterns defined over rainfall seasons and between-site heterogeneity was also a significant source of variation for SLA, LDMC and foliar concentrations of K and Mg, LSCG and LFS.
7. In the grasslands, the aggregated traits indicated that the dominance of plants with high foliar concentrations of P, N, Ca, K and Mg, high SLA, large LSCG, low LDMC and short LLS increased in the rainy season and declined towards the dry season, and most of these trait characteristics were also maintained at the

start of the dry season. In the late dry season the dominant community functional parameters were low foliar Ca, K and Mg concentrations, low capacity for clonal growth and short duration of the reproduction period.

8. In contrast to the effect of the temporal patterns of rainfall seasonality on an important set of morpho-phenological traits, no evidence of variation was found for aggregated RD. Although this result did not invalidate the hypothesis that RD responds plastically to soils properties, nor contradicts the findings by Nieppert and Knapp (2007) that identified different water use strategies between C3 and C4 species when upper soil horizons become dry, it suggests that this functional trait needs to be studied incorporating measures of species' RD variation during the seasonal periods or the time window of interest.

INTERPRETATION OF KEY FINDINGS AND CONSIDERATIONS

The examination of a relatively small set of species in dry seasonally semi-natural grasslands in Central America has shown species and community dimensions of morpho-phenological differentiation. It is necessary, however, to consider that some of the species dimensions/axes of functional variation had a relatively loose internal structure and that LLS and RD were associated with two dimensions of species trait differentiation. Another aspect to consider here is that the three independent species dimensions of trait differentiation imply that there is an important opportunity (space) for species functional differentiation amongst herbaceous and woody species in these grasslands, if the different species dimensions defined here do imply different ecological functions (key findings 3, 4 and 5 above).

The main axis of species differentiation is based on primary or key foliar traits, which provides the evidence of a predominant resource economy axis in these grasslands, matching well with worldwide patterns of leaf trait variation (Wilson *et al.* 1999; Wright *et al.* 2005; Freschet *et al.* 2010). Beyond the lack of the negative correlation of leaf P concentration with LLS and SLA, it is possible to interpret that the negative correlations of foliar nutrients and of SLA with LLS represent the evolutionary and/or biophysical constraints on leaf structure and function that has been mentioned by Reich *et al.* (2003) (key finding 3).

Finding species strategies or dimensions of trait variation and correlation is an important aim of functional ecology (Westoby 1998; Westoby *et al.* 2002). In this study, I focused on species strategies of resource use and growth that may influence above-ground net primary productivity of semi-natural grasslands communities growing under strong rainfall seasonality. I explored a set of traits with less well-documented evidence about their correspondence with the resource acquisition-conservation axis. The trends of variation of leaf K concentration, whole plant traits (LSCG and RD) and the phenological traits (SFP and LFS) were in general governed by differences in life-history and life forms among the species, but patterns are weak when the variability between these major groups are not taken into account (see Appendix 1 in Chapter 3 and also: Mamolos *et al.* 1995; Grime *et al.* 1997; Wright *et al.* 2005; Nieppert and Knapp 2007; Ansquer *et al.* 2009). However, across perennial species, the positive correlation found in this study between LSCG and RD with leaf N concentration indicates that both LSCG and RD, show that high rates of lateral spread and branching are common in fertile conditions and vice versa, in agreement with findings about the differentiation in

nutrient and water use strategy within different life forms and life cycles (Mamolos *et al.* 1995; Nippert and Knapp 2007) and the recent findings of Rusch *et al.* (2010), showing that high rates of lateral spread and branching are common in fertile conditions and vice versa. Further analysis, including mixing of legumes and non-legume data sets, as well as new empirical data comprising a wide number of annual and perennials species common in tropical grasslands are still much needed to clarify this issue.

The results failed to show a negative association of LDMC, the representative trait of leaf structural investment in this study, with leaf N concentration. This result contrasts with the significant correlations found by Duru *et al.* (2005) and Gross *et al.* (2007) for LDMC and leaf N concentration across species in alpine and sub-alpine communities, indicating that, with respect to those grasslands some biome-wise differences appear to exist. The complex relationship of LDMC with leaf N concentration could be related to strategies of both resource use and resistance to biomass loss. The results indicated that perennials with a trend of having a relatively high LDMC are not presenting a significant low leaf N concentration, therefore LDMC could confer protection against herbivory in species with otherwise highly nutritious tissues. Another corresponding explanation is that a trend of a relatively high leaf N concentration between perennials could be part of a strategy for water conservation as was referred to by Wright *et al.* (2004) in studies with diverse sclerophyllous species in eastern Australia (key findings 3, 4 and 6).

The aggregated morpho-phenological traits, i.e. the community functional parameters in this study, showed that the dominance of plants with high foliar concentrations of P, N,

Ca, K and Mg, high SLA, long LSCG, low LDMC and short LLS increased in the rainy season and declined towards the dry season. This result gives evidence that species in these grassland assemblages could separate according to the general species level trade-offs between resource acquisition and conservation, in this case based on the seasonal gradient of water and (indirectly) resource supply. This agrees with studies showing that under high levels of resource supply, species exhibit high SLA, leaf N and P concentrations, while traits such as high LLS and LDMC are more important under low levels of resources (nutrients and water) (Ryser and Urbas 2000; Cornelissen *et al.* 2003; Garnier *et al.* 2004) (key findings 7 and 8).

Although not a central issue of this study, I found that plot (site) heterogeneity was a clear source of variation for all the aggregated traits, except for PH. The gradient of physical properties, and to a lesser extent the chemical properties of the soils explained an important part of the inter-site differences. Additionally, an uncontrolled source of variation affecting patterns of aggregated traits was differences among sites in the composition of the major perennial life forms. Between-plot differences were the only source of variation for aggregated RD and SFP, however neither the physical or chemical soil gradients corresponded well with the variation of the aggregated RD. Other between-plot differences not considered in this study, for example specific patterns of floristic composition could also be relevant sources of variation not only for aggregated RD but for other aggregated traits. Specifically, for aggregated LS, foliar N, Ca and Mg concentrations, LLS, LSCG and LFS, between-plot differences appeared to be related to the particular floristic patterns present in plot 15.

Another, uncontrolled source of variation could be within-species differences in trait expression. Garnier *et al.* (2007) mentioned that generally, species differentiation is large compared to within-species differences, and therefore they justified the calculation of CWM based on trait values averaged within-species without considering the trait variability. When within-species trait variability is large, they suggest the incorporation of within-species variation, i.e. through population or case-specific averages (Garnier *et al.* 2004; Garnier *et al.* 2007; Reiss *et al.* 2010), key findings 7 and 8. In relation with this issue, although trait plasticity was controlled using methodological parameter of 30 replicates for foliar traits with well recognized phenotypic variation, it is necessary to incorporate the measurement of species' traits variation during the relevant period of the study, not only during a particular seasonal period.

This study has brought forward a new and basic ecological knowledge for a set of herbaceous and woody species which represent the dominant species cover and a substantial proportion of the above-ground primary productivity of the semi-natural grasslands in Central Nicaragua. Ecological information of these species and communities has been otherwise very limited.

I also studied the temporal variability of the ANPP in these grasslands and compared it to that of sown pastures. That study not only identified seasonal and annual values of ANPP, and the temporal variability of the production, but also the relationships between ANPP and the cumulative rainfall in 22-day periods in both types of vegetation. The study of these variables and their relations contributes to the understanding of the

consequences of the massive replacement of semi-natural grasslands in the region and ecological risks associated with that practice (key findings 1 and 2).

In chapter 2, I argue that the introduction of sown pastures in the area of the study does not appear to be an effective means to increase ANPP, since at least during the study period, there were no significant gains on an above-ground annual basis. Furthermore, ANPP of sown pastures was more variable within seasons than for grasslands, and there was an indication that sown pastures were more sensitive to variation in the distribution of rainfall. Sown pastures were slightly more productive during the rainy season than grasslands, with the surplus produced in this period potentially being used in the dry season if harvested and stored. If this practice is not implemented, less even seasonal production is likely to impose greater challenges in grazing management for sown pastures than grasslands. Considering that both sown pastures and grasslands in the region were equally unproductive in the latter part of the dry season it is possible to vindicate the widespread local practice of retaining trees in semi-natural grasslands and pastures when opportunities arise.

Overall, the gains of replacing grasslands with sown pastures are questionable, and need to be weighed against increased costs and the ecological and environmental risks of reducing the diversity of the grasslands. These suggestions are based entirely on above-ground biomass measurements, but there could also be differences in nutritive value and the palatability between sown pastures of *Brachiaria brizantha* and the grasslands.

OVERALL SYNTHESIS AND RECOMMENDATIONS

In this section I set out a brief synthesis of what has been found in the chapters, each with different aspects of the linkages among main patterns of morpho-phenological trait differentiation and plant community functional properties that vary seasonally according to rainfall, and than can affect the above-ground net primary productivity in semi-natural grasslands.

In sub-humid and seasonally dry climates, savannas and semi-natural grasslands are being replaced by monocultures of sown pastures to increase the amount and quality of forage offered to cattle (Dias-Filho 2007; Cruz *et al.* 2010). In the region studied, this conversion involves the replacement of vegetation cover by sown pastures. Semi-natural grasslands in the neotropics are the most wide spread type of vegetation derived from forest after clearing (Fisher *et al.* 1994; Maass 1995) and in sub-humid areas occur in a systems with temporal shifts in resource availability.

After this research, it is evident that semi-natural grasslands in seasonally dry ecosystems in Central Nicaragua are mainly explained by a main dimension of plant trait variation that separate species, in correspondence with, the resource use plant strategies. Herbaceous species with high concentrations of Ca, Mg, N, and P in their leaves, and high values of specific leaf area and short leaf longevity are representative of the acquisitive plant resource use strategy, while species with the opposite traits represent a conservative plant resource use strategy. Semi-natural grasslands were importantly explained by a second dimension of trait variation that must be carefully analyzed given the weakness of the variation patterns when the differences between annuals and

perennials, and amongst life-forms, are not taken into account. This was the case of the variation in traits such as, leaf K concentration, lateral spread by vegetative growth, root depth, start of flowering period and the length of the period from flowering to seed shed. Furthermore, within the second dimension of trait variation, semi-natural grasslands comprise perennials with the trend that having a relatively high leaf dry mass investment does not present significant low foliar N concentrations; we interpreted this as a protection mechanism (resistance) against herbivores, clearly contrasting with studies where foliar nitrogen, or crude protein and leaf dry matter contents underline a significant negative relationship (Duru *et al.* 2005; Gross *et al.* 2007).

Summarizing, the morpho-phenological trait differentiation suggests that species in semi-natural grasslands according with the resource availability in dry seasonal climates are able to be distributed along the resource axis from strategies of high acquisition and circulation of nutrients, to strategies of conservation and low circulation of nutrients. Additionally, as semi-natural grasslands are comprised of annuals, perennials and a diverse group of life-forms there is an opportunity (space) for other dimensions of trait variation to be present, related with leaf K concentration, the lateral spread by clonal growth, the root depth and the phenological traits, that are interpretable only into particular groups of species (life forms or life cycles). It is relevant to consider that a number of perennial species in semi-natural grasslands potentially have a resistance mechanism to herbivores that is based on the trend of species having leaves with relatively high N concentrations and also high dry matter content.

A relevant question is if the species in semi-natural grasslands assemblages are capable of segregating, as they did along the resource axis, from strategies of high acquisition of nutrients to the conservation of them. However, now, the question has basis on the seasonal gradient of water and (indirectly) resource supply, and contributes to improve our understanding of the shifts in the plant trait dominance in environments with temporal variation in the resource supply. Some early findings in neotropical savannas suggest that co-existing species reach peak biomass at different times of the rainy season (Sarmiento 1984; Sarmiento 1992).

For this study with measurement of traits weighted by species cover as a proxy of species dominance, it is apparent that the dominance of plants with high foliar concentrations of P, N, Ca, K and Mg, high SLA, high capacity for clonal growth, low LDMC and short LLS increases in the rainy season and declines towards the dry season. These findings clearly support the relevance of the rainfall seasonality to the functional properties of semi-natural grasslands in the region because at the community level they are able to be distributed along the resource axis from strategies of high acquisition, when nutrients and water are available in the rainy season, in some cases up to the early part of the dry season, to the conservation of nutrients and water, in the dry season. Leaf P and K concentrations, and leaf longevity are traits that in the dominant species are particularly responsive to seasonal shifts. For example, the foliar nutrients, P and K decreased their concentrations, and increased their foliar longevity, from the late rainy season to the early in the dry season.

Whilst, the previous question confirmed the presence of seasonal changes in the composition of a set of traits at community level, there are other clear observations to be presented. For instance, only in the cases of aggregated leaf longevity and period from flowering to seed shed, the seasonal variation was the stronger source of variation in comparison to the between-site variation, the other important source of variation considered in the study, which greatly affected shifts in the plant trait dominance of all aggregated traits, except PH.

Semi-natural grasslands present an important between site-soil heterogeneity that in this study was found to be related to textural variation, and secondly to a chemical gradient of variation. On the other hand, the presence of floristic patterns of major life form groups among sites was an omission that in the study was masked by this variation.

This research provides results that support the establishment of links as to how semi-natural grassland composition, at species and community level impact the temporal distribution of the above-ground net primary productivity in seasonally dry lands. With the study of the community functional properties in environments with temporal variation in resource supply, the message is that during the rainy season there is a concentration of dominant species with high concentrations of foliar nutrients (P, N, Ca, K and Mg), and high SLA, low LDMC, short LLS and high capacity for LSCG. From these characteristics it can be expected a high growth rate with lower invest in structural defences than toward the dry season. These seasonal shifts, in the dominant species, concur with the more stable seasonal biomass in semi-natural grasslands rather than in the sown pastures of *Brachiaria brizantha*. Taken together, these results for semi-natural

grasslands are attributable to the presence of dominant species with a wide set of resource use strategies; the dominant species respond to the extended period of growth beyond the late rainy season up to the early part of the dry season. It is clear that foliar nutrients (N, Ca and Mg) and high SLA, short LLS, and high capacity for LSCG are able to be present up to the early dry season because the dominant species strategy in this period make use of scarce resources, in contrast, the decrease in the concentration of the foliar nutrients, P and K, and an increase in the structural defences, occurs from late in the rainy season to early in the dry season, when the availability of those nutrients in the soil becomes scarce and rainfall amount decreases.

This study makes a direct contribution to the advances in our understanding of semi-natural grasslands, ecology and above-ground net primary productivity in dry seasonal lands; it contributes to the advances in our understanding of the integrated functioning and the diverse plant resource-use strategies of co-existing species in semi-natural grasslands. Descriptions, protocols and analyses of morpho-phenological plant traits of semi-natural grasslands species reported in this study provide a quantitative description to develop mechanistic hypotheses of ecological processes operating at the level of a plot (paddock) in livestock environments. In addition, data from this study may be used to improve protocols for plant functional traits measurements, or the calibration of simulation models of tropical semi-natural grasslands with respect to productivity and seasonality.

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