Universidade Federal de São Carlos

Programa de Pós-Graduação em Ecologia e Recursos Naturais

# Determinant factors to the dominance of African grasses in two tropical seasonally dry ecosystems

Candidato – Rafael de Oliveira Xavier

Orientador – Dalva Maria da Silva Matos

Tese apresentada ao Programa de Pós-Graduação em Ecologia e Recursos Naturais, como parte dos pré-requisitos para a obtenção do título de Doutor em Ecologia.

São Carlos, 2015

Ficha catalográfica elaborada pelo DePT da Biblioteca Comunitária UFSCar
Processamento Técnico
com os dados fornecidos pelo(a) autor(a)

X3d

Xavier, Rafael de Oliveira
Determinant factors to the dominance of African grasses in two tropical seasonally dry ecosystems / Rafael de Oliveira Xavier. -- São Carlos : UFSCar, 2015.
135 p.

Tese (Doutorado) -- Universidade Federal de São Carlos, 2015.

1. Plasticidade. 2. Heterogeneidade ambiental. 3. Fenologia. 4. Espécies invasoras. 5. Estresse . I. Título.

**UNIVERSIDADE FEDERAL DE SÃO CARLOS**

Centro de Ciências Biológicas e da Saúde
Programa de Pós-Graduação em Ecologia e Recursos Naturais

## Folha de Aprovação

Assinaturas dos membros da comissão examinadora que avaliou e aprovou a Defesa de Tese de Doutorado do candidato Rafael de Oliveira Xavier, realizada em 26/02/2015:

Dalva Maria da Silva Matos
Profa. Dra. Dalva Maria da Silva Matos
UFSCar

Prof. Dr. Alexander Vicente Christianini
UFSCar

Profa. Dra. Vânia Regina Pivello
USP

Heloisa
Profa. Dra. Heloísa Sinatora Miranda
UnB

Gustavo
Prof. Dr. Gustavo Habermann
UNESP

# Agradecimentos

-à FAPESP pelo suporte financeiro durante dois anos do doutorado, e à Capes pelo fornecimento da bolsa de doutorado no primeiro ano e de doutorado sanduíche no último ano.

-aos gestores da Estação Ecológica de Itirapina pelas informações e suporte oferecido durante todo o trabalho de campo, especialmente ao Ms Paulo H. P. Ruffino e à Dra. Denise Zanquetta.

-aos gestores da Estação Ecológica de Assis pelo suporte técnico e espréstimo de equipamento para o monitoramento do lençol freático.

-a atuais e ex-integrantes do Laboratório de Ecologia e Conservação, pelo apoio no trabalho de campo e pelas discussões que geraram contribuições importantes a esse trabalho. Fernanda, Talita, Renata M., Renata V, Raquel N, Raquel M, Rosana, Carol, Wagner, Luís e Adélcio.

- a Dillon Polito, pela amizade e ajuda em campo e nas atividades de laboratório, que permitiram que eu tivesse todos os dados enquanto estive no exterior.

-a Luiz Aparecido Joaquim, pela amizade, disposição e grande responsabilidade durante o trabalho de campo.

- Pavel Dodonov, pela amizade e ajudam várias fases de execução do projeto, sobretudo com conselhos valiosos sobre a análise dos dados, além de vários momentos de descontração.

-a Marcelo Boccia Leite, que me convidou para trabalhar em conjunto e teve a ideia de trabalhar com lençol freático, e com quem estive lado a lado durante três anos de

intenso trabalho de campo. Obrigado pela paciência com meu mau humor matinal, suprimento infinito de tereré e por fornecer seus nobres veículos para incursões nas estradas traiçoeiras da Estação Ecologica de Itirapina, entre várias outras contribuições.

-aos amigos de São Carlos, que me ouviram pacientemente reclamar da sorte e suportaram meus acessos de entusiasmo.

-aos amigos da república quasecem%, meu lar durante a maior parte desse trabalho, que permitiram que a casa fosse uma extensão do trabalho de campo.

-à minha família, que me incentivou a estudar desde muito cedo, e sempre compreendeu minha ausência e me apoiou nos momentos mais difíceis nesses anos de trabalho.

- à minha namorada Adriana, que eu ainda não conhecia quando iniciei essa pesquisa, mas que mudou minha vida e acompanhou com paciência e compreensão os momentos de dificuldade.

-aos professores que me atenderam prontamente quando os procurei insistentemente em busca de viabilizar teórica e empiricamente meus interesses de pesquisa, sobretudo ao Dr. Marcel Rejmanek, Dr. Vânia Pivello and Dr. Francisco Dubbern.

-à Dr. Carla D'Antonio, que idealizou, financiou e participou ativamente da redação de um dos capítulos, além de ter fornecido excelentes conselhos sobre como deveria conduzir a fase final da pesquisa, e abriu as portas para a experiência no exterior e me mostrou que, mas do que tudo, humildade e trabalho duro levam ao sucesso.

-à minha orientadora Dalva Maria da Silva Matos, que acreditou em mim desde o início de nossa parceria quase 10 anos atrás, e me forneceu amizade, carinho e orientação durante todos esses anos.  Sem essa oportunidade não teria chegado até aqui.

# Summary

**LIST OF FIGURES** **III**

**LIST OF TABLES** **VI**

**GENERAL ABSTRACT** **1**

**RESUMO GERAL** **3**

**GENERAL INTRODUCTION** **5**

**CHAPTER 1 - THE ROLE OF WATER STRESS IN THE OCCURRENCE AND PERFORMANCE OF NATIVE AND AFRICAN GRASSES IN A BRAZILIAN CERRADO** **11**

**ABSTRACT** **12**

**1. Introduction** **13**

**2. Material and Methods** **16**
  2.1. Study site 16
  2.2. Species description 17
  2.3. Methods 18
  1.2. Data analysis 25

**2. Results** **28**
  2.1. Correlation between abiotic variables. 28
  2.2. Species occurrence related to abiotic variables 29
  2.3. Species density and abiotic variation 31
  2.4. Tiller production and abiotic variation 35

**3. Discussion** **38**

**4. Conclusion** **43**

**CHAPTER 2 – PHENOLOGICAL SIMILARITY AND PLASTICITY BETWEEN AFRICAN AND NATIVE GRASSES IN THE BRAZILIAN CERRADO** **44**

**ABSTRACT** **45**

**1. Introduction** **46**

**2. Material and methods 49**
- 2.1. Study site and species 49
- 2.2. Sampling 51
- 2.3. Data analysis 52

**3. Results 53**
- 3.1. Distribution of phenophases 54
- 3.2. Phenological overlap 61
- 3.3. Temporal and environmental effects on phenology 61

**4. Discussion 65**

**5. Conclusion 71**

**CHAPTER 3 –DISTRIBUTION OF EXOTIC AND NATIVE GRASSES ACROSS MICROHABITAT HETEROGENEITY AND ENVIRONMENTAL GRADIENTS IN HAWAI'I 72**

**ABSTRACT 73**

**1. Introduction 74**

**2. Material and methods 77**
- 2.1. Study site 77
- 2.2. Focal Species 78
- 2.3. Site selection 79
- 2.4. Sampling methods 80
- 2.5. Data analysis 86

**3. Results 89**
- 3.1. Presence/absence & local and regional variables 89
- 3.2. Performance related to environmental factors 92
- 3.3. Effect of environmental factors on leaf stoichiometry 96
- 3.4. Comparative soil depth requirement 99
- 3.5. Comparison of soil depth and percent cover among co-occurring C4 grasses 99

**4. Discussion 103**

**5. Conclusion 110**

**GENERAL CONCLUSION 111**

**REFERENCES 117**

List of figures

Figure 1 – Savanna types in the Itirapina Ecological Station (IES). upper- campo sujo, middle – campo cerrado. lower-cerrado *sensu stricto*……………………………….……20

Figure 2 –Grassland types of the Itirapina Ecological Station (IES) during the rainy season. upper- marshy grassland dominated by *A. bicornis*. Lower- wet grassland dominated by *Loudetiopsis chrisothrix*. ……………………………………………….21

Figure 3 – Sites of the Itirapina Ecological Station severely invaded by African grasses in the. upper- *campo ceerrado* invaded by *Urochloa decumbens*. lower- *campo cerrado* invaded by *Melinis minutiflora*…………………………………………………………22

Figure 4 – Sampling methods. Upper – experimental 10 x 10 plot with 1 x 1 subplots. Lower left – Drilling to the water table. Lower right – Measurement of water potential in one plot, also showing the PVC pipe into which the tape was introduced to measure the depth to the water table……………………………………………………………24

Figure 5- Relationship between two-year mean of the minimum annual depth to water table depth and the density (ind/m$^2$) of three African (a) R$^2$adj=0.054, c) R$^2$adj=0.283, e)R$^2$adj=0.034.) and three native grasses (b) R$^2$adj=0.011, d) R$^2$adj=0.244, f) R$^2$adj=0.293) across grasslands and savannas of cerrado in the Itirapina Ecological Station. Smoothing lines were obtained by means of Generalized additive mixed models (GAMM) with the mgcv package in R statistical environment. ……….………………34

Figure 6- Relationship between two-year mean of the minimum annual depth to water table depth and the individual tiller production of three African (a) $R^2$adj=0.209, c) $R^2$adj=0.356, e) $R^2$adj=0.343.) and three native grasses (b) $R^2$adj=0.010, d) $R^2$adj=0.022, f) $R^2$adj=0.318) across grasslands and savannas of cerrado in the Itirapina Ecological Station. Smoothing lines were obtained by means of Generalized additive mixed models (GAMM) with the mgcv package in R statistical environment. ...……..36

Figure 7- Smoothing lines showing the relationship between canopy cover and individual tiller production of three African (a) *M. minutiflora*, $R^2$adj=0.244, c) *U. decumbens*, $R^2$adj=0.161. e) *M. repens*, $R^2$adj=0.038) and two native grasses (b) *L. chrisothrix*, $R^2$adj=-0.042. d) *T. leiostachya*, $R^2$adj=0.001,) across grasslands and savannas of cerrado in the Itirapina Ecological Station. Smoothing lines were obtained by means of Generalized additive mixed models (GAMM) with the mgcv package in R statistical environment. ………………………………………………………...…….37

Figure 8 – Climographs of the Itirapina Ecological Station from 2011 to 2013……….50

Figure 9 - Reproductive phenology of *Urochloa decumbens* in the two sampling years. Bars are the percent of individuals in each month, vectors are the mean period for each phenophase with confidence interval. r= lengh of the mean vector. a) Flower bud. b) Flower. c) Fruit.…..55

Figure 10 - Reproductive phenology of *Melinis minutiflora* in the two years…………56

Figure 11- Reproductive phenology of *Melinis repens* in the two years ……….……...57

Figure 12 - Reproductive phenology of *L. chrisothrix* in the two years. …………….....….58

Figure 13 - Reproductive phenology of *Tristachya leiostachya* in the two years ……..59

Figure 14 - Reproductive phenology of *Andropogon bicornis* in the two years …….….60

Figure 15 –African grasses in sites originated from 750-3000 years old lava flows in the submontane zone of HAVO. up –*M. minutiflora*. center– site dominated by a mixed community of *M. minutiflora* and *M. repens*. bottom- path dominated by *H. rufa*……82

Figure 16 – Image of one exotic (up- *A. virginicus*) and one native grass (center – *H. contortus*) in low elevation sites originated from 250-750 years old lava flows in HAVO, and the exotic *S. condensatum* (bottom) in a higher elevation site with 750-3000 years old soil…………………………………………………………………...83

Figura 17 – Image of one of eight 8 m transects placed in each study site (up), and view of one 1 x 1 m plot where was estimated the percent cover of exotic and native grasses, divided by a line along of which we obtained evenly spaced soil depth measurements (bottom)………………………………………………………………………………..85

Figure 18 –Non-parametric smoothing lines obtained from spline cubic regression, showing the relationship between percent cover on the vegetation and the mean soil depth where individuals are established for six C4 grasses. Results were obtained from GAMM models performed in the R package mgcv on data of the species in 15 sites across environmental gradients in the Hawai'i Volcanoes National Park……………...95

Figure 19 – Smoothing curves showing the relationship between individual height and the mean soil depth where individuals are established for six C4 grasses. Results were obtained from GAMM models performed based on data of the species in 15 sites across environmental gradients in the Hawai'i Volcanoes National Park…………..………...97

Figure 20 – Significant fixed effects of abiotic variables on the C : N ratio of leaves of C4 grasses in the Hawai'i Volcanoes National Park. Effects were estimated by parametric coefficients (a and c) and cubic spline regression (b) in a Generalized Additive Mixed Model. *** significant differences at p <0.001………………………98

Figure 21 – Boxplots showing the variation in soil depth of five C4 grasses inside experimental plots, obtained from the difference between the mean soil depth under established individuals of each species and the mean of random soil depth measurements in those same plots. Generalized Mixed Models showed significant fixed effects related to species, and lowercase letters indicate significant interspecific differences (pairwise *a posteriori* comparisons – p<0.05). Species names: Mre- *Melinis repens*, Mmi- *Melinis minutiflora*, Avi- *Andropogon virginicus,* Sco- *Schizachyrium condensatum*, Hco- *Heteropogon contortus*, Hru-*Hyparrhenia rufa.)*.............................101

Figure 22 – Boxplots of percent cover and soil depth of established individuals between pairs of co-occurring C4 grasses in 15 costal and submontane sites in the Hawai'i Volcanoes National Park. Different letter indicate significant differences between pairs of species (Wilcoxon rank test – p<0.05).Species: Mre- *Melinis repens,* Mmi- *Melinis minutiflora*, Avi- *Andropogon virginicus,* Sco- *Schizachyrium condensatum*, Hco- *Heteropogon contortus.*……………102

Figure 23 – Diagram including the major abiotic factors affecting the occurrence and performance of native and exotic grasses both in the seasonally dry vegetation in the island of Hawai'i and in a reserve of cerrado in the southeast Brazil. Native species in each ecosystem are showed in bold letters……………………………………………………………………….112

## List of tables

Table 1 –Description of each study site in the Itirapina Ecological Station, including the two years mean of the minimum water table level, annual period (%) of the soil saturation (soil moisture=0 kPa)., dominant vegetation (marshy grassland, wet grassland and savanna) and the most common exotic and native C4 grass between the six target species. Ude- *Urochloa decumbens*, Mmi- *Melinis minutiflora*, Mre- *Melinis repens*, Lch- *Loudetiopsis chrisothrix*, Tle- *Tristachya leiostachya*, Abi- *Andropogon bicornis*…………………………………………………………….…………...27

Table 2 – Mean (± standard deviation) density (ind/m$^2$) of three native and three grassed from cerrado in 20 sites of five vegetation types of cerrado (marshy – marshy grassland, wet – wet grassland, c. sujo – campo sujo, c.cerrado- campo cerrado, c. s. stricto – cerrado *sensu stricto*) in the Itirapina Ecological Station. Each value is the mean of the density of each species in six 10 x 10 m plots…………………………………………30

Table 3 –Number of plots where each C4 grasses was found within 15 sites in Itirapina Ecological Station, divided by the annual period of soil moisture saturation (months) and canopy cover (%). Observed data (obs) were compared with expected frequency (exp), the proportional number of plots only based on the total number of plots on each categorical level, in an approximate Pearson Chi-square distribution with a Yates' correction for continuity. * significant differences at p=0.05, **differences at p<0.001.
……………………………………………………………………………………….31

Table 4– Overall explanation and fixed effects from parametric and non- parametric components (cubic spline regression) in Generalized Additive Mixed Models (GAMM). Response variables are the density (ind/m$^2$) and individual tiller production of three native and three exotic grasses. Models including the water table level were performed in 20 sites, including both grasslands and savannas, and those including canopy cover were only performed in savannas (11 sites). Est-estimated effects, edf-estimated degrees of freedom. Significance: * p<0.05, **p<0.01, ***p<0.001…………………………...33

Table 5 - Pianka’s niche overlap index (0-1) calculated to all combination of two invasive and exotic species, based on the number of individuals in three different phenophases (bud- flower bud, flow- flower, fru-fruits) in two consecutive sampling years (year 1 –Nov/2011-Oct/2012. year 2 : Nov/20120Oct/2013)…………………....63

Table 6 - Mean angle vectors (±length of the vector r) of three reproductive stages (flower bud, flower and fruit) in three African and three native grasses, followed during two consecutive years in two water regimes (positive or negative water stress) and two canopy cover categories (0-40 and 40-80%). Different capital and small letters indicate significant differences between water regimes in each sampling year and between sampling years in each water regime, respectively……………………..……………...64

Table 7 –Environmental characteristics of each of the 15 study sites. Data on rainfall are the mean annual precipitation. Fire events were from NPS records where N=no recorded fires since Park founding (1916). …………………………………..…………….....84

Table 8 – Number of plots where each C4 grasses was found within 15 sites in Hawai’i Volcanoes National Park, divided by soil age, fire occurrence, annual rainfall, elevation and mean soil depth. These observed data (obs) were compared with expected frequency (exp), the proportional number of plots was based on the total number of

plots on each categorical level, in an approximate Pearson Chi-square distribution with a Yates' correction for continuity.  * significant differences at $p=0.05$, **differences at $p<0.001$……………………………………………………………………….……...91

Table 9 – Fixed effects from parametric (soil age, annual rainfall, fire occurrence) and smoothing components (mean soil depth beneath established plants and percent rock cover) in the best GAMM for the performance of C4 grasses. Response variables were the percent cover and maximum height of six grasses across 15 sites in the Hawai'i Volcanoes National Park. Est- estimated effects, edf-estimated degrees of freedom. BIC- Bayesian Information Criteria values. Adj $R^2$- model adjusted $R^2$. Significance: * $p<0.05$, **$p<0.01$, ***$p<0.001$……………………………...………………………94

# GENERAL ABSTRACT

While grasses have a large ecological and economical relevance all over the world, they also may be aggressive invaders. Many African grasses are invaders in other Neotropical seasonally dry ecosystems. Positive response to disturbance and superior competitive ability had been associated with this success, but other species attributes and community features are expected to be important. Here we compare the effect of abiotic stress in the dominance of exotic over native grasses between a reserve of cerrado in the Southeast Brazil and in seasonally dry and early sucessional sites in the Hawai'i Island. Because of the low richness of native grasses in Hawai'i, mostly caused by geographical isolation and short evolutionary history, stressful sites should be more susceptible to invasion there than in the cerrado. We sampled the abundance and performance of these species in multiple sites in both ecosystems, where we also measured major abiotic variables that could cause plant stress. Moreover, we followed the reproductive phenology of species in the cerrado for two years. We estimated the effect of environmental heterogeneity on occurrence and performance of grasses with Chi-square tests and Generalized Additive Mixed Models, and described and compared the phenology of grasses with circular analysis and Pianka's niche overlap coefficient. In addition to water and disturbance regime, local variation in soil availability and canopy cover were important in Hawaii and in the cerrado, respectively. In both sites exotic grasses were either species that perform very well in a small range of conditions (*Urochloa decumbens* in the cerrado, and *Schyzachyrium condensatum* and *Hypaerrhaenia rufa* in Hawaii), or more generalist species tolerant to intermediate stress (*Melinis minutiflora* and *Melinis repens*). Phenological plasticity in *U. decumbens* and in lesser extent *M. minutiflora* may contribute with their success in the cerrado, *e*specially because native grasses had high phenological overlap with each other and with two African grasses, and were weakly responsive to environmental variation. While lower rainfall and elevation and newer soils in Hawai'i limited the occurrence of *M. minutiflora*, they favored *M. repens* and a native grass. One stress tolerant exotic grass (*Andropogon virginicus*) was favored in wetter and undisturbed sites with young soils. In contrast, the grasses from cerrado were highly tolerant (*Andropogon bicornis*), intolerant (*T. leiostachya*) or generalist species (*Loudetipsis chrisothrix*) across the gradient of soil waterlogging, but in general were less affected by higher canopy cover than African grasses. Fire could indirectly favor African grasses in both ecosystems, either by increasing N soil content or decreasing canopy cover. Our findings highlight the superior performance of African grasses in mesic condition, but suggest that plastic species as *M. minutiflora* could succeed at intermediate levels of abiotic stress, especially in Hawai'i. Further studies should address physiological mechanisms of African versus native grasses under stress, and compare ecological and genetic attributes of African grasses in introduced sites and in the native range. Climatic change may favor or constrain different African grasses depending on the region, but the current scenarios of increasing global mean temperatures and spread of arid zones should favor these species. Local predictive models could help to prevent further spread of these species, altogether with fire avoidance and suppression of early invasions.

Key-words- plasticity, environmental heterogeneity, phenology, stress, *Melinis minutiflora*, *Urochloa decumbens*.

# RESUMO GERAL

Apesar da grande importância ecológica e econômica das gramíneas em todo o mundo, muitas espécies são invasores agressivos. Gramíneas africanas causam impactos intensos em outros ecossistemas sazonais neotropicais, principalmente por terem grande habilidade competitiva. Nesse estudo comparamos a influência do estresse abiótico no sucesso dessas espécies em relação a gramíneas nativas em uma reserva de cerrado no sudeste do Brasil e em áreas em sucessão primária na ilha do Hawaii. Amostramos a abundância e desempenho das gramíneas nativas e exóticas mais comuns nesses dois ambientes, bem como as variáveis abióticas que poderiam causar estresse nessas espécies. No cerrado também acompanhamos a fenologia dessas espécies por dois anos. Estimamos o efeito da heterogeneidade ambiental por meio de teste de Chi-quadrado e modelos generalizados aditivos mistos, e descrevemos e comparamos os dados fenológicos com estatística circular e o coeficiente de sobreposição de nicho de Pianka. Além do regime hídrico e histórico de perturbações, a variação local em profundidade do solo e cobertura do dossel foi importante respectivamente no Havaí e no cerrado. Nos dois ambientes as gramíneas exóticas foram espécies competitivas em um espectro menor de condições (*Urochloa decumbens* no cerrado, e *Schyzachyrium condensatum* e *Hypaerrhaenia rufa* no Havaí), ou espécies generalistas tolerantes a estresse intermediário (*Melinis minutiflora* e *Melinis repens*). No cerrado, enquanto a fenologia aparentemente é importante para o sucesso de *M. minutiflora*, e principalmente *U. decumbens*, ela pode limitar a presença de *M. repens*. No Havaí, a única gramínea nativa encontrada se restringiu a áreas com intenso déficit hídrico, que também favoreceu *M. repens* e restringiu *M. minutiflora,* enquanto gramíneas exóticas tolerantes ao estresse (*Andropogon virginicus*) poderiam ser favorecidas em áreas mais úmidas, não perturbadas e com solos mais recentes. Por outro lado, no cerrado as gramíneas nativas foram altamente tolerantes ao estresse (*Andropogon bicornis*), intolerantes (*T. leiostachya*) ou generalistas (*Loudetipsis chrisothrix*) ao longo do gradiente de encharcamento do solo, mas foram menos afetas pelo dossel mais fechado em relação a gramíneas africanas. Entretanto, a fenologia foi muito similar entre essas espécies, e respondeu fracamente à variação ambiental.  O fogo pode favorecer indiretamente as gramíneas Africanas nos dois ecossistemas, seja por meio do aumento do teor de N no solo ou pela diminuição da cobertura do dossel. Nossos resultados reiteram o desempenho superior de gramíneas africanas em condições abióticas amenas, e também sugerem que espécies plásticas como *M. minutiflora* podem ser bem sucedidas mesmo em estresse intermediário, especialmente no Havaí. Estudos futuros deveriam comparar mecanismos fisiológicos e outros atributos ecológicos e genéticos de gramíneas africanas em áreas introduzidas e no habitat original. Mudanças climáticas podem favorecer ou restringir diferentes gramíneas africanas dependendo da região, mas os cenários de aumento das temperaturas médias e surgimento de mais zonas áridas devem favorecer a expansão dessas espécies. Modelos preditivos locais, aliados à prevenção de incêndios e supressão de invasões iniciais, podem a ajudar a prevenir ou limitar essa expansão no futuro.

Palavras-chave- plasticidade, heterogeneidade ambiental, fenologia, estresse, *Melinis minutiflora, Urochloa decumbens*.

# GENERAL INTRODUCTION

The grasses are among the most widespread and successful vascular plants on Earth. They are found in a wide range of ecosystems and dominate many natural communities, and are cultivated as food source by human civilizations since early as 12,000 years ago (Glémin & Bataillon 2009). Hence they have been introduced elsewhere for a variety of purposes during centuries, although a greater movement began at the late XX century supported by the globalization and the improved transportation system (Crowl et al. 2008). Many of these introduced species succeed in these new environments and spread to natural communities, where became aggressive invaders (D'Antonio et al. 2010). However, grass invasions apparently follow a biogeographical pattern, since most species are native from Eurasia and Africa, whereas most invaded ecosystems are in the America and Oceania. For instance, many annual grasses native from the Mediterranean Basin are aggressive invaders in the Californian chaparral (Carol Adair & Burke 2010; Balch et al. 2013), and a number of grasses from African savannas are a major problem in seasonally dry ecosystems in South America (Pivello et al. 1999a; Williams & Baruch 2000).

Numerous perennial C4 grasses from Africa have been introduced in other tropical regions since the XVII century, although more precise identification date from XIX century (Parsons 1972). While many of these first introductions were likely either isolated or accidental, subsequent introductions aiming improve cattle productivity occurred until the XX century (Parsons 1972). Brazil was an important destination of these species, which during centuries were successfully cultivated in areas before occupied by the Atlantic Rainforest (Parsons 1972). More recently, some of these species were either introduced or naturally spread to the more inner savannas in the Brazilian cerrado, in which they dominate large sites and has highly negative impacts on native species (Pivello et al. 1999a; Pivello et al. 1999b; Almeida-Neto et al. 2004; Hoffmann et al. 2004; Hoffmann & Haridasan 2008). In addition, many species were also introduced and rapidly spread in Northern South America (Baruch & Fernandez 1993), Caribbean islands (Parsons 1972), Hawaiian Islands (Hughes et al. 1991; Daehler & Carino 1998) and Australian savannas (Lonsdale 1994; Rossiter et al. 2003). Biogeographical studies comparing the performance of invaders in the invaded sites versus the native range are valuable for assess the factor that drive the dominance of exotic species in such multiple circumstances (Hierro et al. 2005). Despite the scarcity of these studies with invasive African grasses, some of these aggressive invaders are either uncommon or occur in mixed communities in their native range (Parsons 1972; Benzie 1986; Parmentier 2003). Accordingly, is largely accepted that particularities in the evolutionary history of many African grasses, such as subjection to high pasture pressure and aridity (Forrestel et al. 2015), are the major factors for the high invasiveness of these species.

Even though the species often differ among ecosystems, the success of African grasses as invaders could be associated with two major attributes. First, many African grasses has been showed to have high photosynthetic capacity (Baruch et al. 1985; Baruch & Goldstein 1999) and effective nutrient and water use (Baruch 1994; Baruch & Goldstein 1999), which support a much higher biomass production than native grasses (Daubenmire 1972; Williams & Baruch 2000; Silva & Haridasan 2007). Second, they both resprout quickly from underground organs and are resistant to fire and grazing (Hughes et al. 1991; Williams & Baruch 2000; Baruch & Jackson 2005; Forrestel et al. 2015). Hence they may colonize more efficiently the richer post-disturbance environment than native species. A larger amount of biomass in the system could change the fire regime, which in turn would provide a positive feedback on the presence of African grasses. This dynamic suggest that African grasses may induce a grass-fire cycle, as originally proposed by D’Antonio and Vitousek (1992), as has been reported in a number of tropical seasonally dry ecosystems (Hughes et al. 1991; Rossiter et al. 2003). Even though these attributes must play an important role on invasion by these species, benefits from disturbance may differ among different African grasses and introduced ecosystems.  For example, Klink (1994) found no clear differences in the benefit of the aboveground biomass clipping in the tillering of African grasses compared to grasses native from cerrado. Likewise, Belsky (1986) reported that grazing strongly favored the dominance of only one of many African grasses. These mixed results suggest that both other attributes of these species and specific characteristics of the invaded communities may contribute for their success as invaders.

Considering the high variety of habitats in the original range of many African grasses, we believe that stress tolerance and phenotypic plasticity could also affect the success of these species as invaders.  Regardless of the changes in the distribution of African vegetation in the last millenia, for a long time the continent has comprised biomes as diverse as deserts, savannas, grasslands and tropical rainforest (Jolly et al. 1998), which are associated with the occurrence of multiple precipitation regimes (Nicholson 2000). Moreover, a number of African grasses experience large spatial heterogeneity even within each of these vegetation types, for example induced by edaphic, altitudinal or water regime variation (Belsky 1986; Roux & Bariac 1998; Zimmermann et al. 2010).  Accordingly, African grasses are either more widespread in the continent or dominant in some specific vegetation types, and they likely could occur either as naturalized or invasive species across similar abiotic gradients in the introduced range, including stressful conditions.

Although the term stress is largely used in plant science, it has been defined in a number of ways (Lichtenthaler 1996; Otte 2001). Here, we used the definition proposed by Larcher (2003), according to which stress occur when the plant is dislocated from habitual functions, which may be followed by either a normalization and resistance phase, or permanent damage and death whether the limits of tolerance are exceeded. Plants may have a number of specific adaptations to overcome unsuitable conditions, so that could in effect experience no stress (Otte 2001). In Neotropical and seasonally dry ecosystems, edaphic and water stress drive the occurrence of difference types of vegetation (Coutinho 1978; Sarmiento 1984; Vitousek et al. 1993; Batalha et al. 2005). Accordingly, many perennial grasses native from these environments are expected to be either tolerant to stress or perform physiological or morphological changes to alleviate its negative effects (Ludlow 1980).

A number of ecophysiological studies have addressed how Neotropical grasses respond to water stress compared to African grasses (Baruch & Fernandez 1993; Williams & Black 1994; Baruch & Jackson 2005). These studies have identified that African grasses may avoid or tolerate water deficit, thus achieving higher productivity than native species even at less suitable conditions (Williams & Black 1994; Baruch & Jackson 2005). Nevertheless, it have been rarely assessed how native and exotic perennial grasses co-occur across environmental gradients in natural conditions. Extreme environments are expected to have lower invasibility and dominance of few stress tolerant native species (Alpert et al. 2000; Davis et al. 2000). However, while exotic species may be rare in a certain stressful condition, they would spread whether dispose of mechanisms to achieve larger productivity and seed output during short favorable periods, such as a plastic phenology or high resource use efficiency. Consequently, invasion resistance could rely on the stress level, which often is spatially and temporally heterogeneous, and on the relative performance of native and exotic species under stress, which is associated with the evolutionary history of each species.

In this study, we compare the response of native and exotic co-occurring grasses to abiotic heterogeneity found in seasonally dry and young sites in the island of Hawai'i and in different types of cerrado in the southeast of Brazil. Vegetation in this region of Hawai'i is driven by altitudinal and precipitation gradients (D'Antonio et al. 2000), altogether with variation in soil age and fertility related to with volcanic lava flows (Vitousek et al. 1993). The Hawaiian Islands have been geographically isolated since its formation (26 to 0.4 Ma), and in turn likely few natural plant introduction events occurred, so that it has a peculiar vegetation composed by a large number of endemic shrubs and trees (Price 2004). The Hawaiian flora currently is dominated by exotic species (Wagner et al. 2012), because of both this geographic isolation and the high propagule pressure of exotic species (Daehler 2006). Moreover, it includes only 65 species of native grasses, over 50% belonging to the genus *Panicum* (Rundel 1980). In contrast, over 400 exotics grasses occur in the islands, among naturalized and invasive species (Rundel 1980; Wagner et al. 2012).

The separation of the current South America from Africa dates as early as the Cretaceous period, but the South American savannas only originated in the late Oligocene or in the Oligocene/Miocene boundary (23 Ma) (van der Hammen 1974; Gottsberger & Silberbauer-Gottsberger 2006). Many large herbivores lived in these savannas, but most disappeared after the large extinction in the late Pleistocene (11,500 years ago) (Patterson & Pascual 1968). Although some species persisted until about 3000 years ago, few of these species were grazers (Owen-Smith 2013). The origin of the cerrado from these savannas is uncertain (Pinheiro & Monteiro 2010), despite that the oldest palynologic records of cerrado species date of about 32,000 years ago (Ledru

2002). Unlike Hawaii, most of the 2 million km$^2$ of the cerrado (Ratter et al. 1997) occurs across a relatively similar climate and soil type, even though abiotic variation emerges, for instance, from differences in the water regime, soil depth and in the canopy openness (Coutinho 1978). Also, the cerrado typically has an almost continuous and often diverse herbaceous layer, especially composed by grasses and sedges (Coutinho 1978; Gottsberger & Silberbauer-Gottsberger 2006).

Regardless structural and evolutionary differences, both cerrado (Pivello et al. 1999a; Hoffmann & Haridasan 2008) and seasonally dry ecosystems in the Hawai'i island (Daehler & Carino 1998; D'Antonio et al. 2011) that have been highly invaded by exotic species, especially African grasses. Nevertheless, we expect that these invaders are differently distributed across major environmental factors on these ecosystems. We hypothesize that in Hawai'i different invasive grasses could dominate even in extremely unsuitable abiotic conditions, considering that the evolution of grasses specially adapted to this environment is unlikely under the island's weaker selective pressure and long geographic isolation. On the other hand, perennial grasses with a longer evolutionary history such as those from cerrado would be more adapted to stress, hence African grasses only could dominate in more mesic conditions. However, we expect that a number of these exotic grasses in both ecosystems have high phenotypic plasticity, which could support persistence in spatially and temporally heterogeneous environments (Schlichting & Levin 1986).

# Chapter 1 - The role of water stress in the occurrence and performance of native and African grasses in a Brazilian cerrado

# ABSTRACT

Even though communities subject to stress often have low invasibility, they may be dominated by plastic exotic species. African grasses are major invasive species in the cerrado, but it is poorly known how less suitable conditions affect their dominance. Here we assess the presence and performance of African grasses and grasses native from cerrado across gradients of soil water content and canopy openness in savannas and grasslands of cerrado. We obtained density and tiller production of three African grasses (*Melinis minutiflora, Melinis repens* and *Urochloa decumbens*) and three native grasses (*Loudetiopsis chrisothrix, Tristachya leiostachya* and *Andropogon bicornis*) in 20 sites, where we also determined depth to the water table, soil moisture and canopy cover. Savannas had short water deficit period and heterogeneous canopy cover, whereas grasslands had varying periods of soil waterlogging. *A. bicornis* was the commonest species and showed optimal performance in flooded grasslands. *T. leiostachya* was limited to savannas and *L. chrisothrix* maintained similar performance throughout the gradient. *M. minutiflora* and *M. repens* seem to have enough plasticity to persist in wet grasslands subject to an intermediate period of soil waterlogging. *U. decumbens* had low density and tiller production at these sites, but responded quickly to a less severe stress. Notwithstanding the higher invasibility in savannas, microsites with high canopy cover could resist more to invasion, as exotic grasses had lower tiller production at this condition. Likewise, waterlogging and competition with tolerant native species would decrease invasion risk by *M. minutiflora* in wet grasslands. While scenarios of lower rainfall could favor the spread of African grasses at these grasslands, suppression of incipient invasions could be an effective management strategy.

Keywords: stress tolerance, canopy cover, invasibility, waterlogging, *Urochloa decumbens*, *Melinis minutiflora*.

## 1. Introduction

Reviews have reported recurrent attributes among the most aggressive invasive plant species, such as effective dispersion and resource use (Van Kleunen et al. 2009; Dawson et al. 2011). However, few studies have quantitatively assessed how invasiveness is modified under stress (MacDougall et al. 2006; Matesanz et al. 2008). According to the invasion theory, communities subjected to severe stresses are less susceptible to invasion, considering that a low number of species is expected to be successful under unsuitable conditions(Alpert et al. 2000). Although a number of stress tolerant exotic species may be aggressive invasive species in some of these ecosystems (Crawford & Rudgers 1996; Whitcraft et al. 2007), this prediction has been confirmed in a number of vegetation types (Alpert et al. 2000). Nevertheless, communities often occur across environmental gradients (Whittaker 1956), so that some widespread exotic species to some extent should persist under stress. These exotic species are more likely to fail under severe stress, in which more tolerant native species should be dominant (Crawford & Braendle 1996; Alpert et al. 2000; MacDougall et al. 2006). In contrast, they could tolerate slightly unsuitable conditions, in which they may dominate because of the weaker competition under stress (Alpert et al. 2000; Grime 2001). They may be also considered generalist species that perform poorly at intermediate stress level, resulting in their persistence as naturalized species. Even though in this last situation naturalized native species would have little or no impact under native species, novel abiotic conditions could allow an advance in the invasion process and increasing impacts. As environmental stress is often driven by local conditions subjected to periodic variation, colonizing these sites could favor the spread of these more tolerant exotic species.

The distribution of species across a gradient of water stress drives the occurrence of a number of Neotropical grasslands and savannas. Tropical savannas typically occur under seasonal dry climates, which requires from the local community mechanisms to overcome an annual period of water deficit (Sarmiento 1984). However, Neotropical savannas may be classified from well-drained seasonal savannas subject to negative water stress during the whole dry season to savannas with waterlogged soil during most of the rainy season (Sarmiento 1984). Certain intermediate regimes may occur, such as the hyperseasonal savannas and grasslands found in the Central Brazil (Batalha et al. 2005), Venezuela (Sarmiento 1984) and Bolivia (Haase 1990), in which there is water deficit in the dry season and waterlogging during the rainy season (Sarmiento 1984). Communities that are subject to waterlogging often have lower richness and diversity than well-drained sites (Sarmiento & Pinillos 2001; Cianciaruso et al. 2005). It is believed that this is a consequence of the many ways as the excess of water on soil may cause stress in plants, such as total or partial deprivation of oxygen, production of toxic ions and facilitation of microbial pathogens (Crawford & Braendle 1996). A number of wetland species has been showed to have specific physiological mechanisms to colonize sites that are subject to long periods of soil waterlogging (Blokhina et al. 2003), especially those associated with a reduction of the negative effects of oxygen depletion on the root system (Crawford & Braendle 1996). A previous experimental study found significant differences in tolerance to flooding between certain C4 foraging grasses (Baruch 1994). However, it is unknown how the period of soil water saturation could affect the distribution of exotic grasses in tropical savannas and grasslands and how these grasses may interact with other abiotic constrains.

In addition to water availability, sunlight availability may also affect the distribution of invasive and exotic grasses in savannas. The C4 photosynthetic metabolism enhances carbon assimilation at higher temperatures, full sunlight and low water availability, because it reduces photorespiration to zero; consequently, enough $CO_2$ is obtained even with low stomatal aperture (Pearcy & Ehleringer 1984). Accordingly, C4 grasses are prevalent at the herbaceous layer of savannas (Sarmiento 1984). Nevertheless, the Brazilian cerrado maintains floristic similarity across a gradient of tree and shrubs density from grasslands to forests, with savannas being the most common vegetation (Coutinho 1978). Despite the prevalence of C4 grasses in the cerrado, we expect that they will perform worst in savannas with greater canopy cover (Silva & Klink 2001).

Even though African grasses are widespread and aggressive invasive species in the cerrado (Pivello et al. 1999a), it is poorly known how their dominance is affected by the above-mentioned heterogeneity in water and light availability. Here we assess the influence of water regime and canopy cover on the occurrence and performance of three native and three African grasses across a cerrado gradient from grasslands to closed savannas in the southeast Brazil. Grasslands occur in depressions with periodically flooded or waterlogged soil, whereas savannas occur in well-drained sites. The exotic species studied were the three most common exotic grasses in these areas, two aggressive African invaders and one naturalized species. Native grasses included one species that occurred throughout the vegetation gradient, one species common in savannas and one species common in marshy and wet grasslands. The vegetation structure and water regime in Neotropical hyperseasonal grasslands and savannas was previously studied (Haase 1990; Cianciaruso et al. 2005). However, from our knowledge it has not yet been studied how the water regime may interact with canopy cover to drive the occurrence and performance of native and exotic grasses across a gradient of vegetation. We specifically aimed at answering the following questions: i)

Do the invasive and exotic grasses occur throughout the gradient of water stress and canopy cover? ii) Are the abundance and performance of African grasses consistently lower under water stress? iii) Which environments have the highest and lowest invasibility, based on their suitability to African grasses? We expect that high canopy cover limit more the performance of African grasses than grasses from cerrado, since they are native from more open savannas (Parsons 1972; Williams & Baruch 2000). Likewise, the stress associated with waterlogging will constrain the occurrence of African grasses in sites subject to a period of flooding (marshy grasslands). Nevertheless, we believe that some of these species may occur in lower density or poorly perform in sites whose soil is waterlogged during the rainy season, or in which canopy cover is intermediate to high.

## 2. Material and Methods

### 2.1. Study site

We carried out this study in the Itirapina Ecological Station (IES), a 2,300 ha reserve located on the center of São Paulo state, Brazil. The climate in the region is Cwa (Köppen), with a wet period from October to March and a dry season from April to September. The mean annual rainfall and temperature in the last three years were 1,613 mm and 20.6 °C, respectively. The prevalent soil is entisol (Silva 2005), composed by over 90% of sand. The dominant vegetation is savanna, including three vegetation types across a gradient that ranges from sites with a dominant herbaceous layer and scattered shrubs and trees (*campo sujo*), to a more closed savanna with higher tree density and discontinuous canopy (cerrado *sensu stricto*), including an intermediate type (*campo cerrado*) (Coutinho 1978) (Figure 1).

In lower and poorly-drained sites of the IES the water table is shallow, and the depth to the water table drives a gradient of grassland vegetation from marshy seasonally flooded grasslands (*campo umido*) to wet grasslands with waterlogged soil during part of the rainy season (wet *campo limpo*) (Figure 2). Soils at these grasslands typically have higher accumulation of organic matter and better quality, although some well drained savannas with high canopy cover may also have slightly high fertility. Since most of the cerrado in the São Paulo state had been deforested (Durigan et al. 2007), these sites are some of the last native grasslands in the region. Even though the area that currently comprises the IES has a long history of human impacts, the management policy in the park has been total fire suppression, so that only one of our study sites, with *campo sujo* vegetation, burned in the last two decades.

### 2.2. Species description

As native species we chose three common C4 perennial grasses in the IES. *Andropogon bicornis* L. is a 1 to 2 m perennial cespitose grass widespread in Neotropical savannas and grasslands, especially in floodplains. *Loudetiopsis chrysothrix* (Nees) Conert, is a 50 to 80 cm native grass that occurs in Neotropical savannas and grasslands (Goodland & Ferri 1979; Klink & Joly 1989; Cianciaruso et al. 2005), including hyperseasonal sites (Cianciaruso et al. 2005), as well as in West Africa, particularly in shallow-soiled sites (Benzie 1986; Grote et al. 2009). *Tristachya leiostachya* Nees is a 1 to 2 m-tall grass native to southeastern and central-western Brazil; it is often a dominant species in this latter region, in which it may produce a high amount of biomass that is believed to create a grass-fire cycle to the detriment of other native species (França et al. 2007).

As exotic grasses we selected *Melinis minutiflora* P. Beauv. and *U. decumbens* (Stapf), two C4 grasses that dominate large areas in the IES (Figure 3), and the C4 grass

*M. repens* (Willd.), which is widespread but rarely dominant. *M. minutiflora* or molasses grass is a perennial grass native from center-southern Africa (Parsons 1972; Sarwatt & Mollel 2006). It is an aggressive invasive species in many tropical and seasonally dry ecosystems (Williams & Baruch 2000; D'Antonio et al. 2001), including the cerrado (Pivello et al. 1999a; Hoffmann et al. 2004) and other Neotropical savannas (Baruch & Jackson 2005). *U. decumbens* is a perennial grass more recently introduced into the Neotropical region (Williams & Baruch 2000). It may achieve high vegetative growth rate and change the vegetation structure and fire regime, and is considered a major threat to the conservation of cerrado (Pivello et al. 1999a) . *Melinis repens*, is a short-term perennial grass native from Africa. Currently it is a naturalized species in the cerrado (Goodland & Ferri 1979) and in other tropical and subtropical ecosystems, and an invasive species in some disturbed sites (Possley & Maschinski 2006) (David & Menges 2011). It occurs with low abundance throughout the reserve. None of these African grasses has been reported as tolerant to either shading or soil waterlogging.

## 2.3. Methods

### 2.3.1. Species occurrence and performance

We selected in the IEE four sites fully occupied by each of three types of savanna (cerrado *sensu stricto*, *campo cerrado, campo sujo)* and two types of grassland (wet grassland and marshy grassland). These sites were spatially distributed so that areas with the same vegetation type were at least 500 m distant from each other. These vegetation type were visually identified based on the classification system of Coutinho (1978) (Table 1), whereas were considered as marshy grasslands those sites that are subject to flooding during part of the wet season. In savannas, each site had 1.0 ha, whereas in wet and marshy grasslands they had 0.5 and 0.2 ha, respectively. Irrespective of these size differences, all sites consisted of grids composed by 10 x 10 m plots.

We randomly obtained five 10 x 10 m plots in each site, and in each one we established six 1 x 1 m subplots, always 2 m apart from each other. During the dry season of 2012 we identified all established individuals of the six target species within the subplots (Figure 4). We assumed that all stems connected above ground or at the soil level were the same individual. Even though this distinction is not so clear for *M. minutiflora*, it was facilitated by its low density at most sites.

In November 2011 we randomly marked one individual of each species around each 10 x 10 m plot, always less than 5m distant from the boundary of the plot. We monthly counted the number of reproductive tillers on each individual until October 2013 (Silva & Ataroff 1985). We only considered the tillers that originated flowers or fruits during the reproductive period, including those developed laterally in other tillers. Individuals that showed no photosynthetic active structures after three consecutive measurements were no longer measured and excluded from analysis.

Figure 1 – Savanna types found in the Itirapina Ecological Station (IES) and sampled in this study. up- campo sujo, center– campo cerrado. bottom-cerrado *sensu stricto*.

Figure 2 –Grassland types of the Itirapina Ecological Station (IES) during the rainy season. up- marshy grassland dominated by *A. bicornis*. bottom- wet grassland dominated by *Loudetiopsis chrisothrix*.

Figure 3 – Sites of the Itirapina Ecological Station severely invaded by African grasses in the. up- *campo ceerrado* invaded by *Urochloa decumbens*. bottom- *campo cerrado* invaded by *Melinis minutiflora*.

### 1.1.1. Abiotic environmental variables

During the dry season peak (August) of 2011 we drilled a narrow hole down to the water table in the corner of each 10 x 10 m plot (Figure 4). At the sites where the water table level was deeper than the maximum drilling capacity of our equipment (7 m) we drilled holes in only two plots. We inserted into each hole a 40 mm-wide plastic pipe with a series of 1cm holes and a geotextile coat in the last 1m; this structure allowed water intake without soil entrance into the pipe. From October 2011 to October 2013 we measured fortnightly the water table depth in each well using a Solinst Water Level Meter 102 500' measuring tape (Figure 4).

We used Watermark soil moisture sensors (Larson Co., Santa Barbara, California) to estimate soil water availability in the plot with the intermediate elevation in each site; each sensor was placed at a depth of 30 cm. This sensor measures the electrical resistance to water absorption (from 0 (water-saturated soil) to 200 (dry soil) kPa) and gives a reliable estimation of available water when there is little difference in soil texture (Spaans & Baker 1992). We attached the sensors to PVC pipes to avoid water intake, and measured the soil moisture on each site fortnightly from November 2012 to October 2013 (Figure 4).

In late December 2013 we estimated canopy cover from hemispherical photographs made over each 1 x 1m subplot and each individual marked for assessment of tiller production. We choose this period because it matches the middle of the rainy season, when we expected canopy cover to be intermediate between the more open canopy of the late dry season and the more closed canopy of the late rainy season (Lemos-Filho et al. 2010). Each photograph was made with a Nikon Coolpix 4500 placed horizontally at 40 cm height. Canopy cover was calculated from each original

Figure 4 – Images of sampling methods in the Itirapina Ecological Station. up – experimental 10 x 10 plot with 1 x 1 subplots. bottom left – Drilling to the water table. bottom right – Measurement of water potential in one plot, also showing the PVC pipe into which the tape was introduced to measure the depth to the water table.

image with Gap Light Analyzer v.2.0 (Frazer et al. 1999), after applying a blue filter for a better contrast between open sky and canopy.

### 1.2. Data analysis

We tested the null hypothesis of independence between the presence of C4 grasses and variation in canopy cover and soil water saturation by means of a Pearson's Chi-squared Test, with Yates's correction for continuity. Contingency tables were based on the occurrence of each species during three annual periods of water soil saturation (0, 4-8 and 8-12 months) and three levels of canopy cover (0-25, 25-50 and 50-75%). We based these levels on the response of most grasses to both variables and on the need of have a similar number of observations within each category. All models were generated in the software R v. 3.1.2. (R.Development.Core.Team 2014). Because Chi-square tests often find significant differences based on very small changes in frequency of occurrence, we assumed a 1% level of significance on these analysis.

We used generalized additive mixed models (GAMM) to estimate the effect of water table depth, canopy cover and soil chemical composition on the density and tiller production of exotic and native grasses. Response variables were either the density of each species into each 10 x 10 m plot, based on the abundance in six 1 x 1 subplots, or the number of tillers produced by the individuals of each species during the two years of monitoring. Random effects were estimated from the variation between plots on each study site assuming random intercept models and including a correlation structure of plots nested on each site, with the R package nlme (Pinheiro et al. 2014). Fixed effects of continuous variables were estimated by non-parametric smoothing with the R package mgcv; smoothers were based on a penalized regression spline approach with cross-validation, and the final smooth terms were built from a k=-1 dimension basis

(Wood 2011). We estimated fixed and random effects together with a single model run for each species in the mgcv R package, since it automatically calls the nlme package in mixed additive models (Wood 2011). The effect of the water table depth was based on the average of the minimum annual water table level on each plot after two years of measurements. Canopy cover was the mean from six values obtained on each 1 x 1m subplot in the GAMM models having density as response variable, and canopy cover above each individual in models of tiller production.

We identified a direct correlation between canopy cover and the minimum water table level, since soil waterlogging in general also prevented the presence of woody species, so that in these sites occur grasslands with no canopy cover (Table 1). Consequently, we also avoided colinearity by assessing effects of canopy cover in the occurrence and tiller production of C4 grasses only in savannas and without including the minimum water table level as an explanatory variable. In this model, we were able to account for small-scale variation in luminosity by using canopy cover and abundance data obtained from the 1 x 1-m subplots. The higher variation in canopy cover in these data also allowed us to only use data from plots where each species occurred, so that effects on density rather than occurrence could be estimated. One savanna site that had a superficial water table was not included in this analysis to avoid confounding effects in the model. By contrast, we assessed the responses of grasses across the whole water table gradient by means of similar models without canopy cover. Based on the relationship between water table and soil moisture, we could expect that any explanation provided for minimum water table levels always higher than 1.5 m should be low or an indirect effect of an increase in canopy cover. However, we used this approach because the very low abundance of invasive grasses in grasslands prevented us of estimating separately fixed effects for sites with more superficial water table.

Table 1 – Study sites descriptions, including the dominant vegetation (marshy grassland, wet grassland and savanna) and the commonest exotic and native C4 grass between the six target species, two years mean of the minimum water table level, annual period (%) of the soil saturation (soil moisture=0 MPa) and water deficit (>0.2 MPa). Species: Ude- *Urochloa decumbens*, Mmi- *Melinis minutiflora*, Mre- *Melinis repens*, Lch- *Loudetiopsis chrisothrix*, Tle- *Tristachya leiostachya*, Abi- *Andropogon bicornis*.

| site | vegetation | commonest native grass | commonest exotic grass | Minimum water table level (cm) | waterlogging (months) | Water deficit (months) | Canopy cover (%) |
|---|---|---|---|---|---|---|---|
| 1 | marshy | *Abi* | - | -27 | 9 | 0 | 0.0 |
| 2 | marshy | *Abi* | *Ude* | 37 | 6 | 0 | 0.0 |
| 3 | marshy | *Abi* | - | 8 | 8.5 | 0 | 0.0 |
| 4 | marshy | *Abi* | - | -21 | 7.5 | 0 | 0.0 |
| 5 | wet | *Lch* | *Mmi* | 10 | 4 | 0 | 0.0 |
| 6 | wet | *Lch* | - | 2 | 6.5 | 0 | 0.0 |
| 7 | wet | *Lch* | *Mre* | 13 | 8 | 0 | 0.0 |
| 8 | wet | *Lch* | *Ude* | 10 | 11.5 | 0 | 0.0 |
| 9 | c.sujo | *Lch* | *Ude* | 120 | 0 | 0.5 | 9.5 |
| 10 | c.sujo | *Lch* | *Mmi* | 172 | 0 | 1 | 10.3 |
| 11 | c.sujo | *Lch* | *Ude* | 269 | 0 | 0.5 | 8.8 |
| 12 | c.sujo | *Lch* | *Ude* | 537 | 0 | 1 | 18.7 |
| 13 | c.cerrado | *Lch* | *Mre* | 24 | 0 | 0 | 22.7 |
| 14 | c.cerrado | *Lch* | *Mre/Mmi* | 395 | 0 | 1 | 28.8 |
| 15 | c.cerrado | *Lch* | *Ude* | 750 | 0 | 1 | 37.1 |
| 16 | c.cerrado | *Lch* | *Ude* | 643 | 0 | 0.5 | 23.1 |
| 17 | c.s.*stricto* | *Lch* | *Mre* | 532 | 0 | 1 | 44.9 |
| 18 | c.s.*stricto* | *Lch* | *Mmi* | 750 | 0 | 1.5 | 55.9 |
| 19 | c.s.*stricto* | *Tle* | *Mmi* | 750 | 0 | 0 | 70.0 |
| 20 | c.s.*stricto* | *Lch* | *Ude* | 438 | 0 | 2 | 59.8 |

## 2. Results

### 2.1. Correlation between abiotic variables.

In general, the average of the minimum water table level was lower than 40 cm in grasslands and higher than 1.5 m in savannas. Only two sites of savanna did not followed this pattern, one of which had water table levels similar to grasslands (Cc1) and other which showed values intermediate between grasslands and savannas (Cs1) (Table 1). A single site of savanna was subject to a period of soil water saturation (Cc1), whereas the duration of this period was mixed in grasslands (Table 1).

There was high correlation between soil water potential and water table when the minimum depth to water table was lower than 1.5m (Spearman correlation - rs=0.849) but no relationship at deeper levels ( rs=-0.003). Nevertheless, the soil water availability in savannas always was in sufficient level (10-30 kPa) during the wet season, and in the dry season never remained under water deficit (> 100 kPa) during more than two discontinuous months (Table 1). In contrast, wet grasslands never were subject to water deficit, and remained waterlogged (0-10 kPa) during at least four months (Table 1). The soil was water saturated during the whole year in three grasslands, including two marshy grasslands subject to seasonal flooding (Cu1 and Cu4) and the wet grassland with the shallowest water table (Cl4). One of the marshy grassland was similar to wet grasslands, whereas the other had a longer period of waterlogging without flooding (Table 1).

### 2.2. Species occurrence related to abiotic variables

We found about 2700 individuals of the six grasses throughout the IES. *L. chrisothrix* occurred in all vegetation types and was the most abundant species, and also had the highest mean density among the three native grasses in all wet grasslands and in most savannas (Table 1, Table 2). *A. bicornis* was almost exclusive of grasslands, and had the highest density in all marshy grasslands (Table 1, Table 2). In contrast, *T. leiostachya* only occurred in savannas, and was the commonest native grass in one single site (Table 2). African grasses were only absent from marshy grasslands. *U. decumbens* was the most common species and exhibited the highest mean density among the exotic grasses in five savanna sites (Table 1, Table 2), whereas *M. minutiflora* and *M. repens* had the highest density in five sites (Table 1; Table 2).

According to Chi-square analysis, all species were less common in sites subject to 8 to 12 months of stress, except *A. bicornis*, which was more common under this condition (Table 3). Two exotic (*U. decumbens* and *M. minutiflora*) and one native grass (*T. leiostachya*) were either rare or absent of plots subject to 4 to 8 months of waterlogging, whereas the other species were equally frequent (*M. repens* and *L. chrisothrix*) or more common (*A. bicornis*) (Table 3). There was no significant effect of the canopy cover occurred on the frequency of occurrence (Table 3).

Table 2 – Mean (± standard deviation) density (ind/m$^2$) of three native and three grassed from cerrado in 20 sites of five vegetation types of cerrado (marshy – marshy grassland, wet – wet grassland, c. sujo – campo sujo, c.cerrado- campo cerrado, c. s. stricto – cerrado *sensu stricto*) in the Itirapina Ecological Station. Each value is the mean of the density of each species in six 10 x 10 m plots.

| site | vegetation | African grasses | | | native grasses | | |
|---|---|---|---|---|---|---|---|
| | | *U. decumbens* | *M. minutiflora* | *M. repens* | *L. crhisothrix* | *T. leiostachya* | *A. bicornis* |
| 1 | marshy | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 1.9 ± 0.5 |
| 2 | marshy | 0.2 ± 0.5 | 0.0 ± 0.0 | 0.6 ± 0.8 | 1.8 ± 1.0 | 0.0 ± 0.0 | 3.0 ± 1.1 |
| 3 | marshy | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 1.1 ± 1.2 | 0.0 ± 0.0 | 1.5 ± 1.2 |
| 4 | marshy | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 2.4 ± 2.1 |
| 5 | wet | 0.0 ± 0.0 | 1.9 ± 0.1 | 0.1 ± 0.1 | 2.2 ± 1.6 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| 6 | wet | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 5.1 ± 2.8 | 0.0 ± 0.0 | 0.6 ± 0.4 |
| 7 | wet | 0.2 ± 0.4 | 0.0 ± 0.0 | 0.4 ± 0.9 | 2.5 ± 0.8 | 0.0 ± 0.0 | 1.9 ± 1.1 |
| 8 | wet | 0.0 ± 0.1 | 0.0 ± 0.0 | 0.0 ± 0.0 | 2.3 ± 2.4 | 0.0 ± 0.0 | 0.0 ± 0.1 |
| 9 | c.sujo | 8.4 ± 6.1 | 0.0 ± 0.0 | 1.2 ± 1.6 | 0.5 ± 0.6 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| 10 | c.sujo | 0.1 ± 0.3 | 1.8 ± 1.4 | 1.6 ± 3.0 | 0.4 ± 0.6 | 0.1 ± 0.2 | 0.0 ± 0.0 |
| 11 | c.sujo | 0.7 ± 1.2 | 0.0 ± 0.0 | 0.2 ± 0.3 | 2.0 ± 1.0 | 1.2 ± 0.6 | 0.0 ± 0.1 |
| 12 | c.sujo | 1.9 ± 2.0 | 0.4 ± 1.1 | 1.1 ± 1.1 | 1.2 ± 0.8 | 1.4 ± 1.3 | 0.0 ± 0.0 |
| 13 | c. cerrado | 0.2 ± 0.4 | 0.0 ± 0.0 | 0.3 ± 0.6 | 2.0 ± 0.8 | 0.0 ± 0.0 | 0.1 ± 0.2 |
| 14 | c. cerrado | 0.5 ± 1.1 | 1.3 ± 1.0 | 1.3 ± 1.0 | 0.9 ± 0.7 | 0.5 ± 0.43 | 0.0 ± 0.0 |
| 15 | c. cerrado | 1.1 ± 1.6 | 1.3 ± 0.3 | 0.5 ± 0.3 | 2.1 ± 1.6 | 1.7 ± 1.5 | 0.0 ± 0.0 |
| 16 | c. cerrado | 1.2 ± 1.1 | 0.1 ± 0.4 | 0.2 ± 0.4 | 2.2 ± 1.3 | 0.5 ± 0.5 | 0.0 ± 0.0 |
| 17 | c.s.stricto | 0.3 ± 0.4 | 0.6 ± 0.7 | 0.9 ± 1.0 | 0.7 ± 0.3 | 0.6 ± 0.5 | 0.0 ± 0.0 |
| 18 | c.s.stricto | 1.3 ± 1.9 | 1.4 ± 1.6 | 0.9 ± 2.3 | 0.8 ± 0.5 | 0.1 ± 0.1 | 0.1 ± 0.1 |
| 19 | c.s.stricto | 0.7 ± 1.0 | 0.9 ± 0.5 | 0.0 ± 0.1 | 0.1 ± 0.1 | 0.2 ± 0.2 | 0.0 ± 0.0 |
| 20 | c.s.stricto | 0.9 ± 1.1 | 0.0 ± 0.0 | 0.0 ± 0.1 | 1.4 ± 1.5 | 0.7 ± 0.7 | 0.0 ± 0.0 |

Table 3 –Number of plots where each C4 grasses was found within 15 sites in Itirapina Ecological Station, divided by the annual period of soil moisture saturation (months) and canopy cover (%). Observed data (obs) were compared with expected frequency (exp), the proportional number of plots only based on the total number of plots on each categorical level, in an approximate Pearson Chi-square distribution with a Yates' correction for continuity. * significant differences at p=0.05, **differences at p<0.001.

| | *U. decumbens* | | *M. minutiflora* | | *M. repens* | | *L. chrisothrix* | | *T. leiostachya* | | *A. bicornis* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | obs | exp | obs | exp | obs | exp | obs | exp | obs | exp | obs | exp |
| waterlogging period (months) | | | | | | | | | | | | |
| 0 | 38*** | 24 | 28*** | 18 | 34*** | 25 | 57*** | 52 | 44*** | 24 | 2*** | 23 |
| 4-8 | 5*** | 13 | 5*** | 10 | 11 | 13 | 30 | 29 | 0*** | 13 | 27*** | 13 |
| 8-12 | 1*** | 7 | 0** | 5 | 0*** | 7 | 8*** | 14 | 0*** | 7 | 13*** | 6 |
| conopy cover (%) | | | | | | | | | | | | |
| 0-25 | 17 | 16 | 7 | 12 | 18 | 14 | 24 | 24 | 15 | 18 | - | - |
| 25-50 | 12 | 11 | 9 | 8 | 10 | 10 | 18 | 16 | 16 | 11 | - | - |
| 50-75 | 9 | 11 | 12 | 8 | 6 | 10 | 15 | 16 | 13 | 11 | - | - |

## 2.3. Species density and abiotic variation

The soil N content provided no additional variation explanation and increased the BIC values of GAMM models, which therefore included only either canopy cover (models for savannas) or minimum water table level (models for all vegetation types) as explanatory variables (Table 4). Consistent with occurrence data, smoothing lines showed that most species had much lower density in sites subject to seasonal flooding (minimum water table <0 cm), whereas deeper levels (>2 m) had no effect on grass density (Figure 5). Nevertheless, the response across the whole gradient was mixed (Figure 5). Fixed effects related to the water table depth either were not significant or explained a low amount of variation in density of *M. repens, M. minutiflora* and *L. chrisothrix*, which apparently was weakly affected even when the minimum water table was close to soil surface (Table 4; Figure 5). In contrast, these effects were highly significant and explained higher variation to *U. decumbens* and *T. leiostachya*. *U. decumbens* had lower density at superficial water table (< 0.50 cm deep) and peaked at a level lower than the threshold to affect the superficial soil moisture (1.5 m) (Table 4). In contrast, the density of *T. leiostachya* was lower when the minimum water table level was as deep as 2 m. Even though *A. bicornis* only occurred where the minimum water was lower than 0.50 m, its density was much higher in flooded plots (Figure 5e).

Table 4– Overall explanation and fixed effects from parametric and non- parametric components (cubic spline regression) in Generalized Additive Mixed Models (GAMM). Response variables are the density (ind/m$^2$) and individual tiller production of three native and three exotic grasses. Models including the water table level were performed in 20 sites, including both grasslands and savannas, and those including canopy cover were only performed in savannas (11 sites). Est-estimated effects, edf-estimated degrees of freedom. Significance: * p<0.05, **p<0.01, ***p<0.001.

| Vegetation | savanna | | | | | | | | | savanna + grassland |
|---|---|---|---|---|---|---|---|---|---|---|
| Component | parametric intercept | | non-parametric canopy cover | | model adj R$^2$ | parametric intercept | | non-parametric water table | | model adj R$^2$ |
| | est | t | edf | F | | est | t | edf | F | (%) |
| *U. decumbens* | | | | | | | | | | |
| density | 4.77 | 11.12*** | 1 | 13.91 *** | 0.08 | 0.89 | 4.90*** | 7.19 | 5.62*** | 0.28 |
| tiller production | 116.2 | 8.71*** | 1 | 13.12*** | 0.16 | 92.71 | 8.94*** | 4.23 | 6.85*** | 0.36 |
| *M. minutiflora* | | | | | | | | | | |
| density | 3.22 | 14.19*** | 1 | 0.92 | <0.01 | 0.47 | 4.85*** | 1 | 7.07** | 0.05 |
| tiller production | 75.55 | 5.40*** | 1.58 | 4.20* | 0.24 | 92.66 | 7.37*** | 1 | 12.58*** | 0.21 |
| *M. repens* | | | | | | | | | | |
| density | 2.89 | 7.83*** | 1 | 0.05 | -0.01 | 0.47 | 4.73*** | 1.74 | 2.56 | 0.03 |
| tiller production | 39.43 | 4.08*** | 1 | 0.56 | 0.04 | 33.24 | 6.05*** | 2.85 | 7.11*** | 0.34 |
| *L. chrisothrix* | | | | | | | | | | |
| density | 2.27 | 19.9*** | 1 | 0.013*** | 0.03 | 1.46 | 10.03*** | 1 | 2.37 | 0.01 |
| tiller production | >1000 | 5957*** | 1 | >100*** | -0.04 | 15.15 | 8.36*** | 1 | 0.06 | -0.01 |
| *T. leiostachya* | | | | | | | | | | |
| density | 2.31 | 13.39*** | 1 | 0.35** | <0.01 | 0.35 | 6.2 | 2.17 | 17.96*** | 0.24 |
| tiller production | 6.38 | 7.82*** | 1 | 8.14** | <0.01 | 6.5 | 8.46*** | 1 | 0.02 | -0.02 |
| *A. bicornis* | | | | | | | | | | |
| density | - | - | - | - | - | 0.57 | 6.25 | 2.61 | 18.74*** | 0.29 |
| tiller production | - | - | - | - | - | 19.74 | 8.64*** | 2.87 | 7.84*** | 0.32 |

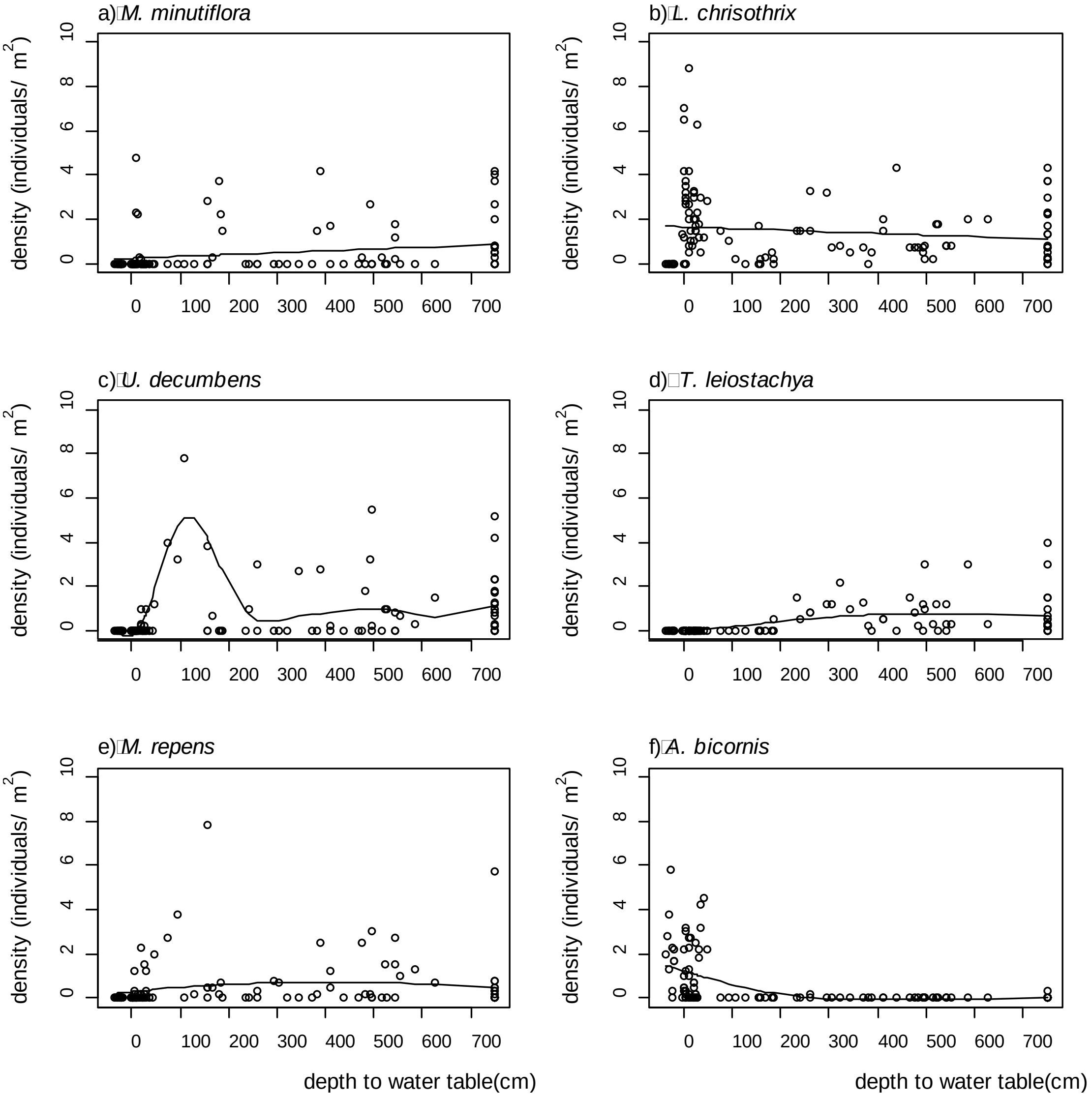


Figure 5- Relationship between two-year mean of the minimum annual depth to water table depth and the density (ind/m$^2$) of three African (a) $R^2$adj=0.054, c) $R^2$adj=0.283, e)$R^2$adj=0.034.) and three native grasses (b) $R^2$adj=0.011, d) $R^2$adj=0.244, f) $R^2$adj=0.293) across grasslands and savannas of cerrado in the Itirapina Ecological Station. Smoothing lines were obtained by means of generalized additive mixed models (GAMM) with the mgcv package in R statistical environment.

The GAMM Models estimating fixed effects of canopy cover in savannas were not significant for two native and two exotic grasses (Table 3). Models for *L. chrisothrix* were marginally significant and showed a very weak negative linear effect of canopy cover on density, whereas this effect was larger in *U. decumbens* (Table 3).

### 2.4. Tiller production and abiotic variation

Since there were high interspecific differences in commonness across vegetation types, we followed the tiller production of more individuals of *L. chrisothrix* (n=97) than of other native (*T. leiostachya*-n=45; *A. bicornis*- n=48) and exotic grasses (U. decumbens-n= 63; *M. minutiflora*-n=44; M. repens-n=35). The water table level also had mixed effects in the total tiller production. While fixed effects on the tillering of *T. leiostachya* and *L. chrisothrix* were not significant, *A. bicornis* produced more tillers when water table surfaced (Table 4, Figure 6). Our model provided a good explanation for *U. decumbens*, whose tiller production peaked at deeper water table level than the density (Table 4, Figure 6). *M. repens* also had maximum tiller production at this water table depth, but it decreased in deeper levels (Table 4, Figure 6). Likewise, the tiller production of *M. minutiflora* linearly decreased with deeper water table, apparently because major influence of a lower production at very deep level (Table 4, Figure 6).

The canopy cover had mixed effects on the tiller production of exotic and native grasses (Table 4, Figure 7). It had a significant effect but explained almost no variation on *L. chrisothix* and *T. leiostachya*, (Table 4, Figure 7). Conversely, for all exotic grasses tiller production linearly decreased as the canopy cover increased (Table 4, Figure 7), even though the effect size and the overall explanation of this relationship were larger with *M. minutiflora* and *U. decumbens* than with *M. repens* (Table 4, Figure 7).

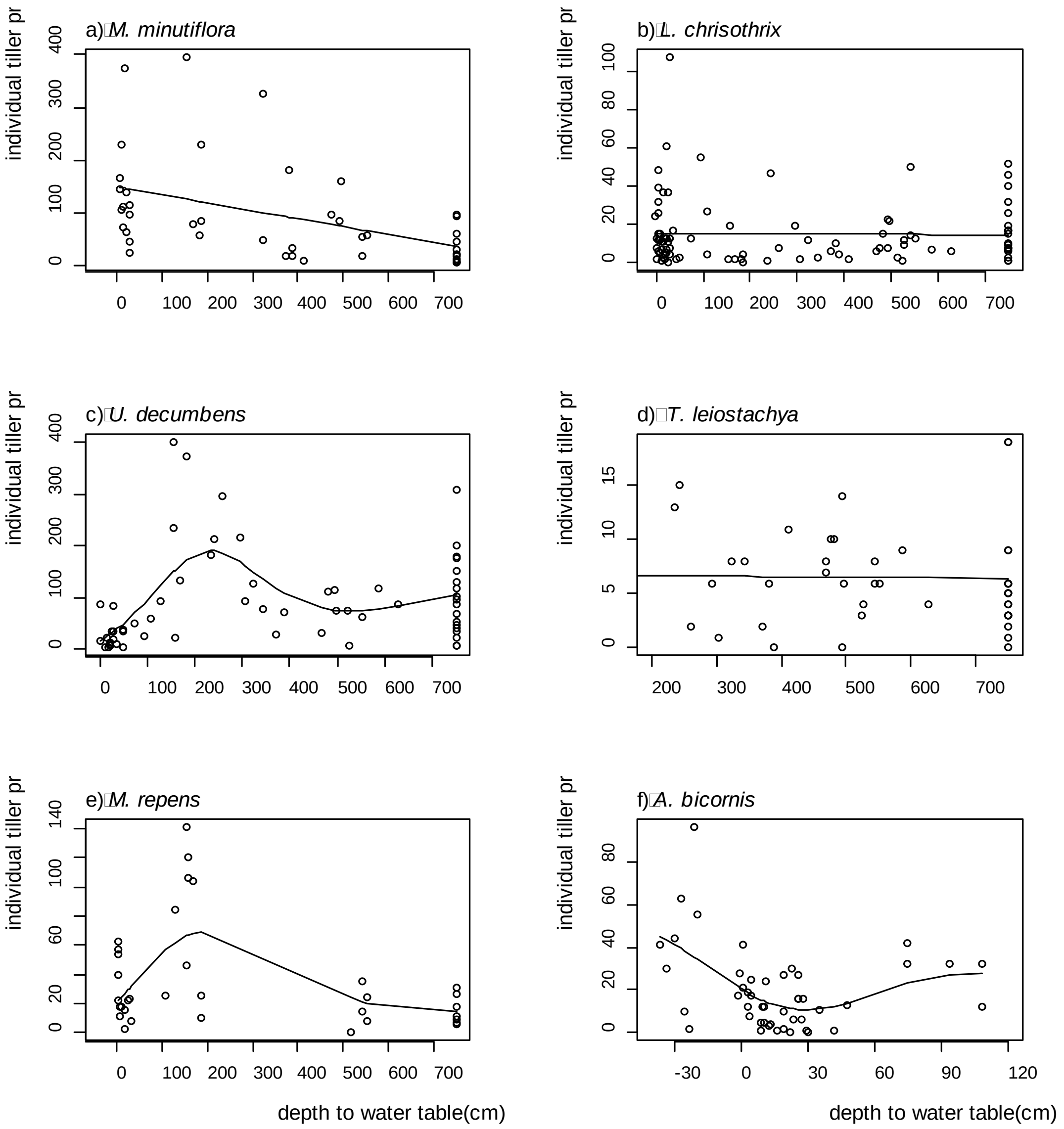


Figure 6- Relationship between two-year mean of the minimum annual depth to water table depth and the individual tiller production of three African (a) $R^2$adj=0.209, c) $R^2$adj=0.356, e) $R^2$adj=0.343.) and three native grasses (b) $R^2$adj=0.010, d) $R^2$adj=0.022, f) $R^2$adj=0.318) across grasslands and savannas of cerrado in the Itirapina Ecological Station. Smoothing lines were obtained by means of Generalized additive mixed models (GAMM) with the mgcv package in R statistical environment.

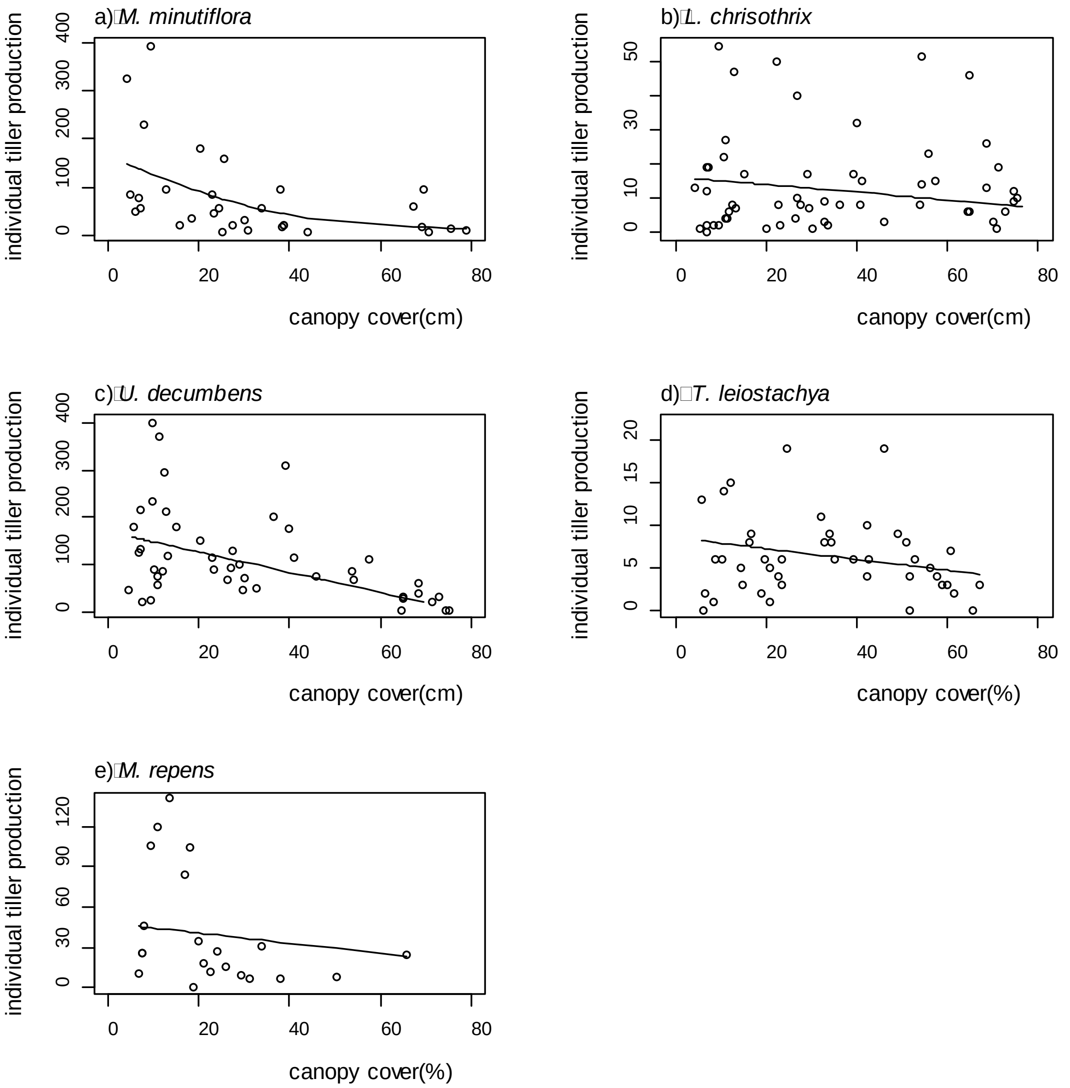


Figure 7- Smoothing lines showing the relationship between canopy cover and individual tiller production of three African (a) *M. minutiflora*, $R^2$adj=0.244, c) *U. decumbens*, $R^2$adj=0.161. e) *M. repens*, $R^2$adj=0.038) and two native grasses (b) *L. chrisothrix*, $R^2$adj=-0.042. d) *T. leiostachya*, $R^2$adj=0.001,) across grasslands and savannas of cerrado in the Itirapina Ecological Station. Smoothing lines were obtained by means of Generalized additive mixed models (GAMM) with the mgcv package in R statistical environment.

## 3. Discussion

Our results suggest that the duration of the period of positive water stress associated with a superficial water table is the major abiotic variable limiting the distribution, abundance and performance of exotic and native grasses across this gradient of cerrado vegetation. Only the stress tolerant *A. bicornis* occurred in grasslands subject to soil waterlogging during a long period of the year (>8 months). When this period was of 4 to 8 months, *U. decumbens* and *T. leiostachya* were absent, rare or performed poorly, whereas *M. minutiflora*, *M. repens* and *L. chrisothrix* achieve density and tiller production similar or higher than found in well drained savannas, suggesting that they are more generalist species. The short period of water shortage in savannas may favor the dominance by African grasses, even though both *L. chrisothrix* and *T. leiostachya* apparently tolerate better a close canopy. This high variety of responses to stress have important consequences for the invasibility of these communities of cerrado.

According to our data, the occurrence of soil waterlogging in wet grasslands of cerrado prevents the spread of *U. decumbens*. Here, occurrence, abundance and individual performance of *U. decumbens* were lower in sites under four or more months of waterlogging in a year. *U. decumbens* was also rare in hyperseasonal grasslands in the Central Brazil, although the authors did not measure the annual soil water saturation (Cianciaruso et al. 2005). The intolerance to waterlogging showed by this species contrasts with certain congeneric species, which are specially adapted to this condition (Michelan et al. 2010), probable because they dispose of specific mechanisms that are absent in *U. decumbens.* Apparently these ecophysiological differences are common in this genus, since it was also reported to have high interspecific variation in tolerance to drought (Guenni et al. 2002). Consequently, under the current water regime this species would be expected to be a minimum concern in wet grasslands. Nevertheless, our data also showed that the density of *U. decumbens* increased from more stressful to more suitable wet grasslands and peaked in a savanna whose minimum water table was as shallow as 1 m. Since this site was never subject to soil saturation, it may have a water table level located slightly under the threshold in which the water table may induce water soil saturation by capillarity (Vervoort & van der Zee 2008). This opportunist response was described to other invasive species with high resources use efficiency, which, despite being less tolerant to stress, could benefit more of slightly suitable abiotic conditions than other species (Richards et al. 2006; Davidson et al. 2011). As the highest tiller production occurred in sites that currently have an only slightly deeper water table, this highlights that a little decrease in precipitation that affected this level could cause a disproportionate increase in the invasibility of wet grasslands. Since *U. decumbens* may spread quickly in the cerrado (Pivello et al. 1999b), this more suitable water regime could severely impact the native vegetation.

Even though *M. minutiflora* and *M. repens* also were less common in sites that are subject to waterlogging, they appear to have enough plasticity to spread on these conditions. *M. minutiflora* was less common when there was a period of waterlogging than in well drained savannas, but achieved density and tiller production similar to savannas in one of the wet grasslands. On the other hand, *M. repens* was equally common in both vegetation types, but had lower density and tiller production in sites with positive water stress. Oxygen scarcity in flooded soils may drastically decrease the growth and vitality of plants (Wegner 2010), and this probably occurred in the superficial soil layer of wet grasslands during at least part of the rainy season. Hence the response of these species was unexpected, since tolerance to soil waterlogging was never reported to these species. We believe that the mechanism behind these responses may be related to physical or chemical changes induced in the root system, which is directly subject to the anoxic or hypoxic conditions in water saturated soils (Crawford & Rudgers 1996). Plasticity to negative water stress was identified in the root system of multiple plant species, including African grasses (Hartnett et al. 2013). Likewise, it is possible that a differential growth response on roots allows *M. minutiflora* and *M. repens* to obtain resources without be exposed to deep and water saturated soils, characterizing an avoidance strategy against stress (Lichtenthaler 1996). The development of adventitious roots was previously proposed as an reliable response to waterlogging in other African grasses (Baruch 1994), and to some extent could have allowed these species to uptake oxygen at soil surface and prevented the damages caused by low oxygen content in the soil. Since *M. minutiflora* and *M. repens* are congeneric species, this may be a general response of plastic grasses without adaptations to persist in soil periodically subject to oxygen scarcity. Alternatively, maintaining a very superficial root system could support resources uptake when water table level was close to soil surface. We believe that this last mechanism could have favored the occurrence of small ruderal grasses as *M. repens*, which often succeeds even under low soil availability (Pratt & Lottermoser 2007).

The absence of water deficit in wet grasslands could also favor the spread of plastic African grasses as *M. minutiflora* and *M. repens*. In hyperseasonal savannas, the period of soil waterlogging in the rainy season is generally followed by a short period of water in adequate level, and then by a period of negative water stress (Sarmiento et al. 2004; Batalha et al. 2005). This water shortage could be an additional environmental filter against species establishment, whose severity would depend on its duration and intensity. *M. minutiflora* was an uncommon species in a hyperseasonal grassland in the Central Brazil (Cianciaruso et al. 2005). Here, there was no period of water shortage in wet grasslands, and the period of positive water stress on the rainy season was followed by a longer period of soil moisture in sufficient level. Accordingly, it is possible that during this period with favorable water balance *M. minutiflora* and *M. repens* produced enough photosynthate to partially compensate the previous stressful period, and sustain the production of reproductive tillers. This could be achieved, for instance, by means of spread of the root system to a deeper soil depth during the period of favorable water balance. Consequently, the success of *M. minutiflora* and *M. repens* on wet grasslands could rely on the extension of this favorable period in comparison with the period of soil waterlogging. On the other hand, the high occurrence, density and consistent tiller production of *L. chrisothrix* in wet grasslands suggest that this species has the best performance in grasslands subject to intermediate periods of soil waterlogging (4-8 months), and could represent a biotic barrier against the spread of these species.

In spite of the superior performance and dominance of African grasses in many savannas, co-existence with native species could interact with positive water stress and/or higher canopy cover in certain sites, and avoid the spread of these species. The stress associated with lower luminosity has the potential of decrease the invasibility of a number of communities (Alpert et al. 2000). However, a previous comparative study found no difference in light use efficiency between a number of invasive and native C4 grasses, including *M. minutiflora* (Baruch et al. 1985). In our study, two sites of the most closed savanna (cerrado *sensu stricto*) had mean canopy cover higher than 60%, whereas many plots and marked individuals of grasses were subject to values as high as 80%. Although the occurrence and abundance of grasses was weakly affected by canopy cover, tiller production of exotic species was consistently lower under higher canopy cover, especially for *U. decumbens* and in lesser extent for *M. minutiflora*. In contrast, canopy cover caused little effects on the tiller production of *T. leiostachya*, and especially *L. chrisothrix*. The former maintained a similar tiller production until about 60% of canopy cover, whereas the latter produced only slightly fewer tillers even over 80% (Figure 3). The tolerance of these native grasses could be a long term adaptation to colonize sites savannas with high spatial variation in light availability, such as may occur in the cerrado (Lemos-Filho et al. 2010). Likewise, these species could be better adapted to use the light that in transmitted through the canopy, which often decreases phytochrome activity and then tiller production of grasses (Deregibus et al. 1983; Deregibus et al. 1985; Wan & Sosebee 1998). This tolerance is not shared with *M. minutiflora* and *U. decumbens*, whose preference by the sunlight borders of cerrado fragments has been reported (Pivello et al. 1999a; Dodonov et al. 2014), and *M. repens*, which was showed to produce less tillers under lower irradiation (Souza et al. 2004). Negative effects of low light availability were also reported to other C4 African grasses in their original habitats, which are mostly open savannas (Belsky 1986). Consequently, perhaps the peculiar response of grasses from the cerrado is associated with specific selective pressures where these species evolved. Many Neotropical savannas support a higher tree density than most savannas, perhaps because have a greater annual precipitation compared to African and Australian savannas (Gottsberger & Silberbauer-Gottsberger 2006). Since the spread of grasses may depend on successful seed production and dispersion, which are associated with tiller production, at higher canopy cover native grasses are more likely to co-occur with exotic grasses. Also, this negative effect of shading on *U. decumbens* could be higher in hyperseasonal savannas, which are also subject to waterlogging.

## 4. Conclusion

We conclude that grasses native from cerrado occur throughout the gradients of water stress and canopy cover in our study site, whereas a period of over 8 months under soil waterlogging prevents the presence of common invasive African grasses in the cerrado. However, unlike we expected, density and tiller production of *M. minutiflora* and *M. repens* in grasslands subject to 4 to 8 months of water waterlogging is similar to well drained savannas, perhaps because a shallow root system or intrinsic attributes. In contrast, *U. decumbens* was intolerant to soil saturation, and is rare or absent under shallow water table (<1m), although the abrupt threshold on the response of this species to soil waterlogging shows that it is favored by a slightly more favorable water regime in wet grasslands. Likewise, the short period of water deficit in savannas in the cerrado seems contributes to the success of African grasses. Canopy cover has no effect on the occurrence of grasses, but the tiller production of native grasses is less affected by high canopy cover (over 60%) than African grasses. Based on these responses we believe that grasslands subject to cover 8 months of waterlogging have the lowest invasibility to African grasses, whereas it is the highest in well drained savannas with low canopy cover. As these species are widespread in most remnants of cerrado, response to water availability could cause either spread of these species from savannas to these grasslands in global change scenarios of lower rainfall, or retraction to savannas under higher rainfall. In addition, canopy disturbance could favor African grasses, since low light availability affects more the tiller production of African than native grasses.

# Chapter 2 – Phenological similarity and plasticity between African and native grasses in the Brazilian cerrado

# ABSTRACT

Phenology has a major role in the life history of plant species, but it is unknown how it contributes to success of invasive species under stress. Here we compare the phenology between African (*Melinis minutiflora*, *Urochloa decumbens* and *Melinis repens*) and native grasses (*Loudetiopsis chrisothrix*, *Tristachya leiostachya* and *Andropogon bicornis*) in different types of Brazilian cerrado, and address how it is affected by annual variation, soil waterlogging and canopy cover. We estimated effects of annual variation and environmental heterogeneity with a Watson-Williams test, and calculated phenological overlap with the Pianka's coefficient. *M. repens*, and in a lesser extent *U. decumbens*, showed a longer reproductive period, which overlapped the short and very similar period of all native grasses, whereas *M. minutiflora* reproduced briefly during the dry season. In the year with less severe dry season, *U. decumbens* and *A. bicornis* had earlier and longer reproduction, whereas *M. repens* showed lower growth. Soil waterlogging and high canopy cover had little effect on the phenology, but seems to have decreased the reproductive period of *M. minutiflora* in the second year. While the phenology of *U. decumbens* matches with its superior performance, it suggests a ruderal strategy for *M. repens*. Unusual phenology and its plasticity under stress could favor *M. minutiflora*. Phenological overlap between native grasses could decrease invasion resistance in the cerrado, whereas phenological stability could be an advantage in stable abiotic conditions. Phenological plasticity could enhance the spread of African grasses at sites of cerrado, especially in global scenarios of either environmental unpredictability or more favorable climate.

Keywords- plasticity, niche overlap, climatic predictability, *U. decumbens, M. minutiflora*.

## 1. Introduction

Many studies have identified recurrent attributes for invasive species, especially those related to effective growth, disturbance response and dispersion (Rejmánek & Richardson 1996; Van Kleunen et al. 2009; van Kleunen et al. 2010). Even though these traits drive many biological invasions, their influence may rely on how they resemble to those found in the local community (Alpert et al. 2000). A number of introduced species may have a locally uncommon strategy to uptake resources, which in turn could enhance the performance of these invaders when compared to native species. For example, the success of *Bromus tectorum*, a Mediterranean grass dominant in many arid and semi-arid North American ecosystems (Ogle et al. 2003), has been associated with effective resource use and high biomass production, but its annual life span and characteristics of root system are also considered important (Carol Adair & Burke 2010). In contrast, the phenological timing of other exotic species may prevent their spread in the introduced range, either because unsuitable abiotic conditions or interaction with native species (Dyer et al. 2012). A number of hypotheses have associated the temporal similarity in the phenology between exotic and native species, hereafter called phonological overlap, with the occurrence of biological invasions (Wolkovich & Cleland 2010). Cleland  et al (2012) proposed that phenology contributes to the success of invasive species whether their reproductive period is temporally distinct (vacancy niches), begins before (priority effects) or is longer than that of native species (niche breadth). Observational studies testing these ideas have found mixed results (Adair & Burke 2010; Dyer et al. 2012), highlighting that specific characteristics of the community may be important.

Phenological distinction may have an importance role on the grass species coexistence and invasibility of savannas. High species diversity is a remarkable attribute of many Neotropical savannas (Sarmiento 1984; Gottsberger & Silberbauer-Gottsberger 2006), and in the cerrado this is in great extent associated with a rich herbaceous community, in general dominated by perennial grasses (Coutinho 1978). Most of these species maintain low vegetative activity throughout the dry season, but species may achieve maximum biomass in different moments of the rainy season (Sarmiento 1992). Likewise, different species may flower and disperse seeds early, in the middle or late in the rainy season, and eventually early in the dry season (Sarmiento 1992). This phenological distinction has been considered important for the coexistence of many perennial grasses at these savannas (Sarmiento 1984; Sarmiento 1992), since it could decrease competition due to a more effective use of available resources. Consequently, according to the hypothesis of resources fluctuation, which that associate community invasibility with the amount of unused resources (Davis et al. 2000), it could also turn these savannas more resistant to invaders.

In addition to intrinsic phenological characteristics, how phenology respond to environmental heterogeneity also affects the success of both native and invasive species in Neotropical savannas. Even though most savannas occur in tropical and seasonally dry climate, perennial grasses may also experience the spatial heterogeneity in water (e.g. waterlogging) (Sarmiento 1984) and light availability (e.g. high canopy cover) (Coutinho 1978). Accordingly, the duration and distribution of the reproductive and vegetative phase of each species may change, so that the most effective use of resources would be achieve at multiple conditions (Volis 2007). On the other hand, phenology may respond to unpredictable abiotic variation associated with extreme climatic events or periodic fluctuation in precipitation and temperature (Jentsch et al. 2009; Richardson et al. 2013). Phenological plasticity may be especially important under stress, which impose additional barriers to survival and reproduction (Aronson et al. 1992). It has been suggested that high phenological plasticity also allows invasive species to take advantage of more suitable abiotic conditions (Dyer et al. 2012). Effective phenological adjustment to this variation could favor exotic over native grasses in Neotropical savannas.

Here we compare the phenology of three African (*Urochloa decumbens, Melinis minutiflora* and *Melinis repens*) and three native grasses from cerrado between sampling years, and their response to water regime and canopy openness. The African grasses *U. decumbens* and *M. minutiflora* are aggressive invaders in the cerrado, and a major threat to its conservation (Pivello et al. 1999a; Pivello et al. 1999b). The displacement of native grasses by these species has been associated with higher resource use efficiency (Williams & Baruch 2000), large biomass production and positive response to fire (Hoffmann et al. 2004). However, the influence of the phenology timing of these species on their success in the cerrado is unknown. Whether phenology plays a important role, we could expect to some extent differences on the allocation of resources to vegetative and reproductive growth across the year between these species and native grasses. We also expect that native grasses will have mixed phenological timing and more concentrated reproductive phenology than most African grasses, since the coexistence of perennial grasses in Neotropical savannas is associated with reproduction in different periods of the rainy season (Sarmiento 1984). Previous studies reported that *U. decumbens* has an early reproduction in the growing season, whereas *Melinis minutiflora* may have the later (Parihar & Pathak 2006). We also believe that spatial heterogeneity in water regime (soil waterlogging x well drained savannas) and canopy cover will affect phenological timing of most species, but annual variation in climate variables could have a relevant effect. The phenology of invasive grasses is more expected to change under this stress, since these species could have less specific adaptations to these conditions. We specifically aimed answer the following questions: i) Are the distribution and duration of the vegetative and reproductive growth of exotic grasses different from native grasses? ii) Is there a lower phenological overlap among native grasses than between native and exotic species? ii) Is there variation on the phenology of African or native grasses related to the soil waterlogging, low canopy openness or between sampling years?

## 2. Material and methods

### 2.1. Study site and species

We performed this study at Itirapina Ecological Station (IES), a 2,300 ha reserve located on the center of São Paulo state, Brazil (see Chapter 1 for environmental description). The mean annual rainfall and temperature de 2011 a 2013 were 1.613 mm and 20.6 °C, respectively, but the precipitation in the dry season of 2012 was higher than expected (Figure 8). The dominant vegetation is the *campo sujo*, but the length of the annual period of soil saturation in lower and poorly drained sites drives the occurrence of wet grasslands (4-8 months) and marshy grasslands (8-12 months) (Chapter 1).

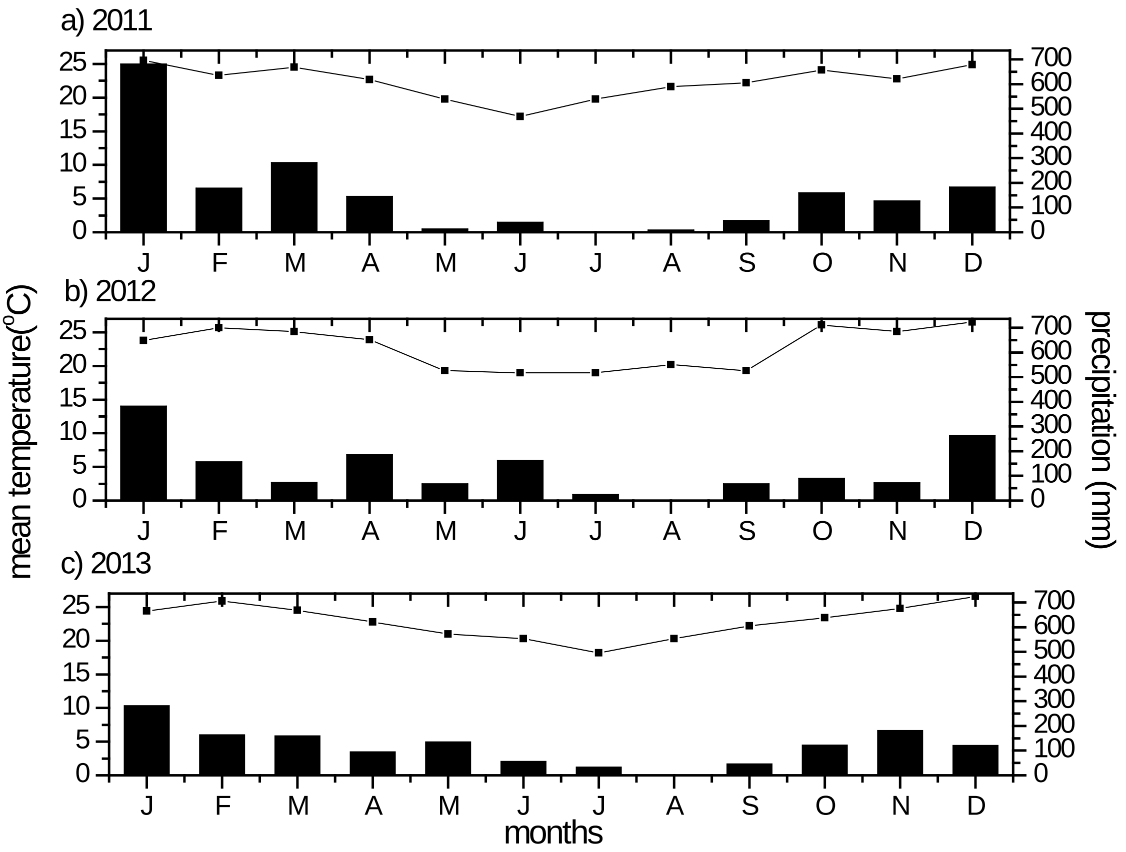


Figure 8 – Climographs of the Itirapina Ecological Station from 2011 to 2013. Bars and lines are monthly precipitation and mean temperature, respectively.

Based on field observations and previous studies in the IES (Tannus & Assis 2004) we choose three of the most common native grasses in the IES to monitor: *Andropogon bicornis* L., *Loudetiopsis chrysothrix* (Nees) Conert and *Tristachya leiostachya* Nees. They are all perennial tussock grasses widespread in Neotropical grasslands and savannas (Goodland & Ferri 1979, Cianciaruso et al. 2005). In the IES, *A. bicornis* and *T. leiostachya* are very common in grasslands and savannas, respectively, whereas *L. chrisothrix* occurs throughout the reserve (Chapter 1). As exotic grasses we selected the perennial decumbent African grasses *M. minutiflora*, *U. decumbens* and *M. repens*. The latter is widespread but occurs in lower density, whereas *M. minutiflora* and *U. decumbens* dominate large sites in the IES (see Chapter 1 for species description and distribution). Previous observations suggest that at least two of these six species always co-occur in the community.

### 2.2. Sampling

In October 2011, we marked individuals of each exotic and native species around 120 plots distributed across 20 sites, the same used for the vegetation survey and abiotic (see Chapter 1 - Table 1). We marked one individual of each species by plot in sites where the species occurred in all six plots. Otherwise, we marked more individuals of a same species by plot, so that all species that occurred in each site had six marked individuals. All individuals were at most 5 m distant from the boundary of the plots, because of the difficult to find individuals very close to the plots. Since the water table level was relatively homogeneous within our study sites (Chapter 1), we believe that this short distance to the plot had minor effect on the water balance of the marked individuals. Using this block approach we were able to easily recognize all marked individuals, and all species were subject to similar environmental heterogeneity. Moreover, we only marked individuals that had both green leaves and traces of reproductive structures from a previous or from the current growth season. Also, we focused on individuals relatively isolated from co-specific, which are more likely to be physiologically independent.

From November 2011 to October 2013 we observed the presence of new stems, new leaves, floral buds, flowers and fruits on each marked individual every month (Silva & Ataroff 1985). During the reproductive season we also sampled the number of reproductive tillers in each reproductive category. We considered as flowering and fruiting tillers containing at least one inflorescence with visible anthers and spikelets, respectively. Inflorescences that emerged laterally from axillary buds of previously present tillers were treated as new tillers. Individuals that showed no green leaves after three consecutive months in the rainy season were considered as non-vegetative. We extended the phenological observation on *M. minutiflora* to January 2014, since its reproductive phenophases in the first sampling year occurred from the middle of the dry season to the middle of the wet season.

In December 2013 we made a 40 cm high hemispherical photography on each marked individual in savanna sites, and each image was used to estimate the canopy cover after the analysis in the software Gap Light Analyzer  v 2.0 (Frazer et al. 1999). We considered the canopy cover above individuals in grassland sites as 0, having in account the absence of canopy on these sites. In addition, we assumed that low interannual fluctuation in the canopy structure would occur during this period, considering that no major disturbance occurred during the period of this study.

### 2.3. Data analysis

We described the phenological pattern of exotic and native grasses by means of circular statistics. Prior to analysis we transformed observation dates to angles (January—30; December-360), and then calculated to all phenophases of each species the mean angle (a), angle deviation (AD) and the length of the vector r, whose length indicates the level of concentration around the mean. We tested the null hypothesis of uniform annual distribution with the Rayleigh test (Z), which was performed to each phenophase of the target species in both sampling years.

As proposed by Ramos et al. (2014), we used the Pianka’s niche overlap index (Winemiller & Pianka 1990) to estimate the intensity of the interspecific phenological overlap, defined as the period of the year during which two species show a same phenophase:

$$O_{12} = O_{21} = \frac{\sum_{i=1}^{x} p_{2i} p_{1i}}{\sqrt{\sum_{i=1}^{x} (p_{2i}^{2})(p_{1i}^{2})}}$$

in which 1 and 2 represent the pair of species, $p_i$ is the proportional use of the resource by a species, here time, and x is the resource state, here the total number of observations by year (12). The index was calculated for each combination of two grasses, in each phenophase and sampling year, in the R package spaa (Zhang 2013). Entry data were matrices containing the number of individuals by species on each phenophase and sampling date. The index describe the annual period in which

We assessed annual variation and the effects of water regime and canopy cover on the phenology by means of the Watson-Williams test, which test the null hypothesis of a similar distribution between two data distribution. We performed these comparisons for each phenophase between the sampling years, sites subject to soil waterlogging (marshy and wet grasslands) and well-drained areas (savannas), and low (0-40%) and high (40-80%) canopy cover. Prior to estimate the effect of water regime on the phenology, we grouped the individuals from the savanna subject to soil waterlogging (Table 1 - Cc1) with those in grasslands. We performed these analyses in the software Oriana v. 2.0.1(Kovach 2004), using as entry values the percentage of individuals in each phenophase. Moreover, we compared the tiller production of each species between sampling years with the Wilcoxon paired rank test, also performed with the software R v 3.1.2 (R.Development.Core.Team 2014)

## 3. Results

One native (*L. chrisothrix*) and one exotic species (*U. decumbens*) occurred in most study sites, so we could observe the phenology of more individuals of these species (Table 1). Mortality was either very low (*T. leiostachya* - 7%; *A. bicornis* - 4%)

or absent (*L. chrisothrix*) among native grasses, whereas in *U. decumbens* (11%) it only occurred in grasslands. Likewise, *M. minutiflora* and *M. repens* had higher mortality in wet grasslands (35 and 53%) than in savannas (16 and 14%), although individuals of *M. repens* only died in the second year.

### 3.1. Distribution of phenophases

In general, all grasses exhibited new leafs and stems throughout both sampling years (Rayleigh test: $p>0.01$), indicating that vegetative growth occurred even during the dry season. The exception was *M. repens*, which had a lower stem production in the end of 2013 (Rayleigh test: $p<0.05$). In contrast, the annual distribution of reproductive stages was never homogeneous (Rayleigh test: $p<0.01$) (Figure 9-14). According to the mean vector length, *M. repens* had the most homogeneous distribution (Figure 11), followed by *U. decumbens* (Figure 9). In contrast, *M. minutiflora* and native grasses had more concentrated reproduction (Figures 10; 12-14), even though *L. chrisothrix* and *T. leiostachya* had longer fructification period (Figures 12-13).

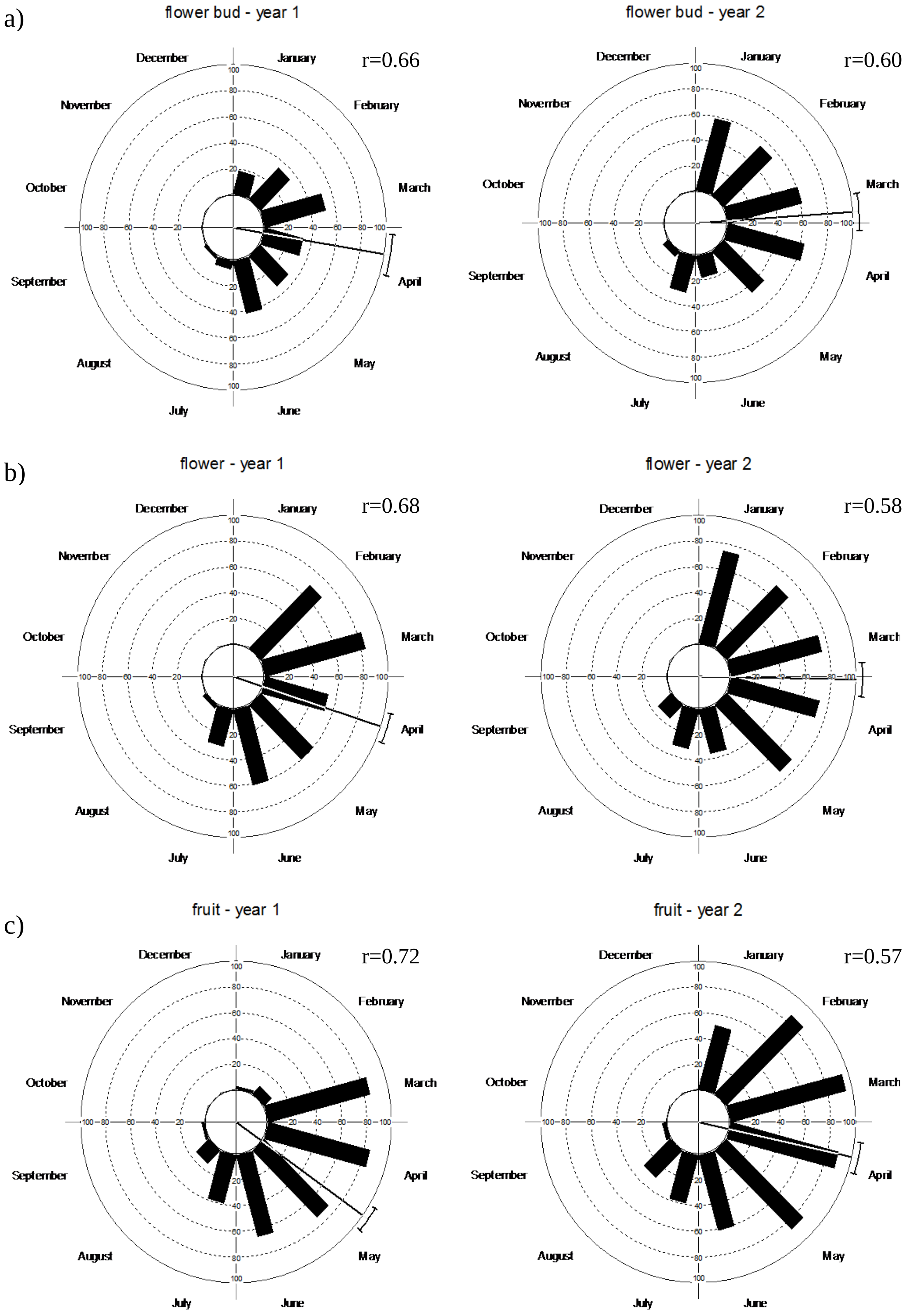


Figure 9 - Reproductive phenology of *Urochloa decumbens* in the two sampling years. Bars are the percent of individuals in each month, vectors are the mean period for each phenophase with confidence interval. r= lengh of the mean vector. a) Flower bud. b) Flower. c) Fruit.

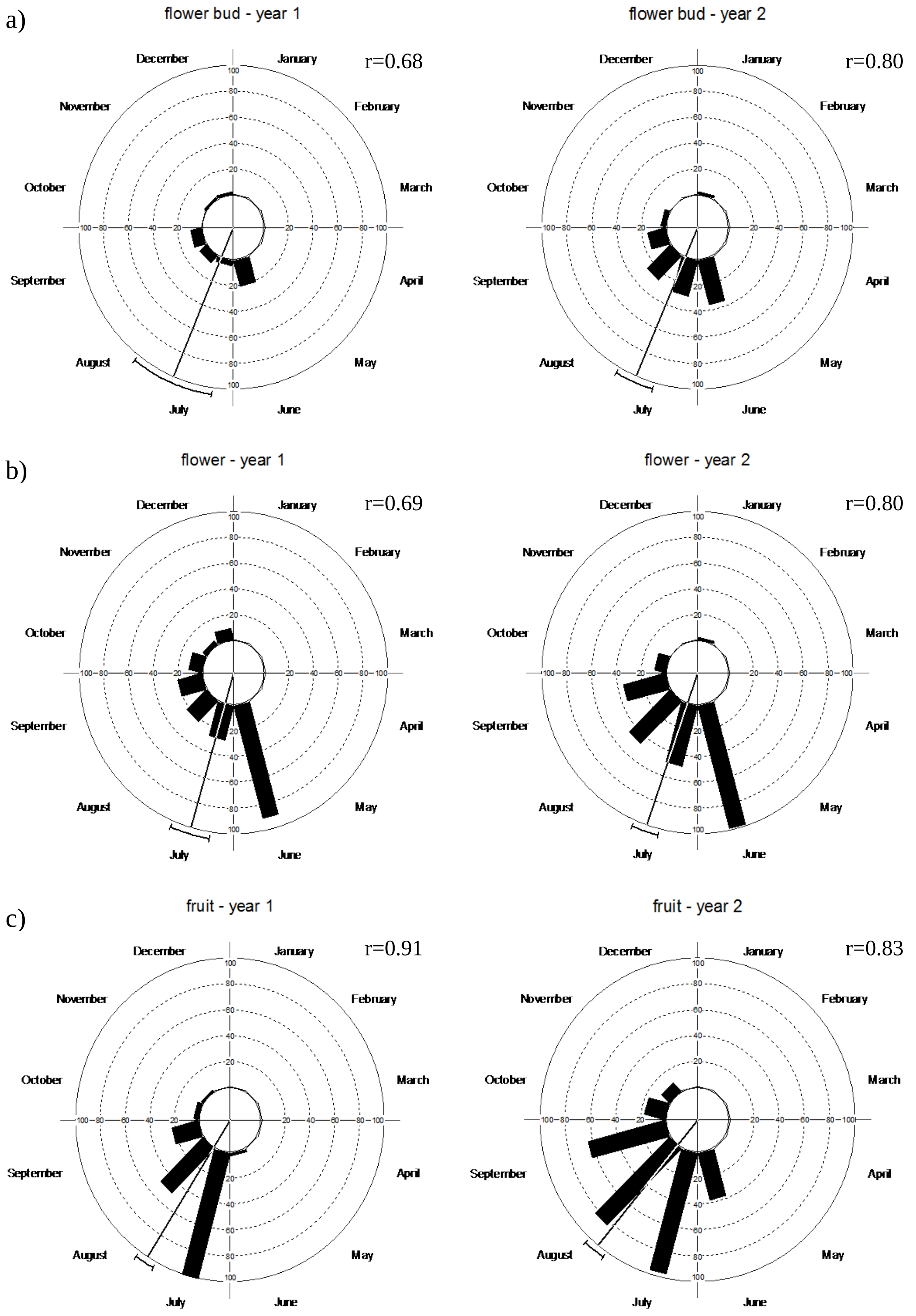


Figure 10 - Distribution of the reproductive phenology of *Melinis minutiflora*. Bars are the percent of individuals in each month, vectors are the mean period for each phenophase with confidence interval. r= lengh of the mean vector. a) Flower bud. b) Flower. c) Fruit.

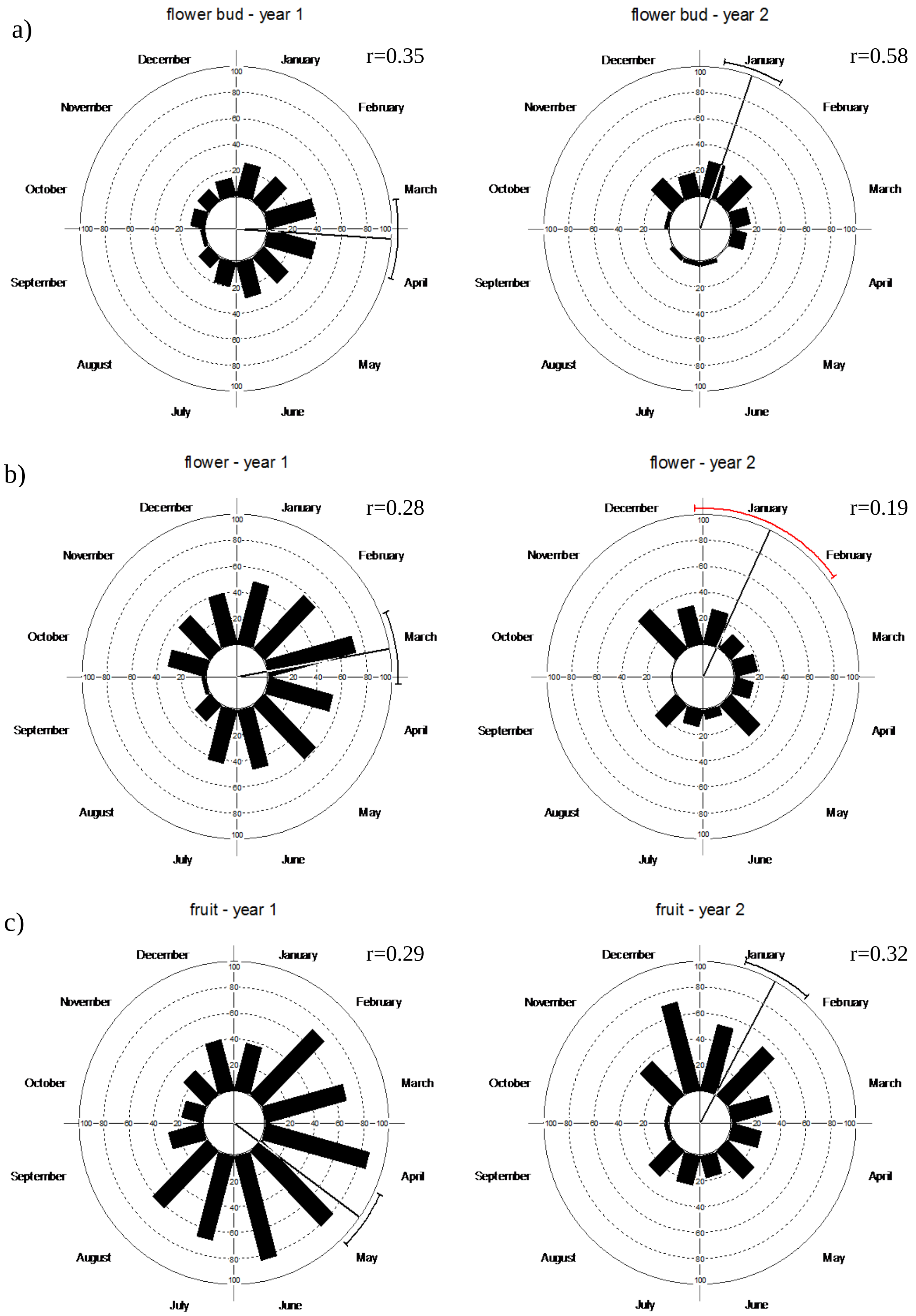
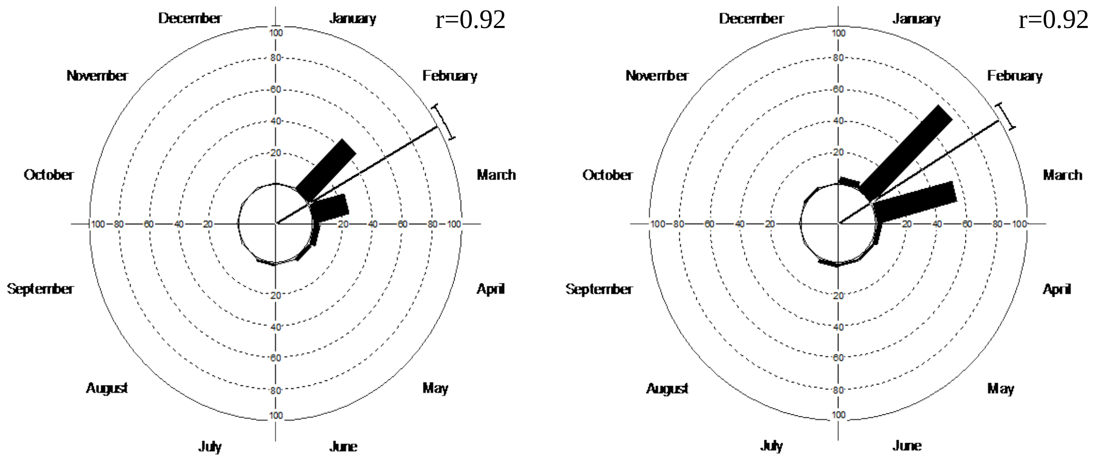
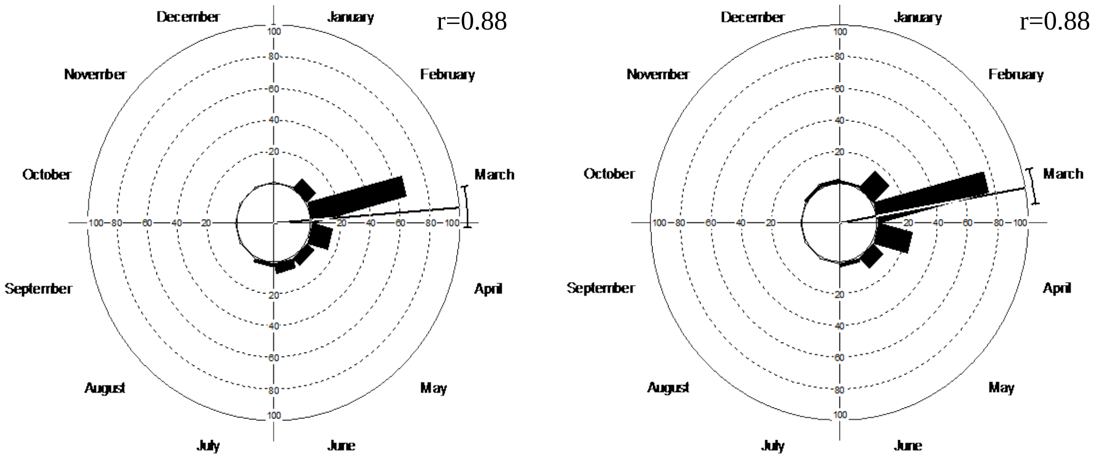


Figure 11 - Distribution of the reproductive phenology of *Melinis repens*. Bars are the percent of individuals in each month, vectors are the mean period for each phenophase with confidence interval. r= lengh of the mean vector. a) Flower bud. b) Flower. c) Fruit.

a) flower bud - year 1 flower bud - year 2



a) flower - year 1 flower - year 2



a) fruit - year 1 fruit - year 2

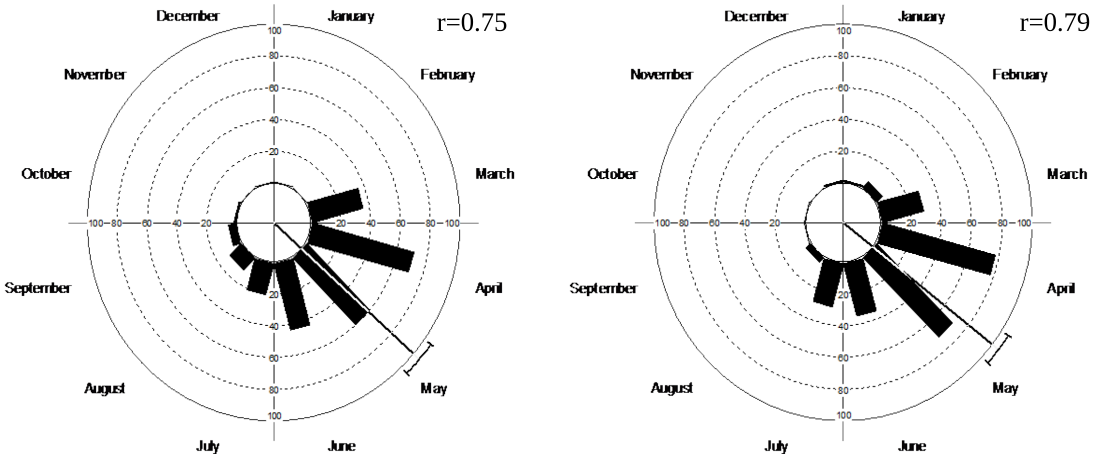


Figure 12 - Distribution of the reproductive phenology of *L. chrisothrix*. Bars are the percent of individuals in each month, vectors are the mean period for each phenophase with confidence interval. r= lengh of the mean vector. a) Flower bud. b) Flower. c) Fruit.

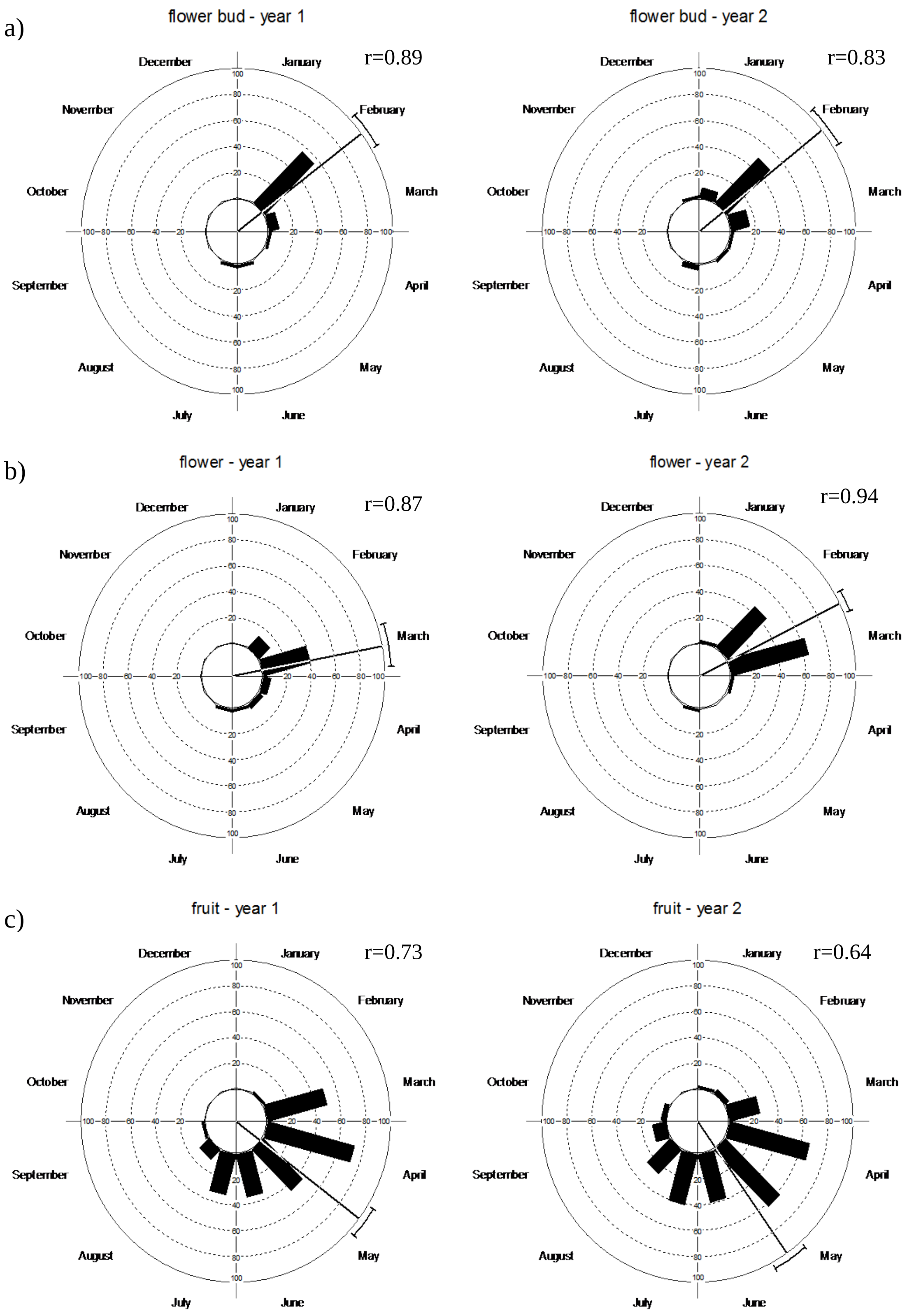


Figure 13 - Distribution of the reproductive phenology of *T.leiostachya* . Bars are the percent of individuals in each month, vectors are the mean period for each phenophase with confidence interval. r= lengh of the mean vector. a) Flower bud. b) Flower. c) Fruit.

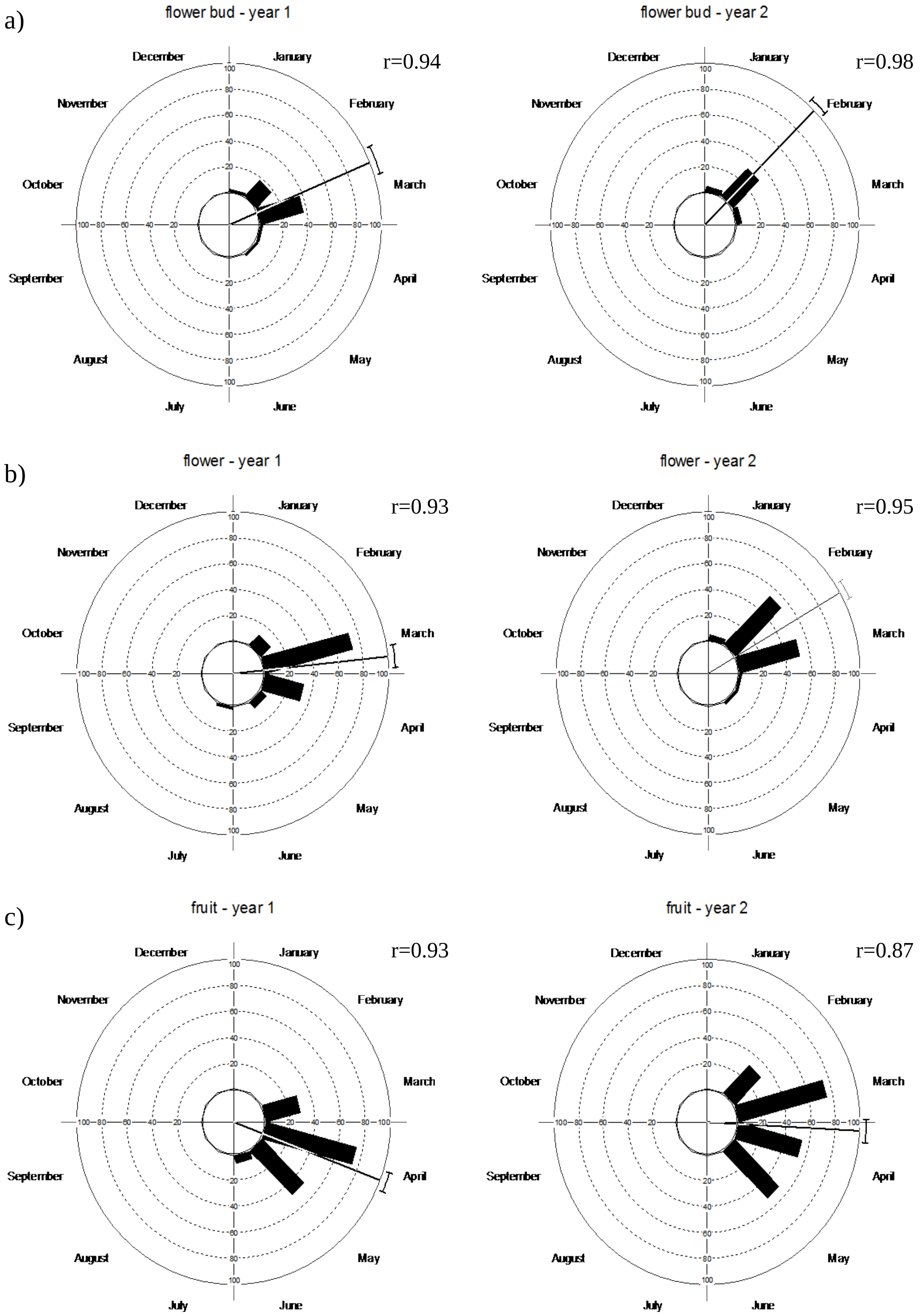


Figure 14 - Distribution of the reproductive phenology of *A. bicornis*. Bars are the percent of individuals in each month, vectors are the mean period for each phenophase with confidence interval. r= lengh of the mean vector. a) Flower bud. b) Flower. c) Fruit.

### 3.2. Phenological overlap

The values of the Pianka's niche overlap index between native grasses suggest a high phenological overlap, especially *T. leiostachya* and *L. chrisothrix* (Table 5). Likewise, these native grasses showed medium to high overlap with *U. decumbens* and *M. repens* (Table 5). In contrast, *M. minutiflora* showed very low overlap with native grasses, and low to intermediate overlap with other exotic grasses (Table 5). *M. repens* exhibited lower overlap with other species in the second year, probably because of its shorter reproductive period in this year (Table 5).

### 3.3. Temporal and environmental effects on phenology

Water regime and canopy cover had mixed effects on the reproductive phenology of exotic grasses, although most species showed annual variation (Tables 6; Figures 5-10). Both water regime and canopy openness had no effect in the mean phenological timing of *U. decumbens* (Table 6), whereas all reproductive phenophases started earlier and were longer in the second sampling year (Table 6, Figure 5). Nevertheless, this extension of the reproductive period was lower in individuals subject to higher canopy cover and positive water stress (Table 6). The response of *M. minutiflora* was mixed (Table 6, Figure 6). In the first sampling year individuals subject to positive water stress and higher canopy cover had significantly later fructification and flowering time, but this difference was lower or absent in the second year (Table 6). In the second year mean flowering and fructification time was early in grasslands under soil waterlogging and later in well drained savannas, whereas it was also later in low canopy cover but no difference occurred in higher canopy cover (Table 6). While the reproduction of *M. repens* was more homogenous in the first year, it was concentrated in the first six months in the second year (Figure 7). We could not quantitatively assess the effect of canopy cover on the phenology of *M.* repens, since most individual of this species occurred in low canopy cover (lower than 40%). Annual differences in tiller production were limited to a larger production by *U. decumbens* (Wilcoxon test: z=4.76, p<0.001) and a lower production by *M. repens* in the second year (z=3.95, p<0.001).

The effect of water regime effect could be only estimated for *L. chrisothrix*, since *T. leiostachya* and *A. bicornis* rarely occurred in grasslands and savannas, respectively. Likewise, canopy cover effect was not estimated for *A. bicornis*, as grasslands had no canopy cover. Nevertheless, both water regime and canopy cover had minor effects in the phenology of native grasses. In contrast, *A. bicornis* and in lower extension *T. leiotachya* reproduced slightly earlier in the second sampling year (p<0.01; Table 6), whereas we found no annual variation in *L. chrisothrix*.

Table 5 - Pianka's niche overlap index (0-1) calculated to all combination of two invasive and exotic species, based on the number of individuals in three different phenophases (bud- flower bud, flow- flower, fru-fruits) in two consecutive sampling years (year 1 –Nov/2011-Oct/2012. year 2 : Nov/2012-Oct/2013).

| | *U. decumbens* | | | *M. minutiflora* | | | *M. repens* | | | *L. chrisothrix* | | | *T. leiostachya* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | bud | flow | fru | bud | flow | fru | bud | flow | fru | bud | flow | Fru | bud | flow | fru |
| year 1 | | | | | | | | | | | | | | | |
| *M. minutiflora* | 0.53 | 0.43 | 0.26 | - | - | - | - | - | - | - | - | - | - | - | - |
| *M. repens* | 0.82 | 0.86 | 0.86 | 0.58 | 0.39 | 0.44 | - | - | - | - | - | - | - | - | - |
| *L. chrisothrix* | 0.79 | 0.74 | 0.97 | 0.22 | 0.09 | 0.22 | 0.65 | 0.63 | 0.83 | - | - | - | - | - | - |
| *T. leiostachya* | 0.79 | 0.77 | 0.98 | 0.45 | 0.06 | 0.31 | 0.65 | 0.64 | 0.84 | 0.80 | 0.98 | 0.97 | - | - | - |
| *A. bicornis* | 0.79 | 0.74 | 0.84 | 0.00 | 0.01 | 0.00 | 0.65 | 0.63 | 0.66 | 0.80 | 0.97 | 0.91 | 0.60 | 0.95 | 0.89 |
| year 2 | | | | | | | | | | | | | | | |
| *M. minutiflora* | 0.50 | 0.27 | 0.29 | - | - | - | - | - | - | - | - | - | - | - | - |
| *M. repens* | 0.78 | 0.67 | 0.70 | 0.78 | 0.28 | 0.27 | - | - | - | - | - | - | - | - | - |
| *L. chrisothrix* | 0.88 | 0.67 | 0.82 | 0.50 | 0.02 | 0.25 | 0.78 | 0.39 | 0.45 | - | - | - | - | - | - |
| *T. leiostachya* | 0.80 | 0.62 | 0.83 | 0.40 | 0.01 | 0.49 | 0.72 | 0.32 | 0.48 | 0.80 | 0.88 | 0.96 | - | - | - |
| *A. bicornis* | 0.61 | 0.64 | 0.86 | 0.20 | 0.00 | 0.00 | 0.55 | 0.34 | 0.51 | 0.61 | 0.79 | 0.78 | 0.65 | 0.98 | 0.71 |

Table 6 - Mean angle vectors (±length of the vector r) of three reproductive stages (flower bud, flower and fruit) in three African and three native grasses, followed during two consecutive years in two water regimes (seasonal waterlogging or water deficit) and two canopy cover categories (0-40 and 40-80%). Different capital and small letters indicate significant differences between water regimes in each sampling year and between sampling years in each water regime, respectively.

| species | stage | 2011/2012 | | | | 2012/2013 | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | waterlogging | | Water deficit | | waterlogging | | Water deficit | |
| *U. decumbens* | flower | 106±0.68 | A,a | 108±0.68 | A,a | 83±0.64 | A,b | 93±0.56 | A,b |
| | bud | 103±0.76 | A,a | 99±0.64 | A,a | 81±0.70 | A,b | 87±0.58 | A,b |
| | fruit | 122±0.77 | A,a | 127±0.72 | A,a | 101±0.69 | A,b | 103±0.53 | A,b |
| *M. minutiflora* | flower | 201±0.55 | A,a | 192±0.72 | A,a | 202±0.80 | A,a | 198±0.82 | A,a |
| | bud | 305±0.51 | A,a | 192±0.79 | B,a | 195±0.87 | A,b | 206±0.82 | A,b |
| | fruit | 219±0.88 | A,a | 208±0.84 | B,a | 207±0.93 | A,b | 224±0.84 | B,b |
| *L. chrisothrix* | flower | 85±0.85 | A,a | 86±0.88 | A,a | 83±0.83 | A,a | 77±0.91 | A,a |
| | bud | 60±0.93 | A,a | 62±0.89 | A,a | 59±0.90 | A,a | 58±0.92 | A,a |
| | fruit | 125±0.73 | A,a | 136±0.78 | B,a | 125±0.76 | A,a | 130±0.81 | A,a |
| | | 0-40 | | 40-80 | | 0-40 | | 40-80 | |
| *U. decumbens* | flower | 109±0.67 | A,a | 104±0.69 | A,a | 94±0.55 | A,b | 90±0.59 | A,b |
| | bud | 96±0.63 | A,a | 105±0.66 | A,a | 85±0.60 | A,b | 94±0.53 | A,b |
| | fruit | 126±0.70 | A,a | 126±0.76 | A,a | 106±0.50 | A,b | 97±0.65 | A,b |
| *M. minutiflora* | flower | 184±0.88 | A,a | 210±0.70 | B,a | 202±0.76 | A,b | 194±0.81 | A,b |
| | bud | 188±0.82 | A,a | 299±0.30 | B,a | 204±0.82 | A,b | 215±0.85 | A,b |
| | fruit | 207±0.95 | A,a | 221±0.87 | A,a | 212±0.89 | A,b | 226±0.86 | A,b |
| *L. chrisothrix* | flower | 87±0.87 | A,a | 85±0.91 | A,a | 78±0.95 | A,a | 74±0.87 | A,b |
| | bud | 68±0.83 | A,a | 55±0.98 | B,a | 59±0.90 | A,a | 57±0.96 | B,a |
| | fruit | 141±0.80 | A,a | 131±0.75 | A,a | 129±0.80 | A,b | 131±0.83 | A,a |
| *T. leiostachya* | flower | 61±0.88 | A,a | 87±0.86 | A,a | 65±0.95 | A,a | 75±0.93 | A,b |
| | bud | 49±0.92 | A,a | 60±0.85 | A,a | 48±0.77 | A,a | 56±0.94 | A,a |
| | fruit | 127±0.76 | A,a | 128±0.71 | A,a | 141±0.66 | A,b | 151±0.64 | A.b |

## 4. Discussion

In this study we addressed whether the phenology of African grasses is temporally distinct and more responsive to abiotic heterogeneity and annual variation in comparison with grasses from the cerrado. Our results showed high phenological overlap between native grasses, and between these species and the African grasses *U. decumbens* and *M. repens*. In contrast, reproductive phenology of *M. minutiflora* was temporally distinct from other species. All species were more influenced by annual variation than by spatial abiotic heterogeneity. As we expected, the phenology of African grasses was more responsive to environmental heterogeneity than in native species, and therefore may contribute to the success of African grasses in the cerrado.

Our data suggest that the reproductive phenology of *U. decumbens* is consistent with its superior performance in Neotropical savannas. As expected, *U. decumbens* displayed an early reproduction in both years; moreover, it also exhibited longer reproductive period compared to native grasses. We believe that this favors this species during the invasion process, both by means of priority effects and wider niche breadth (Cleland et al. 2007). Since early reproduction in the rainy season was found in congeneric species (Parihar & Pathak 2006), it could be a conservative trait on this genus. On the other hand, a long period of flowering and fructification as showed by *U. decumbens* is uncommon in perennial grasses from Neotropical savannas (Sarmiento 1992), and also did not occur in our study. Considering the high energetic costs often involved in reproduction, the longer reproductive period showed by *U. decumbens* must rely on high rates of primary production and efficient use of available resources, which had been reported for this and other African grasses (Williams & Baruch 2000). In turn, perhaps both the intensity of the vegetative growth and the amount of resources in the underground organs of *U. decumbens* is positively associated with the annual period of favorable water balace. Assuming that the extension of the reproductive period of *U. decumbens* is limited by resource availability, it could be longer in our sites of savanna, where the period of water deficit (Chapter 1) is shorter than other Neotropical savannas (Sarmiento 1984) and sites of cerrado in the center of Brazil (Amorim & Batalha 2006; Ramos et al. 2014). Whether these more suitable conditions support a larger seed production of *U. decumbens*, this could be reflected in higher spread by sexual reproduction in our study site.

Unlike *U. decumbens*, spread of *M. minutiflora* could be favored by its unusual reproductive period, especially in wet grasslands. As expected, here *M. minutiflora* showed a later reproduction than other grasses. Moreover, its reproductive period had no overlap with native grasses, and low overlap with other African grasses. A positive relationship between invasibility and the amount of vacant niches is a basic prediction in the general invasion theory (Davis et al. 2000). Consequently, the unusual reproductive period of *M. minutiflora* compared to the commonest native grasses could favor its presence in our study sites, as well as in other similar sites of cerrado. However, grasslands subject to soil waterlogging would be much more susceptible to this invasion for two reasons. First, according to our data *A. bicornis* and *L. chrisothrix*, the commonest species on these sites (Chapter 1), have a very similar reproductive period, which has no overlap with the period of *M minutiflora*. Second, *M. minutiflora* reproduced from June to September, when the water table is in the deepest level and soil moisture in grasslands is in enough level (Chapter 1), which is more suitable to the initial establishment of seedlings (Sarmiento & Pinillos 2001). In turn, *M. minutiflora* would spread along different sites, as a consequence of the large amount small anemocoric seeds produced (Martins et al. 2009).

On the contrary of the other African grasses, the reproductive strategy adopted by *M. repens* may be a barrier to its dominance in the cerrado. Here, *M. repens* reproduced almost continuously during the first year. Since *M. repens* is a prolific producer of windborne seeds (Stokes et al. 2011), which could generate large seed rain and support its spread by sexual reproduction in our study sites. Nevertheless, our data also showed that both vegetative and reproductive growth of *M. repens* decreased in the last six months of the next year, when many individuals died. Since the precipitation pattern was more favorable in this year (Figure 4), we believe that *M. repens* must be a short-term perennial in the cerrado. This type of response is common in ruderal species, which after establishing in favorable microsites quickly mature and invest heavily in seed production, having a short life-cycle (Grime 2001). In turn, exotic ruderal as *M. repens* are not expected to become dominant unless the local disturbance regime comprises large and frequent disturbance, which would support continuous establishment by seeds (David & Menges 2011). This limitation explains why *M. repens* in general is naturalized in more protected vegetation of cerrado and other areas subject to periodic disturbance, whereas it is an invasive species on highly disturbed sites, especially those shaped by human activity (David & Menges 2011). In addition, our data suggest that this species is even less likely to occur in grasslands subject to positive water stress, where many individuals of *M. repens* died before two years of observation. The stress caused by water saturated soils may constrain the primary production due to the lack of specific adaptation to anoxic or hypoxic soil conditions (Rubio et al. 1995). Consequently, in these sites likely occurs a trade-off, in which *M. repens* could respond to the resources shortage by investing in reproductive growth to the detriment of the extension of the individual life cycle. That could be the most expected response for a ruderal species (Grime 2001), even though we could not test this hypothesis here since our marked individuals could not belong to the same cohort.

On the other hand, a phylogenetic constrain may have emerged from the comparison between *L. chrisothrix* and *T. leiostachya*. These species have a close evolutionary history and share a number of morphological features (Alvarez et al. 2008), although they differ in size and appear to have contrasting environmental requirements (Chapter 1). As flowering time is often shared by many related species, it is considered a conservative trait subject to weak selective pressure or neutral selection (Ollerton & Lack 1992). Nevertheless, the adoption of different ecological strategies by congeneric species, such as *M. minutiflora* versus *M. repens,* could drive the selection of contrasting reproductive phenology(Ollerton & Lack 1992). Irrespective of the major causes of the little temporal differences in the phenology of native grasses, the reproductive period of all these species was completely overlapped with that showed by *U. decumbens* and *M. repens*. This overlap may negatively affect the persistence of native grasses in sites invaded sites by *U. decumbens*, since in mesic conditions it produces a large amount of biomass(Williams & Baruch 2000), that may shade reproductive individuals of other species.

While lower reproductive activity of *M. repens* in the second year may emerge from its ecological strategy, early and longer reproduction of most species highlights that phenological changes in grasses may be induced by specific abiotic signals. While the phenology of temperate grasses is, in general, controlled by a combination of temperature and photoperiod (Cleland et al. 2007), in seasonally dry ecosystems the precipitation pattern often has major influence (Chambers et al. 1999). Here, in the second year, mean temperatures in the first months of the rainy season were about $2^{o}$ C higher than in the previous year. Our results therefore are consistent with the early flowering dates that have been associated with the global warming (Cleland et al. 2007; Chambers et al. 2013). Moreover, we also believe that the high precipitation (165 mm) in the middle of the dry season in the first year may have contributed to an early reproduction in the next year. While perennial grasses often maintain low vegetative growth in the dry season because of the water deficit (Sarmiento 1992), this high precipitation would have allowed higher vegetative growth during this period. Consequently, enough resources for reproduction could be achieved early in the rainy season. This hypothesis is supported by the earlier and longer reproduction of *U. decumbens* in the second sampling year, which resulted in higher tiller production. Since this species has a decumbent growth form, in which apical inflorescences emerge from all stems, as well as high efficiency in the use of resources (Williams & Baruch 2000), more suitable conditions could promote higher biomass production, and in turn higher reproductive output. Likewise, in the second year the reproduction was in a lesser extent anticipated in *A. bicornis*, as expected since this species is typical of wetter environments (Chapter 1). In contrast, these responses to annual fluctuation may be less important to *L. chrosothrix* and *T. leiostachya* (Alvarez et al. 2008), in which were reported mechanisms to overcome the water deficit, and *M. minutiflora*, which apparently also responded to spatial abiotic heterogeneity in the water regime.

Differently from what we expected, the phenology of both native and African grasses was only weakly affected by spatial variability in the water regime and canopy cover. *M. minutiflora* was the only species that showed plasticity to differences in the water regime. Previous studies have found mixed phenological responses of grasses to spatial variation in water availability (Jentsch et al. 2009, Dyer et al. 2012). For example, in a experimental study with temperate annual grasses the response was species-specific and related to the precipitation predictability (Aronson et al. 1992).

Unlike the water regime, the canopy cover was firstly associated with changes in the plant reproductive vigour (Wan & Sosebee 1998), such as found in some of our species (Chapter 1). However, it had minor influence on triggering or on the length of the reproductive period of the studied grasses. In our study, *M. minutiflora* responded to the higher precipitation in the second year with a slightly later and longer reproductive period, but only in well drained savannas and in lower canopy cover. We believe that this occurred because the photosynthesis in high canopy cover and soil waterlogging would be limited by light quality (Wan & Sosebee 1998) and low oxygen in the soil (Končalová 1990), respectively, rather than low water availability. In contrast, the absence of response in *U. decumbens* is unexpected, considering that the phenology of this species apparenty responded to a more favorable precipitation pattern. While this could suggest that this species has lower phenotypic plasticity, it is also possible that phenological adjustment to stress had minor importance, since positive water stress and high canopy cover had extensive effects on this species (Chapter 1). Likewise, we believe that the higher tolerance of *L. chrisothrix* to positive water stress and close canopy cover (Chapter 1) matches the stability on the phenology of this species, which could be resulted of a long term evolutionary adjustment.

While our data allow only indirect inferences about the mechanisms behind the phenological responses of African grasses, the high phenological overlap between native and the two African grasses is a clear and important result of this study. This phenological similarity contrast with the temporal differences in the reproductive period often found between perennial grasses in Neotropical savannas (Sarmiento 1992; Ramos et al. 2014). This unexpected result may be associated with two major factors. On the one hand, here we compared a small number of native grasses, although these species may be locally dominant. Despite that *L. christhrix* is a widespread species in our sites, other native grasses are either common in few sites or rare species in a wider range of conditions (personal observation). The best experimental design to address phenological overlap and how it is related to niche sharing should include even theses less common species. Nevertheless, this would be difficult here because of the rarity of these species, and also considering that the other goals of this study required a great sampling effort on each target species.

## 5. Conclusion

The results of this study show that the reproductive phenology of *U. decumbens* and *M. minutiflora* is distinct of common native grasses, and contributes with their success as invaders in the cerrado, whereas the ruderal reproductive pattern of *M. repens* corroborates with its status of naturalized species. Moreover, native grasses showed a higher phenological overlap compared to exotic grasses, which increases the amount of unused resources and the invasibility of the community. The phenology of both native and exotic grasses responded weakly to spatial variability in canopy openness and soil waterlogging, but interaction between high efficiency in the resources use and phenological plasticity to temporal variation in precipitation contributes to the high invasiveness of *U. decumbens*, and in lesser extent *M. minutiflora*. This last ability is also important because the climatic changes in the future must favor species that respond more efficiently to temporal variation in key abiotic variables. In contrast, higher stability in the phenology will favor less competitive native grasses whether global changes increase water shortage in the cerrado. Our study suggests that phenology timing is important for the success of African grasses in the cerrado. Accordingly, management should also incorporate phenological information to achieve a more effective control of these species.

# Chapter 3 –Distribution of exotic and native grasses across microhabitat heterogeneity and environmental gradients in Hawai’i

# ABSTRACT

While biotic homogenization impacts native species, it could also shape novel communities dominated by exotic species. Many attributes that contribute to the dominance of invaders and increase invasibility of communities have been identified (eg. resources use and dispersion efficiency), but have rarely been considered as they interact to drive the dominance of co-occurring invaders across heterogeneous ecosystems. Here we assessed the presence of five overlapping exotic and one native C4 perennial grass across local (microhabitat) and regional (geology, disturbance regime and climate) scale variation in young volcanic sites on Hawai'i Island.  We obtained percent cover, individual height and leaf C : N rate of each species in 15 sites with different fire history and lave flow age across altitudinal and precipitation gradients, and estimated microhabitat heterogeneity from the rock cover and soil depth in which each species established. We estimated the effects of local and regional variables in Chi-square tests and Generalized Additive Mixed Models, and assessed biotic interactions comparing the performance between pairs of co-occurring species. Tolerance to harsh conditions in poorly developed soils, higher competitive ability, and nutrient release after fire explain the prevalence of *M. minutiflora*. Undisturbed sites favor an inferior competitor (*S. condensatum*) in deeper soils and stress tolerant species (*A. virginicus*) in shallower soils, whereas higher temperatures and lower rainfall in low elevation sites favor both a widespread opportunistic species (*M. repens*) and a water deficit tolerator (*H. contortus*). While only the avoidance of fire and new introductions will constrain further spread of exotic grasses, the suppression of less common exotic grasses (e.g. *H. rufa*) and control of early invasions by *M. minutiflora* are reliable restoration strategies.

Keywords: *Melinis minutiflora*, *Andropogon virginicus*, stress, Hawai'i, soil depth, fire.

## 1. Introduction

The increasing research effort related to understanding biological invasions has generated a large amount of information about the impact of individual non-native plant species on native species in environments where they have become dominant (Vilà et al. 2011). However, the distribution of many of these invaders across local environmental gradients are little studied, as well the conditions under which they could rise to dominance, remain rare or be part of a mixed species assemblage. Ecosystems with high invasibility or that have been subjected to a long-term history of species introduction and disturbance often contain many co-occurring non-native species, whose dominance likely shifts across gradients of environmental stress and disturbance and as invaders interact with each other. Despite the prevalence of multiple invaders within many habitats, few studies have looked quantitatively at interactions among these species or how related invaders might distribute themselves across environmental gradients. For example, in Californian grasslands numerous species of annual grasses from Europe co-occur in individual sites, with dominance likely shifting across local and regional environmental gradients e.g.(Jackson & Bartolome 2002). Likewise in Hawai'i, many species of exotic C4 grasses can be found at a single site (Hughes et al. 1991; Daehler & Carino 1998; D'Antonio et al. 2001). It is recognized that individual species dominance changes with the abiotic environment (D'Antonio et al. 2000; Daehler 2003) and depending on the result of competitive interactions (e.g. (Rice & Nagy 2000).

Interactions between exotic species are expected to occur where multiple similar invaders are present (Vitousek et al. 1987a). However, similarly to communities of native species, the role of these interactions in determining species dominance will depend on both regional environmental factors, such as precipitation, temperature and disturbance history, and more local factors such as microhabitat soil conditions.

Likewise, it will depend on the phenotypic plasticity and hence environmental tolerance of the invader species.

Meta-analyses have identified recurrent traits in invasive plant species, which include rapid growth, high productivity and effective spread (Van Kleunen et al. 2009). These traits suggest that successful invaders largely rely on good growing conditions such as that found after disturbance. Yet many invaders also display great phenotypic plasticity (Richards et al. 2006; Zenni et al. 2014), which may allow them to exist within but not dominate sites that are more stressful or where they cannot compete well against other invaders. Invaders that are weak competitors may dominate only in repeatedly disturbed sites, highly stressful sites or microsites where stronger competitors cannot gain dominance. By contrast, invaders that are good competitors in rich conditions may be present but rare where conditions are stressful.

The Hawaiian Islands are characterized by strong environmental gradients and relatively consistent chemistry of parent material, thus allowing evaluation of the role of climate variables in explaining variation in species distributions (Vitousek 2004). They also have weak resistance to invasion (Vitousek et al. 1987a) and a long history of human-driven disturbance, particularly at lower elevations (Cuddihy et al. 1990). As a result, vegetation at low and middle elevation is dominated by non-native species (Vitousek et al. 1987a). Exotic perennial grasses utilizing the C4 photosynthetic pathway are abundant across the Hawaiian Islands, particularly in dry and mesic sites with low to mid elevation (Wagner et al. 2012). The relative distributions of these grasses compared to native C4 and C3 grasses has been well studied (Edwards & Still 2008; Pau & Still 2014), and it has been demonstrated that grass distributions respond to both moisture and temperature gradients (Edwards & Still 2008; Angelo & Daehler 2013). Several studies also point out that many of these species respond positively to the occurrence of fire (Hughes et al. 1991; D'Antonio et al. 2011). In addition, in Hawai'i microhabitat conditions may also play an important role in influencing the performance and distribution of invading C4 grass species has been poorly studied. Because of intrinsic spatial variability in the process of soil formation, landscapes shaped by recent lava flows display a high environmental heterogeneity even at very small spatial scales, where only a some few 'safe sites' containing soil offer suitable growth conditions (Cutler et al. 2008). Under such heterogeneous conditions, local abiotic factors such as soil depth and rockiness could shape which species becomes dominant, especially early in primary succession where soil development is generally poor and site conditions are often harsh (Cutler et al. 2008). Biotic interactions between introduced exotic grasses are also expected to occur even within these unsuitable sites (Tirado & Pugnaire 2005).

Here we assess the distribution of five exotic and one native C4 perennial grass across local and regional abiotic variation in young volcanic sites on Hawai'i Island. The grass species included: (1) two species (*Melinis minutiflora* [P. Beauv] and *Schizachyrium condensatum* [Kunth] Nees) that have been well studied because of their ability to dominate certain mesic habitats and negatively affect native species (Hughes & Vitousek 1993; D'Antonio et al. 1998), (2) two widespread species (*Melinis repens* [Willd.] Zizka and *Andropogon virginicus* [L.]) that seem to have weaker ecological influence, and (3) two currently restricted species one of which is native, *Heteropogon contortus* (L.) P. Beauv, and the other ((*Hyparrhenia rufa*) (Nees) Stapf) is an aggressive invader of Neotropical habitats elsewhere (Pivello et al. 1999a; Williams & Baruch 2000). We sampled grass species occurrence, percent cover and height across an elevation and rainfall gradient, different soil ages and contrasting fire history to evaluate their ability to occupy habitats that vary in their favorability for plant growth.

We expect that the grasses with higher competitive ability, such as *M. minutiflora, S. condensatum* (D'Antonio et al. 2001) and *H. rufa* (Daubenmire 1972) require more suitable conditions, hence should be restricted to, or obtain the greatest cover in sites with deeper soils and higher rainfall (Grime 2001). In contrast, poorer competitors that are stress tolerant species (e.g. *A. virginicus*) should occur across the range of sites, but are expected to be more common under a combination of shallow soils, higher temperatures and lower precipitation, where the performance of competitively superior species is constrained (Grime 2001). Finally, ruderal species (*M. repens*) could be more abundant in more recently disturbed sites or in extremely shallow soils, where other grasses cannot successfully colonize (Grime 2001). We specifically aimed to answer the following questions: i) How does microhabitat condition correlates with occurrence and percent cover of each grass? ii) What is the relative importance of broader-scale environmental variables, including rainfall, elevation, and fire history to each species? and iii) Are some grasses limited to more shallow soils when co-occurring with more competitive species ?

## 2. Material and methods

### 2.1. Study site

We performed this study in Hawai’i Volcanoes National Park (hereafter HAVO), located in the south of the island of Hawai’i, the youngest and largest in the Hawaiian Islands archipelago, USA. Since HAVO includes the currently active Kilauea Volcano, there is much area dominated by open lava and poorly developed soil. This scarcity of substrate constrains the establishment of most vascular plants, and the low amount of N in the soil is a major limiting of plant growth, especially in undisturbed sites (Vitousek et al. 1987b). In the Park there are also altitudinal, temperature and rainfall gradients, ranging from more than 3000 mm of annual rainfall in colder montane sites to around 1000 mm in some sites in the warmer coastal lowlands. The Park was established in 1916, and exotic grass species were reported since the first botanical surveys. However, the major spread of these species occurred after 1975, when feral goats were eliminated (Katahira & Stone 1982). The dominance by these species also caused an increase in fuel continuity and understory biomass. Previous studies associated this higher biomass with the increased fire frequency in the Park, which is believed to favor the dominance of C4 grasses to the detriment of native shrubs and trees (Tunison et al. 2001; D'Antonio et al. 2011).

### 2.2. Focal Species

Sites dominated by *M. minutiflora* in HAVO may be dense, high-biomass, monospecific stands (D'Antonio et al. 2001). The spread of this species has been shown to be positively associated to fire and with fertile soils (Hughes et al. 1991; D'Antonio et al. 2001) (Figure 15).  Its congener, *Melinis repens* (Willd., Natal redtop) is generally more rare in HAVO (personal observation) (Figure 15). *Hyparrhenia rufa* (Nees, jaraguá or thatching grass) is another African perennial bunchgrass, which can grow up to 3 m tall (Daubenmire 1972).  It is a highly fire tolerant species and an aggressive invader in Neotropical savannas (Pivello et al. 1999a; Williams & Baruch 2000), but is less common at the Park (Figure 15). *Schizachyrium condensatum* (Kunth, bush beardgrass) is a 1 to 1.8 m erect bunchgrass native to tropical America, from Mexico to Argentina (Peichoto 2010), which like *M. repens* only reproduces by small and easily wind-dispersed seeds (Peichoto et al. 2008). D’Antonio et al. (1998) demonstrated that it can be competitive against native species, and also that it can outcompete *M. minutiflora* through priority competition in some sites (D'Antonio et al. 2001) (Figure 16).  *Andropogon virginicus* L. (broomsedge) is a perennial bunchgrass native to the southeastern USA, where it may be a persistent dominant species across a range of sites

(Rice 1972). It has become established out its native range in US and other continents, and has been shown to be tolerant to edaphic stress (Ning & Cumming 2001; Ezaki et al. 2008). It is believed that all these species occurred in the region before the Park was established (Figure 16). The most common native species in HAVO is *Heteropogon contortus* L. (pili grass or tanglehead), a pantropical perennial C4 grass (Williams & Black 1994). Currently, in Hawai'i it only occurs in the coastal zones with warmer temperatures (Figure 16), but it is believed that it had a wider distribution now limited by the competition with C4 invasive grasses (Daehler & Carino 1998).

### 2.3. Site selection

Our study sites were entirely located within the coastal lowlands and submontane climatic zones, and were focused along two main roads, (1) Chain of Craters Road, and (2) Hilina Pali Road, which follow the South and East Rift Zone of the Kilauea Volcano, respectively. Sites were selected in consultation with the Resources Management Division of HAVO on the basis of including a wide range of lava flow ages, fire frequency and annual rainfall. We also based the site selection on the possible presence of *Melinis repens*, since this species has a wide but also the most scattered distribution among the C4 grasses. We avoided sites with higher rainfall and elevation in order to control light availability because as rainfall increases above 2000 mm, the density of the dominant tree *Metrosideros polymorpha* increases greatly and grasses become restricted to roadsides. We controlled for substrate type by only sampling sites from lava flows of pahoehoe (smooth surface), because substrate from 'a'a (rough surface) was shown to follow a different pattern of colonization (Aplet et al. 1998), is more difficult to find across the gradients sampled, and is physically difficult to sample. In addition, we fixed the minimum distance between sites as 200m, as well a minimum distance to roads of 30m. Based on these assumptions we selected 15 locations (Table 7). We obtained the soil age, fire frequency and annual precipitation in each site from maps provided by the Resources Management Division of HAVO.

### 2.4. Sampling methods

Since we expected high spatial heterogeneity inside each site due to the complex topography, we generated 8 random GPS coordinates for each site (Figure 17), so that they were at least 20m but no more than 100m apart from each other. In each of these locations we established eight 1 x 1 m adjacent plots placed along alternate sides of an 8m transect oriented from east to west (Figure 17). We visually estimated the percentage cover of all species within each plot, as well the area covered by rock. We also measured the height of the tallest individual of each species, and identified the substrate type (cinder (volcanic debris) or ash soil). In each 1 x 1 m plot we used a 3mm diameter pointed steel rod to measure the soil depth in 6 evenly spaced points along the plot diagonal irrespective of species present. We then also measured the soil depth as close as possible to the base of 6 individuals in each plot, to take into account the high level of small-scale heterogeneity in soil depth. We selected these individuals to represent all species with at least 1% cover, but we sampled in proportion to their cover so that species with high cover had more measured individuals. Our goal was to characterize the soil depth for each species compared to an average depth across the plot.

We randomly selected half of the sampled plots to conduct leaf sampling for % C and N. In each plot we removed one leaf of three individuals of *M. repens* if it was present (to have one consistent species across sites) and of the dominant C4 grass; we only collected fully expanded leaves without visual indications of herbivory or senescence. We also obtained information on the substrate type and measured the soil depth beneath each sampled individual. Each leaf sample was ground to a fine powder with a Wig-L-Bug Amalgamator (Crescent Dental, Utah), and the percentages of C and N were obtained using a Fison's Carlo Erba NA 1500 Nitrogen Analyzer (Fisons Instruments, Saddlebrook, New Jersey, USA).

Figure 15 –African grasses in sites originated from 750-3000 years old lava flows in the submontane zone of HAVO. up –*M. minutiflora*. center– site dominated by a mixed community of *M. minutiflora* and *M. repens*. bottom- path dominated by *H. rufa*.

Figure 16 – One exotic (up- *A. virginicus*) and one native grass (center – *H. contortus*) in low elevation sites originated from 250-750 years old lava flows in HAVO, and the exotic *S. condensatum* (bottom) in a higher elevation site with 750-3000 years old soil.

Table 7 –Environmental characteristics of each of the 15 study sites. Data on rainfall are the mean annual precipitation. Fire events were from NPS records where N=no recorded fires since Park founding (1916).

| Study site | elevation (m) | soil age (years) | Substrate type | rainfall (mm) | Climatic zone | fire events |
|---|---|---|---|---|---|---|
| kipukend | 0 | 400-750 | ash | 1300 | coastal lowlands | N |
| kipbrown | 30 | 400-750 | ash | 1225 | coastal lowlands | N |
| kiproad | 90 | 1500-3000 | ash | 1350 | coastal lowlands | 1970/1999 |
| kipandr | 120 | 400-750 | ash | 1375 | coastal lowlands | N |
| harpim | 360 | 400-750 | ash | 1775 | seasonal | 1972 |
| lastchain | 457 | 400-750 | ash | 1975 | seasonal | 1972/1981/1992 |
| kipkau | 610 | 1500-3000 | ash | 1525 | seasonal | 1987 |
| down | 670 | 400-750 | ash | 1600 | seasonal | N |
| shelter | 670 | 750-1500 | ash | 1600 | seasonal | 1987 |
| lastkipuk | 680 | 200-750 | cinder | 1975 | seasonal | 1969 |
| oldaa | 700 | 750-1500 | ash | 1575 | seasonal | 1987 |
| midpali | 790 | 750-1500 | ash | 1800 | seasonal | 1987 |
| kippali | 850 | 400-750 | ash | 1775 | seasonal | 1987 |
| befnene | 880 | 750-1500 | ash | 1800 | seasonal | 1970/1987 |
| begpali | 970 | 750-1500 | cinder | 1650 | seasonal | N |

Figure 17 – One of the eight 8 m transects placed in each study site (up), and view of one 1 x 1 m plot where we estimated the percent cover of exotic and native grasses, divided by a line along of which we obtained evenly spaced soil depth measurements (bottom).

## 2.5. Data analysis

We divided abiotic variables into two types based on the spatial scale over which they were obtained. Continuous soil depth and rock cover data obtained for each plot were called local variables, whereas broader scale data were obtained for each site based on available maps were called regional variables. Many of our study sites had the same values for two or more of these regional variables, so that we used categorical values for regional variables with two levels per variable.  Fire frequency was categorized as, fire versus no-fire, soil age as 200-750 versus 750-3000 years, rainfall as 1200-1600 versus 1600-2000 mm/year and elevation as 0-500 versus 500-1000 m. We adopted this simplified approach in order to have a similar number of study sites within each level of each regional variable and because sites tended to fall out in a bimodal fashion for these variables.  In addition, this elevation threshold matches with the division between climate zones in Hawaii, coastal lowlands and submontane zone.  Most of our study sites burned during volcanic eruptions early in the 70’s, so no-fire refers to sites that did not burn in the last 40 years. Previous studies have found a stable presence of invasive grasses in burned sites even many decades after fire occurrence, suggesting that it left a positive legacy for these species(D'Antonio et al. 2011).

### 2.5.1. Distribution of C4 grasses

We tested the null hypothesis of independence between the presence of C4 grasses and local and regional variables by means of a Pearson's Chi-squared Test with Yates' correction for continuity. Expected values were the total number of plots where each species was found multiplied by the proportional number of plots sampled on each category. Contingency tables were obtained for each species occurrence as it related to fire, soil age, annual rainfall and elevation assuming the same levels specified before.

We addressed the effect of the soil depth on the presence of each C4 grass dividing the sampled plots in four categories:  very shallow soil (0 – 5 cm depth), shallow soil (5-10 cm depth), intermediate depth soil (10-20 cm depth) and deep soil (>20 cm depth). We based these categories on the response of grass species to soil depth, but also to assemble a similar number of observations within each category. In this analysis, we used the random soil depth measurements on the plot instead of those taken at the sampled individuals.

#### 2.5.2. Performance of C4 grasses

We assessed the effects of soil depth and regional variables in the performance of grasses in a Generalized Additive Mixed Model (GAMM), having as dependent variables the percent cover or maximum height of each species in each 1 x 1 plot. Random effects related to variation between sites could not be estimated in a model including both local and regional abiotic variables because sites are not nested in all the levels of regional variables  (Zuur et al. 2009). This situation is common in landscape data, in which is difficult to find study sites subject to the same combination of environmental variables, and a number of methods have been used to overcome this problem, such as autoregressive and auto-correlation methods that incorporate the spatial structure between locations (Zuur et al. 2009). Here, in order to perform models including all variables without consider them as independent, we incorporated in the GAMM model a spherical spatial autocorrelation error structure (corRSpher function) available in the R package nlme (Pinheiro & Bates 2000). Distance between all plots was obtained from actual GPS coordinates taken on each sampling plot.

In our GAMM model fixed effects related to soil depth and rock cover were estimated by means of cubic spline regression with non-parametrical smoothing functions (Wood 2011). Different values of soil depth were used depending on the response variable being predicted. When the variable was percent cover of each grass species, we used as soil depth values the mean soil depth from locations where individuals of this species were established. In contrast, when the response variable was maximum height, we used the soil depth measured for the tallest individual on the plot. The effect of regional variables was assessed by a parametric linear mixed function. We ran all GAMM models in the gamm function in the R package mgcv (Wood 2011), which incorporates the lme function from the nlme package (Pinheiro et al. 2014) to estimate the parametric fixed effects. The overall model explanation was based on that provided by both parametrical and non-parametrical components.

We also used GAMM models to assess the relationship between the leaf C:N ratio and the percent cover and individual height of the C4 grasses, which was the average of eight plots sampled on each transect. We also assessed the effect of local and regional variables on leaf C : N ratio, in which the soil depth was the mean from the three individuals of each species whose leaves were removed in each transect. In this analysis we only used the plots where data on C and N leaf content were available for each species.

### 2.5.3. Interspecific variation in soil depth requirement

We used a Linear Mixed Model to compare the interspecific variation in the difference between the soil depth found beneath each species versus that randomly obtained on each 1 x 1m plot. In the model we estimated fixed effects related to the C4 grasses and random effects associated with the study sites, by means of the function lmer on the R package lme4 (Bates et al. 2014). When fixed effects were significant, we applied corrected pair-wise comparison between all species in the R package multcomp.

All tests were performed in the R environment v. 3.1.2 (R.Development.Core.Team 2014).

### 2.5.4. Co-occurrence between C4 grasses

We identified all plots where each C4 grass species co-occurred with each of the other grasses in the 15 sampled sites. Hereafter, we compare the cover and mean soil depth under individuals between two C4-grasses occurring in the same 1 x 1m plot by means of a Wilcoxon paired rank test. We performed independent tests for each pair of species to each level of fire frequency, soil age, elevation and annual rainfall. A Holm-Bonferroni correction of the p-values was used for each combination of species, in order to avoid α error associated to the high number of comparisons. All tests were performed in the R. environment v. 3.1.2. (R.Development.Core.Team 2014).

## 3. Results

### 3.1. Presence/absence & local and regional variables

The C4 grass species occurred in over 95% of the sampled plots. Both *M. minutiflora* and *M. repens* occurred in about 60% of the plots, whereas *Andropogon virginicus* occurred in 40%. In contrast, *S. condensatum* and *H. rufa* only occurred in 20% and 7% of the samples, respectively. The single native grass found was *Heteropogon contortus*, which occurred in four sites and 13% of the plots (Table 8).

The Chi-square tests suggest that different combinations of local and regional abiotic factors favor the presence of different C4 grasses. *M. repens* occurred in more plots in lower annual rainfall and lower elevation than would be expected based on the proportional number of plots sampled on these conditions, but its presence was indifferent to soil age, soil depth and fire (Table 8). *M. minutiflora* and *S. condensatum* were more common than expected with higher rainfall, but had mixed responses to the other factors. *M. minutiflora* and *S. condensatum* were more frequent in plots at higher elevation, more rainfall and deeper, older soil. However, occurrence of *M. minutiflora* was strongly favored by fire, whereas it only weakly favored *S. condensatum* (Table 8). *A. virginicus* was more common than expected in unburned areas and also was favored by higher rainfall but younger soils at lower elevation. Although the occurrence of *H. contortus* was independent of soil depth, it occurred exclusively in lower elevation and lower annual rainfall, and was more common in younger and burned plots. *H. rufa* only occurred in higher elevation plots, but was much more common in deep soils, as well as sites that had not burned (Table 8).

Table 8 – Number of plots where each C4 grasses was found within 15 sites in Hawai'i Volcanoes National Park, divided by soil age, fire occurrence, annual rainfall, elevation and mean soil depth. These observed data (obs) were compared with expected frequency (exp), the proportional number of plots was based on the total number of plots on each categorical level, in an approximate Pearson Chi-square distribution with a Yates' correction for continuity. * significant differences at p=0.05, **differences at p<0.001.

| | *M. repens* | | *M. minutiflora* | | *A. virginicus* | | *S. condensatum* | | *H. contortus* | | *H. rufa* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | obs | exp | obs | exp | obs | exp | obs | exp | Obs | exp | obs | exp |
| soil age (years) | | | | | | | | | | | | |
| 200-750 | 321 | 320 | 237** | 318 | 293** | 214 | 56** | 103 | 119** | 67 | 29 | 37 |
| 750-3000 | 279 | 280 | 359** | 278 | 108** | 187 | 138** | 91 | 7** | 59 | 41 | 33 |
| fire frequency | | | | | | | | | | | | |
| burned | 288 | 280 | 401** | 278 | 105** | 187 | 105* | 91 | 76** | 59 | 10** | 33 |
| unburned | 312 | 320 | 195** | 318 | 296** | 214 | 89* | 103 | 50** | 67 | 60** | 38 |
| rainfall | | | | | | | | | | | | |
| 1200-1600 | 450** | 320 | 291** | 318 | 184** | 214 | 52** | 103 | 126** | 67 | 70** | 37 |
| 1600-2000 | 150** | 280 | 305** | 278 | 217** | 187 | 142** | 91 | 0** | 59 | 0** | 33 |
| elevation (m) | | | | | | | | | | | | |
| 0-500 | 270** | 240 | 155** | 238 | 202** | 160 | 0** | 78 | 126** | 50 | 0** | 28 |
| 500-1000 | 330** | 360 | 441** | 358 | 199** | 241 | 194** | 116 | 0** | 75 | 70** | 42 |
| soil depth (cm) | | | | | | | | | | | | |
| 0-5 | 269 | 255 | 182** | 253 | 190** | 170 | 74 | 82 | 54 | 53 | 15** | 30 |
| 5-10 | 128 | 138 | 146 | 137 | 100 | 92 | 29** | 44 | 33 | 29 | 15 | 16 |
| 10-20 | 105 | 105 | 123 | 104 | 70 | 70 | 31 | 34 | 19 | 22 | 19 | 12 |
| >20 | 98 | 102 | 144** | 101 | 41** | 68 | 59** | 33 | 20 | 21 | 21** | 12 |

### 3.2. Performance related to environmental factors

The cover and height of C4 grasses was highly variable even at small spatial scales, particularly in study sites with younger soil. Accordingly, GAMM models provided low ($R^2$<25) to intermediate ($R^2$>60) explanatory power for the variation in cover and height of all species (Table 9). We performed GAMM models for *M. minutiflora* and *M. repens* including also C:N ratio as an explanatory variable, but it provided no additional explanation of variation for percent cover (*M. minutiflora* – F=1.84, p=0.169; *M. repens* – F=0.69, p=0.417) or height (*M. minutiflora* F=1.77, p=0.188; *M. repens* - F=, p= ). Likewise, models including the soil type (ash or cinder) had no significant effect on the cover of *A. virginicus* (F=2.36, p=0.184) and added a very low amount to the variation explained by the model. In addition, a number of regional abiotic factors could not be used as explanatory variable in model runs for *H. contortus* (annual rainfall and elevation), *S. condensatum* (elevation) and *H. rufa* (annual rainfall and elevation), since they are distributed in only one level of each of these factors (Table 7). We also could not assess effects of soil age in models for *H. contortus*, since in older soils it occurred in a single transect in one site (Table 7).

When used as a continuous variable, soil depth had a significant relationship with the cover in the best models for all species except *M. repens* (Table 8). Its effect on cover of *M. minutiflora* was significant and driven by soils less than 10 cm deep (Table 8, Figure 2). *A. virginicus* had lower cover in plots with shallow soils (<5 deep) (Figure 18). By contrast, the cover of *S. condensatum*, *H. rufa* and *H. contortus* showed a positive linear relationship with soil depth, which accounted for most of the explained variation in their cover (Table 8, Figure 18). All species exhibited a weak but significant negative linear (estimated degrees of freedom=1) relationship with rock cover, except *M. repens* (Table 8).

With regard to regional variables, *M. repens* was the single species whose cover showed a significant relationship with soil age (Table 8). It had higher cover on older soils, at lower elevation, and where fires had occurred (Table 8). Nevertheless, when the single site in which it had high percent cover and was the dominant species (kiproad) was excluded from the model, percent cover was significantly higher under lower elevation (t=-3.683, p<0.001), but model explanation was lower ($R^2$adj=0.11) and percent cover was higher in more recent soils (t=-4.488, p<0.001) and unburned sites (t=-2.692, p=0.009). Consistent with occurrence data, *M. minutiflora* had higher cover in burned sites and at higher elevation (Table 9). Likewise consistent with occurrence data, *A. virginicus* had higher cover in unburned sites, in lower elevation sites and at higher rainfall (Table 9). In contrast, the cover of *S. condensatum* was longer in burned sites (Table 9). Because there was no significant effect of regional variables on the cover of *H. rufa* and *H. contortus*, it appears that most variance in space occupancy by these species is related to local abiotic variables (Table 9).

Even though the individual height of all C4 grasses was positively related to soil depth, its importance as a predictor variable for height for each species was mixed (Table 9). It explained only a small amount of the variation in height for *M. repens, H. contortus* and *A. virginicus*. Indeed some of the tallest individuals of these species occurred even in very shallow soils (Table 9, Figure 19). On the other hand, smoothing curves obtained for *M. minutiflora* showed that many individuals were either short or tall in shallow soils (0 to 10 cm deep), but maintained intermediate to high height in deeper soils (Figure 1). In contrast, the tallest individuals *S. condensatum* and *H. rufa* only occurred in soils more than 30 cm deep (Figure 19; Table 9). The relationship with percent cover of rock was only highly significant for *M. minutiflora* and *H. contortus*, and also provided a poor explanation of the variation in height (Table 9).

Table 9 – Fixed effects from parametric (soil age, annual rainfall, fire occurrence) and smoothing components (mean soil depth beneath established plants and percent rock cover) in the best GAMM for the performance of C4 grasses. Response variables were the percent cover and maximum height of six grasses across 15 sites in the Hawai'i Volcanoes National Park. Est- estimated effects, edf-estimated degrees of freedom. BIC- Bayesian Information Criteria values. Adj $R^2$- model adjusted $R^2$. Significance: * p<0.05, **p<0.01, ***p<0.001.

| response | non-parametric | | | | parametric | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| variable | soil depth | | rock cover | | intercept | | soil age | | rainfall | | fire | | elevation | | BIC | adj R2 |
| | edf | F | edf | F | est | t | est | t | est | t | est | t | est | t | | (%) |
| *A. virginicus* | | | | | | | | | | | | | | | | |
| cover | 5.56 | 7.85*** | 1 | 17.58*** | 2.88 | 1.56 | - | - | 10.11 | 6.98*** | 11.16 | 7.23*** | -7.02 | -44.91*** | 2874 | 0.42 |
| height | 3.76 | 3.61** | 1.485 | 4.04* | 31.38 | 26.77*** | - | - | 11.68 | 7.16*** | - | - | - | - | 2947 | 0.26 |
| *M. minutiflora* | | | | | | | | | | | | | | | | |
| cover | 2.95 | 9.23*** | 1 | 30.49*** | 40.43 | 17.78*** | - | - | - | - | -5.52 | -2.6** | -18.96 | -7.62*** | 4920 | 0.25 |
| height | 5.82 | 14.918 | 1.694 | 6.072 | 50.19 | 35.52 | 2.398 | 1.59 | - | - | | | -21.48 | -11.62*** | 4147 | 0.35 |
| *M. repens* | | | | | | | | | | | | | | | | |
| cover | 2.79 | 3.80* | 3.117 | 9.89*** | 26.81 | 10.83*** | 11.73*** | 4.74*** | - | - | -10.07 | -4.21*** | -26.17 | -12.51*** | 4795 | 0.44 |
| height | 5.35 | 7.27*** | 2.326 | 2.76* | 82.08 | 22.86*** | 6.41 | 1.78 | -6.75 | -2.17** | -15.99 | -4.62*** | -32.78 | -11.00*** | 5462 | 0.36 |
| *S. condensatum* | | | | | | | | | | | | | | | | |
| cover | 1.57 | 17.46*** | - | - | -1.89 | -0.83 | 3.41 | 1.92 | 3.89 | 2.09* | 4.18 | 2.57* | - | - | 1185 | 0.24 |
| height | 4.15 | 6.52*** | 1.42 | 4.20* | 55.26 | 6.13*** | 3.93 | 0.6 | 16.27 | 2.21* | -9.87 | -1.67 | - | - | 1201 | 0.47 |
| *H. ruffa* | | | | | | | | | | | | | | | | |
| cover | 1 | 10.68** | 2.967 | 2.72* | 20.98 | 1.8 | -6.17 | -0.81 | - | - | 1.63 | 0.16 | - | - | 576 | 0.35 |
| height | 1 | 22.59*** | - | - | 47.88 | 7.29*** | -7.95 | -1.80 | - | - | 5.37 | 0.94 | - | - | 540 | 0.41 |
| *H. contortus* | | | | | | | | | | | | | | | | |
| cover | 2.06 | 10.98*** | 1 | 49.22*** | 30.53 | 4.52*** | - | - | - | - | -11.09 | -1.60 | - | - | 1056 | 0.51 |
| height | 2.63 | 3.11* | 1 | 31.71*** | 59.85 | 10.43*** | - | - | - | - | -24.12 | -4.10*** | - | - | 894 | 0.38 |

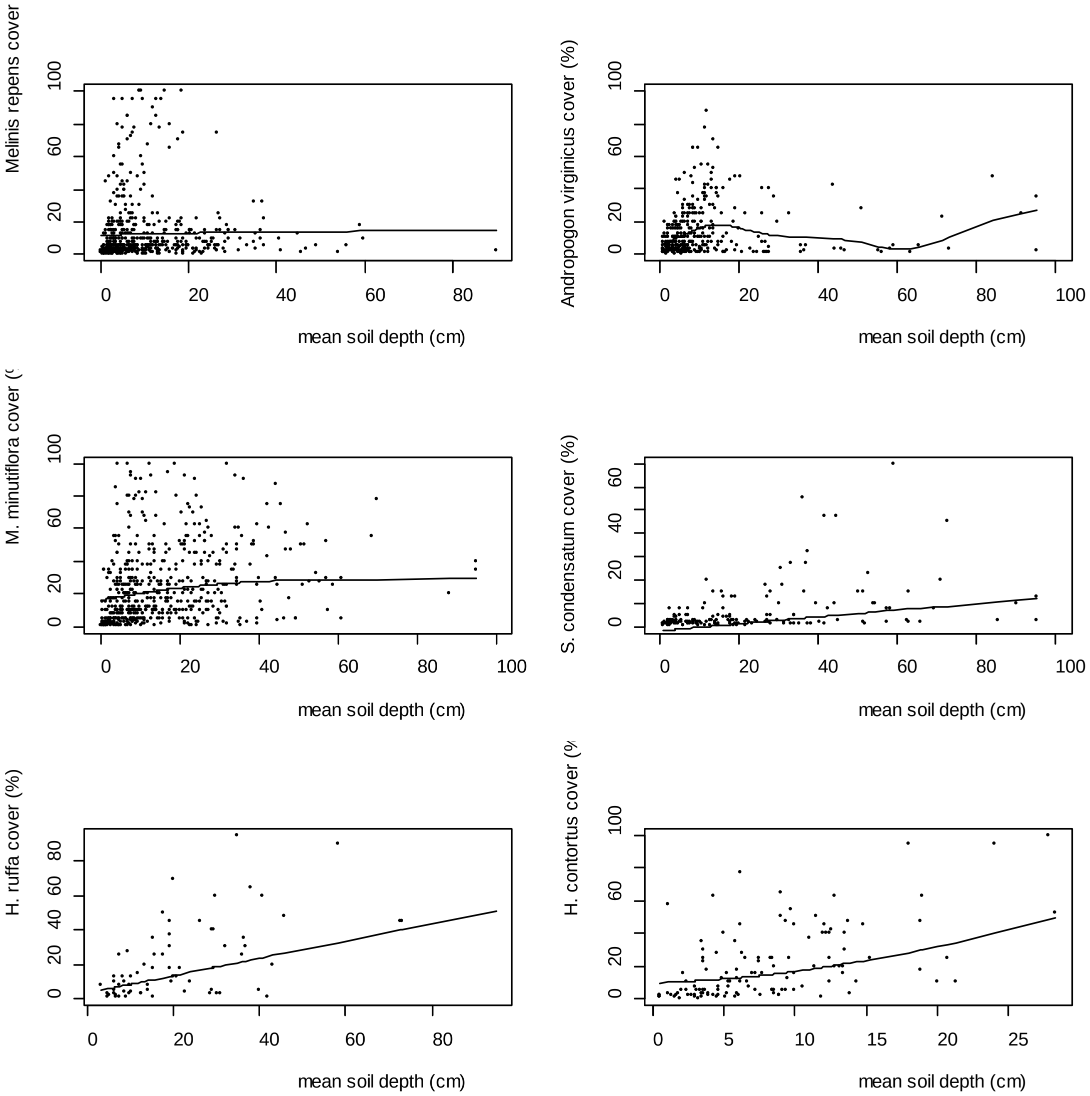


Figure 18 –Non-parametric smoothing lines obtained from spline cubic regression, showing the relationship between percent cover on the vegetation and the mean soil depth where individuals are established for six C4 grasses. Results were obtained from GAMM models performed in the R package mgcv on data of the species in 15 sites across environmental gradients in the Hawai'i Volcanoes National Park.

Height responses to regional variables were similar to responses observed for percent cover data (Table 8). Individuals of *M. repens* were also taller in burned sites, in lower elevation and more recent soils (Table 8). However, when the site with the tallest individuals (kiproad), the same with highest percent cover, was excluded from the analysis elevation was the single significant factor (t=5.22, p<0.001). *A. virginicus* had taller individuals with higher rainfall and lower elevation, and *M. minutiflora* had taller individuals in higher elevation plots (Table 8). *S. condensatum* had a marginally higher individual height with higher rainfall but it occurred in a much narrower window of rainfall than some of the other species. *H. contortus* was taller when it was in burned sites (Table 8). Regional abiotic variables had no significant effect on *H. rufa* height (Table 8).

### 3.3. Effect of environmental factors on leaf stoichiometry

Three C4 grasses (*H. contortus*, *H. rufa* and *S. condensatum*) were the most common species in few transects, hence we did not obtained an enough number of samples of these species to analyze the leaf stoichiometry. The GAMM models were significant to the other species, and the best model always included a major explanatory highly related to C : N ratio. (Figure 20a). *M. minutiflora* showed lower C:N under low rainfall (F=3.36, p=0.002), but it was based in a single site where soils are deeper. In *M. repens* it was positively related to soil depth (F=6.52, p=0.001) (Figure 20b). Leaves of *A. virginicus* had a much higher C : N ratio in soils containing volcanic cinder (t=-9.98, p<0.001), suggesting lower N availability there (Figure 20c). Models having as response variable the leaf percent N instead of leaf C : N ratio showed similar results for these three species, as might be expected given the high linear correlation between these variables (Spearman correlation- *M. repens*- rs=0.916; *M. minutiflora* – rs=0.791; *A. virginicus*- rs=0.991).

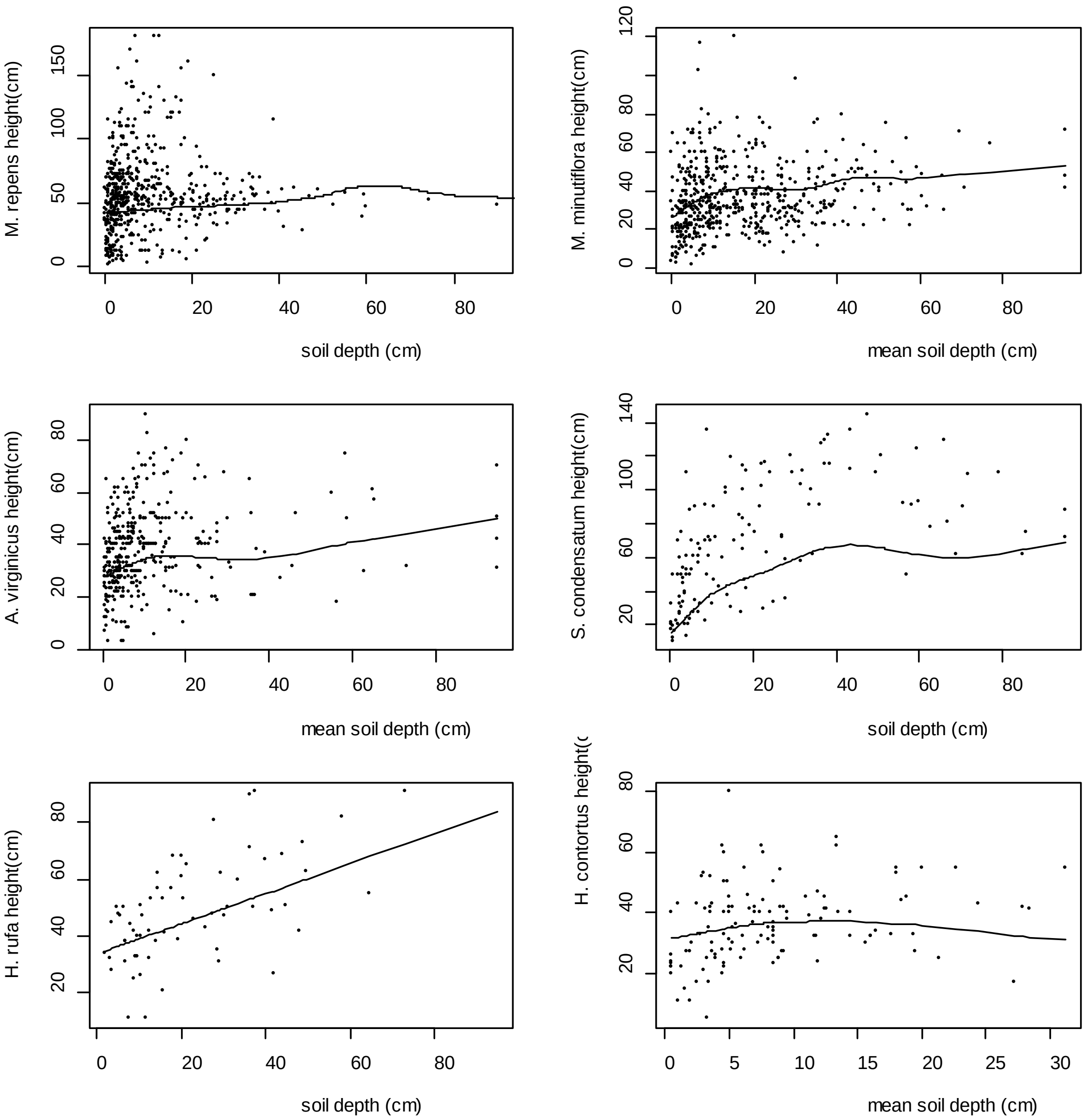


Figure 19 – Smoothing curves showing the relationship between individual height and the mean soil depth where individuals are established for six C4 grasses. Results were obtained from GAMM models performed based on data of the species in 15 sites across environmental gradients in the Hawai'i Volcanoes National Park.

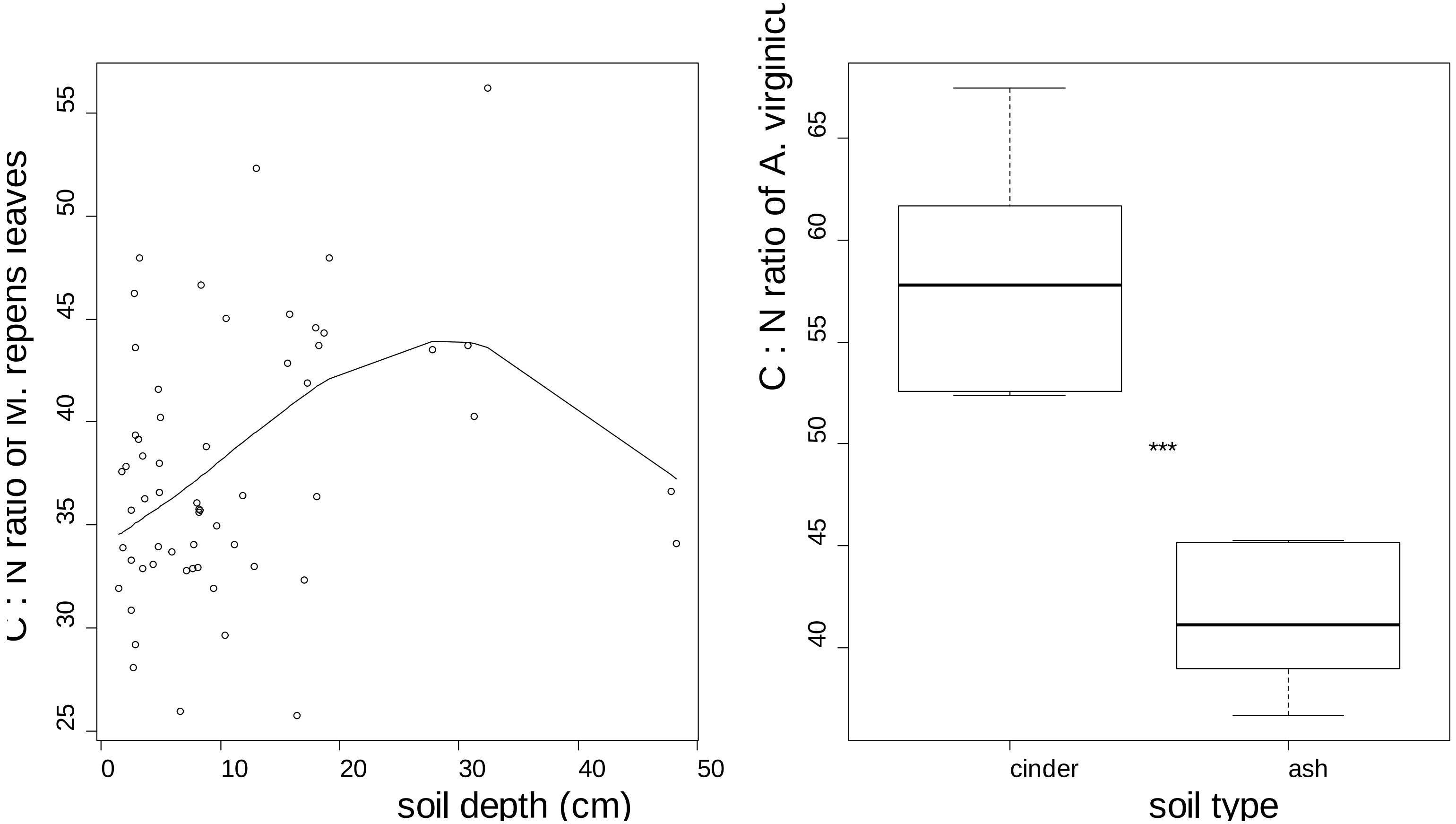


Figure 20 –Boxplots showing the significant fixed effects of abiotic variables on the C : N ratio of leaves of *Melinis repens* (a) and *Andropogon virginicus* (b) in the Hawai’i Volcanoes National Park. Effects were estimated by cubic spline regression (a) parametric coefficients (b) in a Generalized Additive Mixed Model. *** significant differences at p <0.001.

### 3.4. Comparative soil depth requirement

Most species occurred in deeper soil than the average soil depth measured for the plot (Figure 21). The GMM models showed a significant interspecific variation in the difference between the soil depth for a given grass species versus that randomly obtained in the plot ($F=10.43$, $p<0.001$, Figure 4). However, *a posteriori* pairwise comparison found that the whole effect was due to a lower difference for *M. repens* compared to the other species (Figure 21). This is consistent with its occurrence on the shallowest soils (Table 7).

### 3.5. Comparison of soil depth and percent cover among co-occurring C4 grasses

Since some species, such as *H. rufa*, had a very distinctive distribution and rarely co-occurred with other species, we could not perform statistical comparisons between all pairs of species. *H. rufa* could not be compared with other species, and we could not compare *M. minutiflora* and *H. contortus*. In general, *M. minutiflora* had higher cover than all other co-occurring C4 grasses (Figure 22), except when compared to *M. repens* in low elevation (Wilcoxon paired test – $V=379.5$; $p=0.899$). It colonized sites with similar soil depth to *S. condensatum* (Figure 22b) and *A. virginicus* (Figura 22b). Nevertheless, in low elevation sites, it occurred on lower soil depth compared to *A. virginicus* ($V=2241.5$, $p=0.004$). *M. repens* always occurred in shallower soil than other species (Figure 22c), except when compared to *M. minutiflora* in lower elevation plots ($V=335.5$, $p=0.617$), and it had lower cover in comparison with *H. contortus* where they co-occurred (Figure 22d). The percent cover of *A. virginicus* was similar to that of *M. repens* in burned sites ($V=0.292$, $p=0.393$), under lower rainfall ($V=3873$, $p=0.088$) and in older soils ($V=626.5$, $p=0.919$). *S. condensatum* generally had lower cover than other C4 grasses (Figure 22), but there was no difference when it occurred with *A. virginicus* in burned sites (V=110, p=0.865), lower rainfall (V=47, p=0.1677) and in older soils (V=167, p=0.3614), and to *M. repens* with fire (V=298, p=0.098), higher rainfall (V=515, p=0.503) and older soils (V=619.5, p=0.503). Although *H. contortus* occurred in deeper soil than *A. virginicus*, they showed similar percent cover (Figure 22).

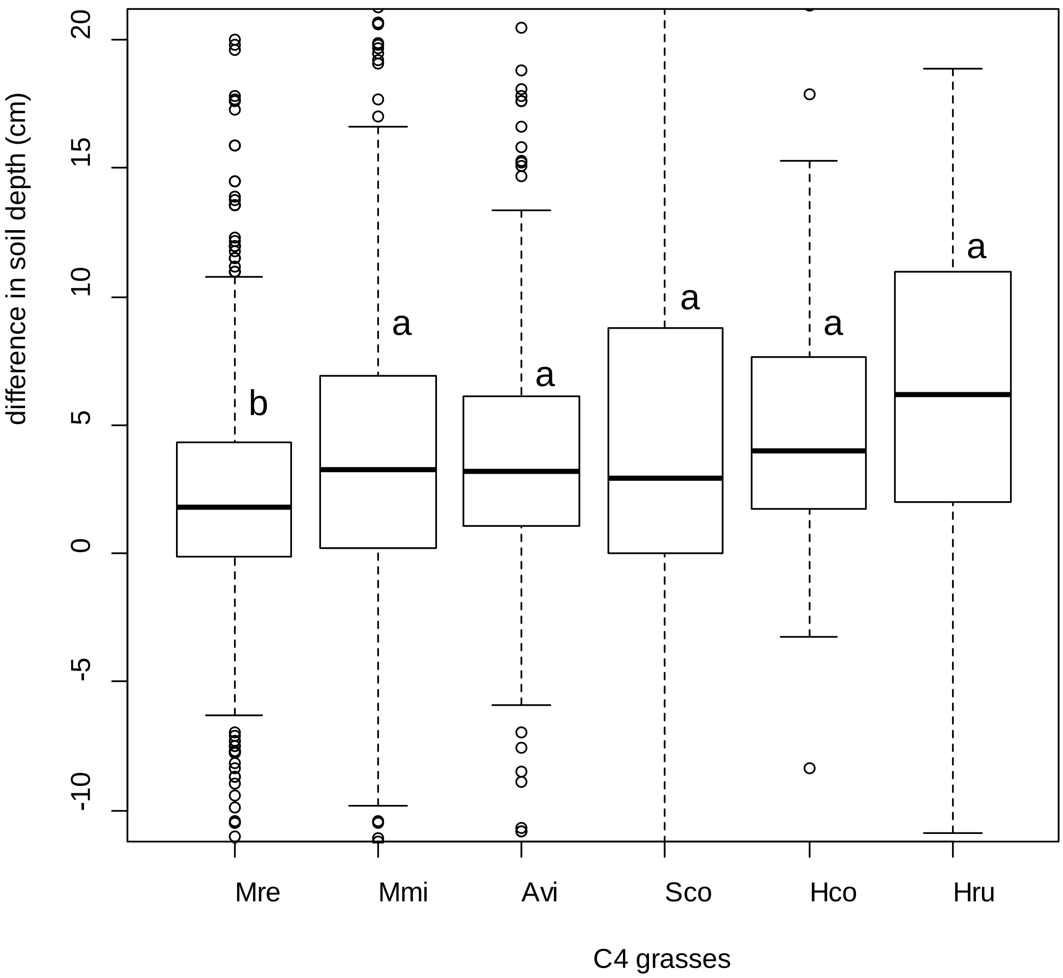


Figure 21 – Boxplots showing the variation in soil depth of five C4 grasses inside experimental plots, obtained from the difference between the mean soil depth under established individuals of each species and the mean of random soil depth measurements in those same plots. Generalized Mixed Models showed significant fixed effects related to species, and lowercase letters indicate significant interspecific differences (pairwise *a posteriori* comparisons – $p<0.05$). Species names: Mre- *Melinis repens*, Mmi- *Melinis minutiflora*, Avi- *Andropogon virginicus*, Sco- *Schizachyrium condensatum*, Hco- *Heteropogon contortus*, Hru-*Hyparrhenia rufa)*

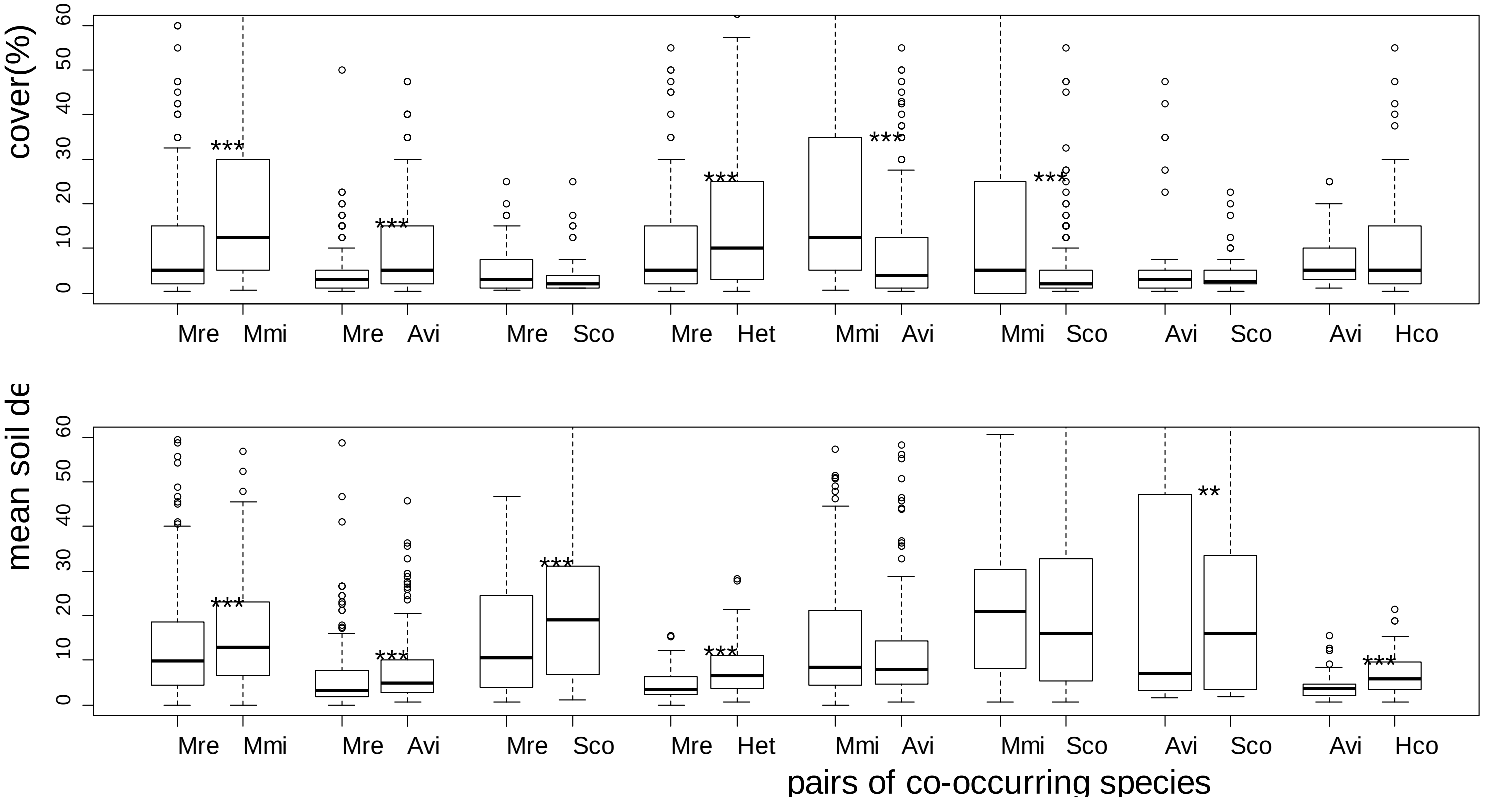


Figure 22 – Boxplots of percent cover and soil depth of established individuals between pairs of co-occurring C4 grasses in 15 costal and submontane sites in the Hawai'i Volcanoes National Park. Different letter indicate significant differences between pairs of species (Wilcoxon rank test – $p<0.05$).Species: Mre- *Melinis repens*, Mmi- *Melinis minutiflora*, Avi- *Andropogon virginicus*, Sco- *Schizachyrium condensatum*, Hco- *Heteropogon contortus.*

## 4. Discussion

In this study we associated the occurrence and performance of exotic and native C4 grasses in some young volcanic sites in Hawai'i with abiotic heterogeneity at local and regional spatial scales. Instead of showing a single response characteristic of typical disturbance responsive 'invaders' (Davis et al. 2000), these species distributed according to individual tolerances to the harsh conditions imposed by both local soil depth and rockiness variables, and more regional abiotic variables. However, competitive ability, historical factors and disturbance regime also explain patterns of occurrence and abundance of some species. Our results suggest that the current distribution of grasses in this portion of Hawai'i is shaped by environmental requirements and how they affect interaction between co-occurring grass species.

We found that two C4 grasses *H. contortus* and *H. rufa* have a very limited distribution in our study site. *H. contortus* is believed to be a native species in coastal habitats (Williams & Black 1994) so it has presumably been in HAVO for millenia. Previous studies suggested that this species originally had a wider distribution, but has been displaced by more competitive introduced C4 grasses (Daehler & Carino 1998). Here, even after extensive sampling we did not find any individuals of *H. contortus* in submontane sites (above 500 m) (Table 7). *H. contortus* is common in sites where a water deficit is expected, and previous work has shown that its seedlings establish more effectively in such sites than co-occurring exotic grasses (Goergen & Daehler 2002). Here, *H. contortus* was more common in soils with intermediate depth and in sites with lower rock cover, and performed better in burned sites suggesting that within the low elevation drier sites where it occurs, it is favored by more rich (intermediate depth and burned) conditions. In addition, when occurring with other C4 grasses, it generally had greater cover and occupied sites with deeper soil than other C4 grasses (*M. repens, A.*

*virginicus*) present in the same plot (Figure 5). Based on these responses, we believe that *H. contortus* has the best performance in the coastal lowlands, where higher temperatures and lower annual rainfall constrain the dominance by most exotic grasses.

In contrast to *H. contortus*, environmental requirements and a relatively shorter period of introduction likely contribute to the current limited distribution of *H. rufa* in HAVO. We expected a larger presence of this species in seasonally dry sites in Hawai'i, since *H. rufa* is considered a highly competitive species, may easily spread after fire and has been showed to be an aggressive invader in many Neotropical savannas (Pivello et al. 1999a; Williams & Baruch 2000). However, the close association between the presence and performance of this species and deeper soils in our study suggests that the prevalence of the shallow soils on recent lava flows in HAVO could limit the presence of *H. rufa* across a large portion of the lowlands of the park. In turn, the interspersing of recent and older lava flows could slow dispersal of this species to suitable sites (e.g. deeper). Even though *H. rufa* seeds have structures that could be dispersed by attachment to large animals, there are no such animals in this part of the Park so most of these larger seeds would be only locally dispersed (Daubenmire 1972). Because of limited sampling sites relative to its current distribution, we could not evaluate the influence of precipitation and elevation on the presence of *H. rufa*. A more extensive sampling in HAVO could more precisely identify the major reasons for the low abundance of this species in the areas sampled here, and help to identify potential limits to its spread. Our findings suggest that, because of site limitations, this species could cause lower impact in our study sites compared to that reported to other ecosystems.

While our results suggest that *H. rufa* and *H. contortus* are good competitors limited by local or regional abiotic variables, *M. repens* and *A. virginicus* appear to be less competitive species tolerant to a wide range of local microhabitats and across a wide elevation gradient. *M. repens* was more common in younger than older soils and was unresponsive to local variation in environmental quality, here estimated by soil depth and rock cover. Even though soil depth and rock cover are related to each other, we believe that they could select different abilities in colonizing species. While shallow soils could select species that may compensate a superficial root system by spreading roots over a larger area, the presence of a high percent of rock cover could limit the establishment to very small portions of surface area. These circumstances are common in young lava flows, particularly in lower rainfall areas, so that only a very small number of 'safe sites' may be present (Cutler et al. 2008). In lava flows with pahoehoe texture these favorable sites are often represented by cracks, which are especially important when the precipitation is lower (Aplet et al. 1998). Despite limited suitable microsites and soils with low N, small grasses as *M. repens* could be more successful because they can complete the life cycle even with a very low investment in vegetative structures (David & Menges 2011). Lower elevation sites in HAVO also have higher mean annual temperatures (D'Antonio et al. 2000), limiting species that require a larger root system or have higher water requirements to grow and reproduce. Nevertheless, certain native shrubs and herbs were present in our lowest and driest sites and those with very little soil (Matson 1990). Even though *M. repens* tolerated these extreme conditions, in most of our sites it rarely had higher cover than other co-occurring C4 grasses and always occupied more shallow soils. These results therefore suggest that competition with tolerant native species could be a real barrier to the presence of *M. repens* in undisturbed sites with recent soils. In effect, the single site where *M. repens* achieved highest cover and individual height has older soils (kiproad, Table 1), has been repeatedly burned in the last decades, and occurs in a region of the coastal lowlands with low elevation and precipitation. This specific combination of circumstances where

*M. repens* would be expected to be dominant, altogether with its lower individual size, may be major reason why this species is widespread but rarely dominant.

Similarly to *M. repens, A. virginicus* is a widespread but rarely dominant C4 grass. However, the leaf stoichiometry and the response to soil depth and fire suggest that *A. virginicus* is perhaps more of a stress tolerator than *M. repens*. *A. virginicus* is a dominant species in many extremely nutrient limited sites in eastern United States (Rice 1972). According to our data, only sites with very shallow soil (0-5 cm deep) constrain the performance of *A. virginicus*. Yet surprisingly, it was also more common under these more extreme conditions than expected, suggesting that phenotypic plasticity allows it to successfully colonize even these unfavorable sites. In addition, it was the dominant species in both of our study sites whose soils contain basaltic tephra or cinder. Tephra is a general denomination for volcanic debris, which may accumulate on the soil surface during explosive volcanic eruptions, (Malin et al. 1983; Grishin et al. 1996). Soils formed from Tephra are typically poor in nutrients and may have very low water holding capacity, depend on the fragment size (Pérez 2000); they should thus be poor sites for grass growth and establishment. Specific studies on *A. virginicus* reported resistance to toxic levels of metals in certain soils (Cumming & Ning 2003; Ezaki et al. 2008), and higher P-use efficiency induced by mycorrhyzical fungi (Ning & Cumming 2001). Thus the prevalence of *A. virginicus* on the Tephra sites and the lack of other grasses is consistent with it being a stress tolerator type species. Previous studies elsewhere have found that the species was excluded or severely reduced after fertilization with N, P and K in sites with severe edaphic nutrient limitation (Peters & Lowance 1974). Likewise, since *A. virginicus* was much less common in burned sites, we believe that the nutrient release following fire favored other more competitive C4 grasses, such as *M. minutiflora*.

Our results suggest that *S. condensatum* may be a more competitive species than *M. repens* and *A. virginicus*, but is limited by local conditions and competition with *M. minutiflora*. *S. condensatum* had the tallest individuals between all C4 grasses, but similarly to *H. rufa* it was less common in shallow soils and its cover and height showed an almost linear positive relationship with the soil depth. Nevertheless, the cover of the species even in deeper soils seems to be negatively affected by fire occurrence. These results corroborate results from previous studies performed in the submontane zone in the HAVO (Hughes et al. 1991; D'Antonio et al. 2001). In sites with relatively deep soil, *S. condensatum* was shown to be replaced by *M. minutiflora* when the fire frequency increased (D'Antonio et al. 2001). This effect seems to be related to high seedling recruitment and vigorous vegetative growth of *M. minutiflora* in the recently burned and presumably more N rich sites (D'Antonio et al. 2001). *S. condensatum* is widespread in Neotropical savannas (Turpe 1984), where fire is recurrent and soils are predominantly nutrient poor (Sarmiento 1984). It can resprout after fires in the submontane zone (D'Antonio et al. 2011) but is eventually outcompeted by *M. minutiflora* if it is present (D’Antonio et al. 2001). Sites invaded by *M. minutiflora* in the submontane zone maintain a similar vegetation structure after many years even without new fire event (D'Antonio et al. 2011). Accordingly, *S. condensatum* is expected to be only an occasional species, eventually only dominant in undisturbed deeper soil or burned sites with deep soil that are lacking *Melinis minutiflora*. Disturbance by feral pigs is also recurrent in the submontane zone (Aplet et al. 1991), and may contribute to the replacement of *S. condensatum* by *M. minutiflora*.

Even though *M. minutiflora* is known to be highly competitive in some seasonally dry ecosystems (Williams & Baruch 2000; D'Antonio et al. 2001), our data also suggest that fire occurrence give additional advantage to this species over other C4 grasses both in deeper and shallow soils. The positive feedback of fire occurrence in the spread of *M. minutiflora* found here agrees with previous studies in the submontane zone in HAVO (D'Antonio et al. 2001), and was also reported in invaded sites in Neotropical savannas (Hoffmann et al. 2004). However, the advantages of fire to this species may be much larger in Hawai'i, where many of the native species are intolerant to or resprout very slowly after fire (D'Antonio et al. 2011). This could explain why we could detect positive effects of fire in the occurrence and abundance of *M. minutiflora* in our study sites, which burned at least 20 years ago. In addition, other competitive C4 grasses resistant to fire as *H. rufa* and *S. condensatum* may have a limited distribution in the HAVO because of the prevalence of shallow soils. Here *A. virginicus* also had a good performance on these soils, so that it could directly compete with *M. minutiflora*. In effect, we found no difference between the percent cover of *A. virginicus* and *M. minutiflora* in undisturbed sites. Nevertheless, *A. virginicus* was much less common and had a much lower cover than *M. minutiflora* in burned sites. This is in contrast with previous studies, which found that *A. virginicus* may resprout and even spread after fire (Hodgkins 1958). We believe that the inferior performance of *A. virginicus* in burned sites with shallow soil emerges from an indirect effect from coexistence with *M. minutiflora*. The establishment and growth of the latter were favored in the past by an post-fire environment more open and richer in nutrients (D'Antonio et al. 2001) and, both because of a priority effect and its superior competitive ability, it could not be displaced by the former even many years after fire occurrence(D'Antonio et al. 2011).

In addition to fire, we believe that phenotypic plasticity is a key factor explaining the wide distribution and abundance of *M. minutiflora*. It seems to be uncommon only in low elevation sites and in soils with cinder suggesting a relationship with water availability. Our data show that *M. minutiflora* had lower cover only in extremely shallow soils with a high amount of rock, and exhibited only slightly lower individual height in shallow (5-20 deep) soils. These results suggest that the soil limitation may have a small effect in the performance of this species, which therefore could be a good competitor even in young soils with a less extensive root system. Even though we do not have data supporting that all individual of *M. minutiflora* belong to the same population, we believe that this generalist response might be achieved by plasticity in the allocation to aboveground and underground biomass, which has been widely reported for other grasses in heterogeneous soils (Fransen et al. 1998). However, our data suggest that this plastic response may not be enough to ensure the spread of *M. minutiflora* to sites at lower elevation and with cinder. We believe that the combination of higher temperatures, low precipitation and young soil in the coastal lowlands imposes an excessive water shortage to *M. minutiflora*. Under these conditions it is an inferior competitor than *H. contortus* in soil with intermediate to high depth, and *M. repens* in shallow soils. Contrasting with the submontane zone, previous studies suggest that fire may not give a large additional advantage to *M. minutiflora* on these more stressful sites (D’Antonio et al. 2000). On the other hand, unlike *A. virginicus M. minutiflora* could not have mechanisms to overcome the water and nutrient shortage in cinder soils. Based on these findings, we hypothesize that even after fire sites subject to these two conditions (shallow soil in low elevation or soils with cinder) are protected of be dominated by *M. minutiflora*.

## 5. Conclusion

This study shows that local and regional environmental heterogeneity have a mixed influence on the distribution and performance of exotic and native grasses in this region of Hawai’i, depending on interactions between abiotic variables and the co-occurring species. Even though all C4 grasses have co-occurred on these early sucessional sites for a very brief period in evolutionary terms, the high susceptibility to invasion in these sites and phenotypic plasticity in the invaders has allowed exotic grasses to dominate extremes environments despite constrains imposed by unfavorable local abiotic conditions. Nevertheless, disturbance and altitudinal gradients seem to also drive the relative abundance of these species. Fire allows *M. minutiflora* to displace other good competitors (*S. condensatum*) and more stress-tolerant species (*A. virginicus*) in middle elevation sites, even over 20 years after fire occurrence, whereas higher temperatures and lower precipitation in low elevation favor an opportunist species (*M. repens*) and a native species tolerant to water deficit (*H. contortus*). Sites with a surface layer of volcanic debris are more protected from invasion by exotic grasses, except *A. virginicus*. The current distribution of these species must persist, unless new volcanic eruptions increase the surface Tephra or changes in the climate result in warmer and drier conditions. However, the high impact of biotic interactions suggested here highlights that further introductions of species or spread of currently rare species (e.g. *H. rufa*) may change the structure of these communities. Our findings reinforce that fire suppression and controlling of new introductions are important in avoiding further spread of exotic grasses in HAVO.

## GENERAL CONCLUSION

The results of this study are in agreement with our initial hypothesis that the abiotic stress would be less effective barrier to invasion in an ecosystem with very low biotic resistance as Hawai'i compared to the cerrado. In Hawai'i, only the uncommon combination of very shallow soils, low disturbance frequency and low elevation prevents the dominance of exotic grasses (Figure 23). In contrast, in the cerrado exotic grasses neither dominated sites that were subject to a period of soil water saturation nor occurred in sites annually subject to eight or more months of soil waterlogging (Figure 23). However, similar strategies occurred in both ecosystems. One species in the cerrado (*U. decumbens*) and two species in Hawaii (*S. condensatum* and *H. ruffa*) are limited by local stress induced by unsuitable soil conditions, represented by shallow soils in the latter and water saturated soils in the former (Figure 23). On the other hand, the species common to both ecosystems (*M. minutiflora* and *M. repens*) were more generalist, whose plasticity allow them to have success even in shorter periods of water soil saturation (lower than 8 months) in the cerrado or in shallow soils (<20 cm) in Hawai'I (Figure 23).

How we hypothesized, some resistance against the dominance by African grasses in more stressful sites in the cerrado may emerge from stress tolerant native species, whereas in Hawaii this could occur in very specific circumstances. The longer evolutionary history and lower isolation of the cerrado seems to have allowed the evolution stress tolerant or more generalist species, such as *A. bicornis* and *L. chrisothrix*, respectively. The superior performance of *Loudetiopsis chrisothrix* in sites subject to soil waterlogging or higher canopy cover, altogether with good performance

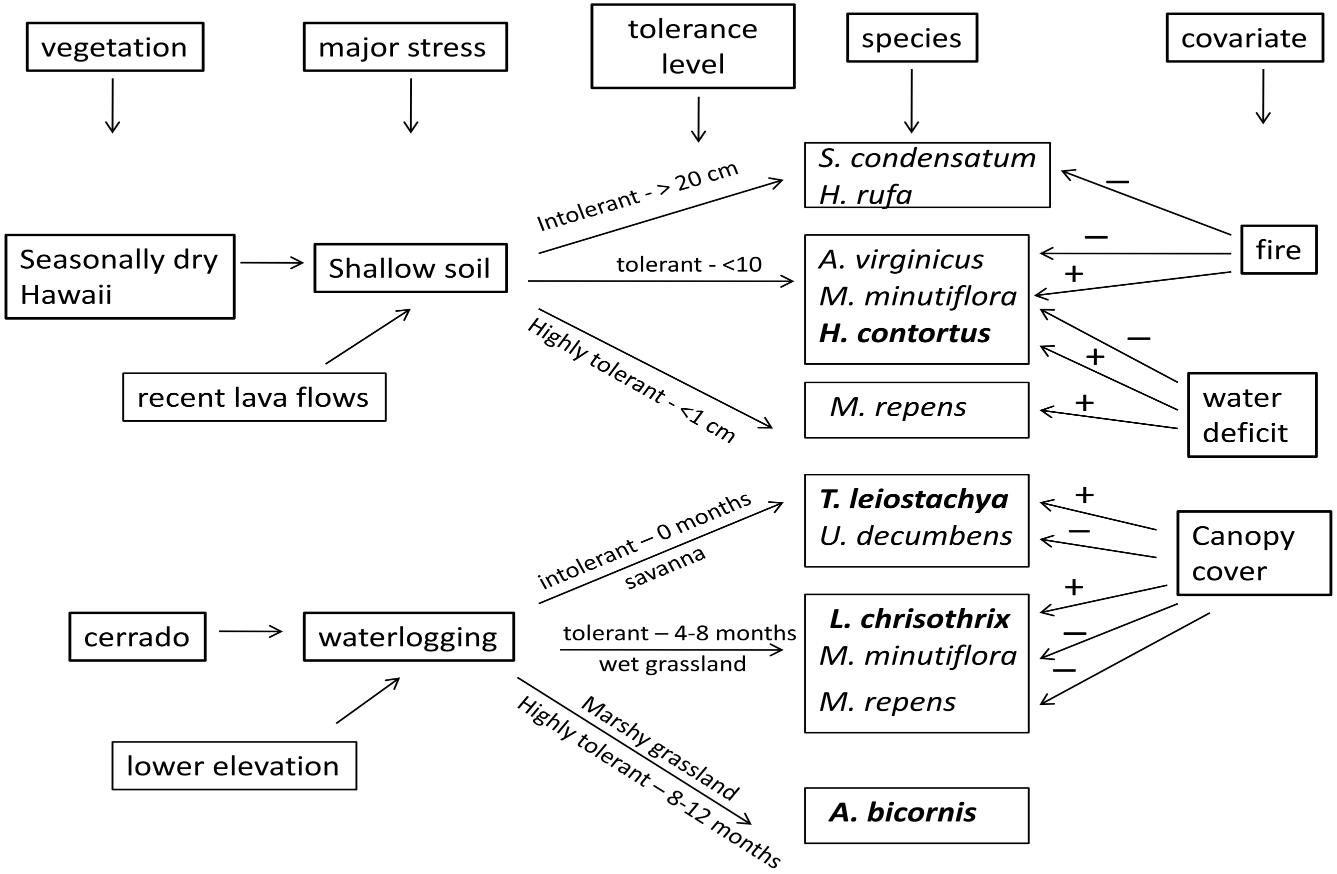


Figure 23 – Diagram including the major abiotic factors that affect the occurrence and performance of native and exotic grasses, both in the seasonally dry vegetation in the Hawai'i island and in the Brazilian cerrado. Native species in each ecosystem are indicated in bold letters.

and ability of co-occur with exotic grasses in more mesic conditions, suggests that this species could be a biotic barrier to the spread of African grasses in the cerrado (Figure 23). In contrast, the single native grass sampled in Hawaii (*Heteropogon contortus*) could represent certain invasion resistance only in warm, dry and shallow-soiled sites, in which most exotic species (e.g. *M. minutiflora*) are very limited (Figure 23). Since *H. contortus* do not occur in other sites either because of intrinsic limitation or competitive exclusion, a biotic resistance induced by native grasses could only occur on this circumstances. We strongly believe that the geographical isolation and short evolutionary history in Hawaii are a major reason for the absence of either stress tolerant or competitive native grasses in Hawai'i. Likewise, our results suggest that African grasses perform better than grasses native from cerrado in mesic conditions. However, this advantage is much higher whether disturbances occur more often, how is indicated by the resistance that *Schyzachyrium condensatum*, a grass native from Neotropical savannas, may imposes to the dominance by *M. minutiflora* in undisturbed sites of Hawaii. This is consistent with the hypothesis that either a very long evolutionaty history or higher selective pressures, especially water deficit and high pasture pressure (Owen-Smith 2013), favored the evolution of superior grass competitors in Africa compared to perennial grasses native from Neotropical savannas.

Our study highlights that the phenological features plays an important role in the success of African grasses as invasive species in the cerrado. Phenology was very similar and less plastic among grasses from the cerrado in comparison with African grasses. Phenological shifts induced by temporal and spatial variability may be a major attribute to convert more available resources in a higher seed output, and hence could enhance the competitive ability of *U. decumbens* and *M. minutiflora*. In contrast, high investment in reproduction seems to decrease the life-cycle of *M. repens*, and therefore should constrain its dominance to severely disturbed sites. Even though we have no phenological data for grasses in Hawai'i, we believe that a similar limitation for *M. repens* occurs in this ecosystem, since it continuously produce inflorescences (personal observation) and is favored by fire and very harsh conditions there. Likewise, *M. minutiflora* could also benefit from an unusual reproductive period, considering that in field observations it also have a later flowering (December) in Hawaii than other grasses (September) (personal observation). In contrast, biotic resistance emerging from a mixed phenology is unlikely in our sites in Hawai'i, since native grasses are currently rare.

Based on our results, is possible outline certain generalizations about the invasive potential of a number of African grasses in Neotropical and seasonally dry ecosystems. Lower competitive ability and a shorter life cycle seems constrain the dominance by *M. repens* to either severely disturbed sites and/or poorly developed soils, such as areas with strong anthropogenic influence. In contrast, the generalist response of *M. minutiflora* indicates that phenotypic plasticity may favor the spread of this species in most tropical and seasonally dry regions in the world. The success of *U. decumbens* is expect to be larger whether the soil is more accessible (e.g. well drained and deep soils) and water regime more mesic (e.g. shorter dry season). Finally, our data suggest that *H. rufa* could depend on deeper soils to be dominant, although further experimental investigations or more extensive surveys need to be performed on this species. Appling this predictions to our study sites, the prevalence of more mesic condition and deep soils in the cerrado could explain the status of *U. decumbens* as the major invasive species in the cerrado, whereas the predominantly shallow soils and more heterogeneous conditions would drive the high impacts of *M. minutiflora* in Hawai'i. Irrespective of these interspecific differences, fire occurrence, perhaps by means of a grass-fire cycle, could favor the spread of all African grasses in both ecosystems, both by sexual (*M. minutiflora*) or vegetative reproduction (*U. decumbens* and *H. rufa*).

Even though African grasses have been reported in both ecosystems for decades, we recognize that propagules limitation could have influenced the distribution of African grasses in our study sites. Accordingly, fine scale information about the propagules pressure of each exotic grass in Hawaii and in the cerrado, as well more precise information about the moment of introduction, could be important to assess spread potential and the factors limiting the distribution of these species. Likewise, the long time since some of these species were introduced could have allowed the evolution of distinctive response to stress in certain regions (e.g. *M. minutiflora* in the cerrado). The interest in the evolution of exotic species has increased recently (e.g. Novak 2007; Zenni et al. 2014), and studies comparing the genotypes of African grasses in the native and in the introduced range could be a valuable tool to address this issue. Finally, controlled experimental studies could manipulate the effect of canopy cover and water regime on the performance of native and exotic grasses, and then identify the physiological mechanisms related to stress tolerance. Altogether with observational studies, all these applied studies could shed light on the problem of the invasion by African grasses in the world, and generate a more effective control of these species.

While more effective disturbance response related to higher evolutionary pasture pressure is considered the major cause of the success of African grasses in the Neotropical region, our study highlights that the ability to quickly respond to environmental heterogeneity may be important for the high invasiveness of these species. Accordingly, changes in the disturbance and water regime could have large consequences in the distribution and performance of African grasses. Predictions from the effects of global change simulations on the South America biomes indicate that savannas are expected to advance over the current tropical rainforest during the XXI century (Salazar et al. 2007), similarly to what occurred in the Pleistocene (van der Hammen 1974), and related changes in the precipitation pattern could convert currently flooded sites into typical seasonally dry savannas. Likewise, the upward shifts on the distribution of species across altitudinal gradients that occurred during the XX century are expect to be intensified in scenarios of sustained global warming (Lenoir et al. 2008), and a upward movement in the distribution of African grasses in the Hawai'i Island was recently described (Angelo & Daehler 2013). Models assessing which sites will become more suitable for these species in the next decades may help to constrain or prevent further invasions.

## REFERENCES

Adair, E.C. & Burke, I.C. 2010. Plant phenology and life span influence soil pool dynamics: Bromus tectorum invasion of perennial C3–C4 grass communities. *Plant and Soil* 335: 255-269.

Almeida-Neto, M.r., Prado, P., Kubota, U., Bariani, J., Aguirre, G. & Lewinsohn, T. 2004. Invasive grasses and native Asteraceae in the Brazilian Cerrado. *Plant Ecology* 209: 109-122.

Alpert, P., Bone, E. & Holzapfel, C. 2000. Invasiveness, invasibility and the role of environmental stress in the spread of non-native plants. *Perspectives in Plant Ecology, Evolution and Systematics* 3: 52-66.

Alvarez, J.M., Rocha, J.F. & Machado, S.R. 2008. Bulliform cells in Loudetiopsis chrysothrix (Nees) Conert and Tristachya leiostachya Nees (Poaceae): Structure in relation to function. *Brazilian Archives of Biology and Technology* 51: 113-119.

Amorim, P.K. & Batalha, M.A. 2006. Soil characteristics of a hyperseasonal cerrado compared to a seasonal cerrado and a floodplain grassland: implications for plant community structure. *Brazilian Journal of Biology* 66: 661-670.

Angelo, C.L. & Daehler, C.C. 2013. Upward expansion of fire-adapted grasses along a warming tropical elevation gradient. *Ecography* 36: 551-559.

Aplet, G.H., Anderson, S.J. & Stone, C.P. 1991. Association between feral pig disturbance and the composition of some alien plant assemblages in Hawaii Volcanoes National Park. *Vegetatio* 95: 55-62.

Aplet, G.H., Hughes, R.F. & Vitousek, P.M. 1998. Ecosystem development on Hawaiian lava flows: biomass and species composition. *Journal of Vegetation Science* 9: 17-26.

Aronson, J., Kigel, J., Shmida, A. & Klein, J. 1992. Adaptive phenology of desert and Mediterranean populations of annual plants grown with and without water stress. *Oecologia* 89: 17-26.

Balch, J.K., Bradley, B.A., D'Antonio, C.M. & Gomez-Dans, J. 2013. Introduced annual grass increases regional fire activity across the arid western USA (1980-2009). *Global Change Biology* 19: 173-183.

Baruch, Z. 1994. Responses to drought and flooding in tropical forage grasses. *Plant and Soil* 164: 97-105.

Baruch, Z. & Fernandez, D.S. 1993. Water relations of native and introduced C4 grasses in a neotropical savanna. *Oecologia* 96: 179-185.

Baruch, Z. & Goldstein, G. 1999. Leaf construction cost, nutrient concentration, and net CO2 assimilation of native and invasive species in Hawaii. *Oecologia* 121: 183-192.

Baruch, Z. & Jackson, R. 2005. Responses of tropical native and invader C4 grasses to water stress, clipping and increased atmospheric CO2 concentration. *Oecologia* 145: 522-532.

Baruch, Z., Ludlow, M.M. & Davis, R. 1985. Photosynthetic responses of native and introduced C4 grasses from Venezuelan savannas. *Oecologia* 67: 388-393.

Batalha, M.A., Cianciaruso, M.V., Silva, I.A. & Delitti, W.B.C. 2005. Hyperseasonal cerrado, a new brazilian vegetation form. *Brazilian Journal of Biology* 65: 735-738.

Bates, D.M., Maechler, M., Bolker, B. & Walker, S. 2014. lme4:Linear mixed-effects models using Eigen and S4. R package version 1.1-6. http:/CRAN.R-project.org/package=lme4.

Belsky, A.J. 1986. Population and Community Processes in a Mosaic Grassland in the Serengeti, Tanzania. *Journal of Ecology* 74: 841-856.

Benzie, J.H. 1986. The distribution, abundance, and the effects of fire on mound building termites (Trinervitermes and Cubitermes spp., Isoptera: Termitidae) in northern guinea savanna West Africa. *Oecologia* 70: 559-567.

Blokhina, O., Virolainen, E. & Fagerstedt, K.V. 2003. Antioxidants, Oxidative Damage and Oxygen Deprivation Stress: a Review. *Annals of Botany* 91: 179-194.

Carol Adair, E. & Burke, I. 2010. Plant phenology and life span influence soil pool dynamics: Bromus tectorum invasion of perennial C3–C4 grass communities. *Plant and Soil* 335: 255-269.

Chambers, L.E., Altwegg, R., Barbraud, C., Barnard, P., Beaumont, L.J., Crawford, R.J.M., Durant, J.M., Hughes, L., Keatley, M.R., Low, M., Morellato, P.C., Poloczanska, E.S., Ruoppolo, V., Vanstreels, R.E.T., Woehler, E.J. & Wolfaardt, A.C. 2013. Phenological Changes in the Southern Hemisphere. *Plos One* 8: e75514.

Chambers, R.M., Meyerson, L.A. & Saltonstall, K. 1999. Expansion of Phragmites australis into tidal wetlands of North America. *Aquatic Botany* 64: 261-273.

Cianciaruso, M.V., Antônio Batalha, M. & Aurélio da Silva, I. 2005. Seasonal variation of a hyperseasonal cerrado in Emas National Park, central Brazil. *Flora - Morphology, Distribution, Functional Ecology of Plants* 200: 345-353.

Cleland, E.E., Chuine, I., Menzel, A., Mooney, H.A. & Schwartz, M.D. 2007. Shifting plant phenology in response to global change. *Trends in Ecology & Evolution* 22: 357-365.

Cleland, E.E., Larios, L. & Suding, K.N. 2012. Strengthening Invasion Filters to Reassemble Native Plant Communities: Soil Resources and Phenological Overlap. *Restoration Ecology*: no-no.

Coutinho, L.M. 1978. O conceito de cerrado. *Revista brasileira de Botânica* 1: 7.

Crawford, K.M. & Rudgers, J.A. 1996. Plant species diversity and genetic diversity within a dominant species interactively affect plant community biomass. *Journal of Ecology*: n/a-n/a.

Crawford, R.M.M. & Braendle, R. 1996. Oxygen deprivation stress in a changing environment. *Journal of Experimental Botany* 47: 145-159.

Crowl, T.A., Crist, T.O., Parmenter, R.R., Belovsky, G. & Lugo, A.E. 2008. The spread of invasive species and infectious disease as drivers of ecosystem change. *Frontiers in Ecology and the Environment* 6: 238-246.

Cuddihy, L.W., Pratt, L.W. & Stone, C.P. 1990. Alteration of native Hawaiian vegetation: effects of humans, their activities and introductions. In: Hawaii, U.o. (ed.), Honolulu.

Cumming, J.R. & Ning, J. 2003. Arbuscular mycorrhizal fungi enhance aluminium resistance of broomsedge (Andropogon virginicus L.). *Journal of Experimental Botany* 54: 1447-1459.

Cutler, N.A., Belyea, L.R. & Dugmore, A.J. 2008. Spatial patterns of microsite colonisation on two young lava flows on Mount Hekla, Iceland. *Journal of Vegetation Science* 19: 277-286.

D'Antonio, C., Stahlheber, K. & Molinari, N. 2010. Grasses and forbs. In: Rejmánek, M. & Simberloff, D. (eds.) *Encyclopedia of Biological Invasions*. University of California Press, Los Angeles.

D'Antonio, C.M., Hughes, R.F., Mack, M., Hitchcock, D. & Vitousek, P.M. 1998. The response of native species to removal of invasive exotic grasses in a seasonally dry Hawaiian woodland. *Journal of Vegetation Science* 9: 699-712.

D'Antonio, C.M., Hughes, R.F. & Tunison, J.T. 2011. Long-term impacts of invasive grasses and subsequent fire in seasonally dry Hawaiian woodlands. *Ecological Applications* 21: 1617-1628.

D'Antonio, C.M., Hughes, R.F. & Vitousek, P.M. 2001. Factors Influencing Dynamics of Two Invasive C4 Grasses in Seasonally Dry Hawaiian Woodlands. *Ecology* 82: 89-104.

D'Antonio, C.M. & Vitousek, P.M. 1992. Biological Invasions by Exotic Grasses, the Grass/Fire Cycle, and Global Change. *Annual Review of Ecology and Systematics* 23: 63-87.

D’Antonio, C.M., Tunison, J.T. & Loh, R.K. 2000. Variation in the impact of exotic grasses on native plant composition in relation to fire across an elevation gradient in Hawaii. *Austral Ecology* 25: 507-522.

Daehler, C.C. 2006. Invasibility of tropical islands by introduced plants: partitioning the influence of isolation and propagule pressure. *Preslia* 78: 389-404.

Daehler, C.C. 2003. Performance Comparisons of Co-Occurring Native and Alien Invasive Plants: Implications for Conservation and Restoration. *Annual Review of Ecology, Evolution, and Systematics* 34: 183-211.

Daehler, C.C. & Carino, D.A. 1998. Recent Replacement of Native Pili Grass (*Heteropogon contortus*) by Invasive Grasses in the Hawaiian Islands. *Pacific Science* 52: 220-227.

Daubenmire, R. 1972. Ecology of Hyparrhenia rufa (Nees) in Derived Savanna in North-Western Costa Rica. *Journal of Applied Ecology* 9: 11-23.

David, A. & Menges, E. 2011. Microhabitat preference constrains invasive spread of non-native natal grass (Melinis repens (Willd.) Zizka). *Biological Invasions* 13: 2309-2322.

Davidson, A.M., Jennions, M. & Nicotra, A.B. 2011. Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. *Ecology Letters* 14: 419-431.

Davis, M.A., Grime, J.P. & Thompson, K. 2000. Fluctuating resources in plant communities: a general theory of invasibility. *Journal of Ecology* 88: 528-534.

Dawson, W., Fischer, M. & van Kleunen, M. 2011. The maximum relative growth rate of common UK plant species is positively associated with their global invasiveness. *Global Ecology and Biogeography* 20: 299-306.

Deregibus, V.A., Sanchez, R.A. & Casal, J.J. 1983. Effects of Light Quality on Tiller Production in Lolium spp. *Plant Physiology* 72: 900-902.

Deregibus, V.A., Sanchez, R.A., Casal, J.J. & Trlica, M.J. 1985. Tillering Responses to Enrichment of Red Light Beneath the Canopy in a Humid Natural Grassland. *Journal of Applied Ecology* 22: 199-206.

Dodonov, P., Xavier, R.d.O., Tiberio, F.C.d.S., Lucena, I.C.d., Zanelli, C.B. & Silva Matos, D.M.d. 2014. Driving factors of small-scale variability in a savanna plant population after a fire. *Acta Oecologica* 56: 47-55.

Durigan, G., Siqueira, M.F.d. & Franco, G.A.D.C. 2007. Threats to the Cerrado remnants of the state of São Paulo, Brazil. *Scientia Agricola* 64: 355-363.

Dyer, A.R., Hardison, J.L. & Rice, K.J. 2012. Phenology constrains opportunistic growth response in Bromus tectorum L. *Plant Ecology* 213: 103-112.

Edwards, E.J. & Still, C.J. 2008. Climate, phylogeny and the ecological distribution of C4 grasses. *Ecology Letters* 11: 266-276.

Ezaki, B., Nagao, E., Yamamoto, Y., Nakashima, S. & Enomoto, T. 2008. Wild plants, Andropogon virginicus L. and Miscanthus sinensis Anders, are tolerant to multiple stresses including aluminum, heavy metals and oxidative stresses. *Plant Cell Reports* 27: 951-961.

Forrestel, E.J., Donoghue, M.J. & Smith, M.D. 2015. Functional differences between dominant grasses drive divergent responses to large herbivore loss in mesic savanna grasslands of North America and South Africa. *Journal of Ecology* 103: 714-724.

França, H., Ramos Neto, M.B. & Setzer, A. 2007. *O fogo no Parque Nacional das Emas*. Ministério do Meio Ambiente, Brasília.

Fransen, B., de Kroon, H. & Berendse, F. 1998. Root morphological plasticity and nutrient acquisition of perennial grass species from habitats of different nutrient availability. *Oecologia* 115: 351-358.

Frazer, G.W., Canham, C.D. & Lertzman, K.P. 1999. Gap Light Analyzer (GLA), Version 2.0: Imaging software to extract canopy structure and gap light transmission indices from true-colour fisheye photographs, users manual and program documentation. In. Simon Fraser University/ Institute of Ecosystem Studies, Burnaby/Millbrook, .

Glémin, S. & Bataillon, T. 2009. A comparative view of the evolution of grasses under domestication. *New Phytologist* 183: 273-290.

Goergen, E. & Daehler, C. 2002. Factors affecting seedling recruitment in an invasive grass (Pennisetum setaceum) and a native grass (Heteropogon contortus) in the Hawaiian Islands. *Plant Ecology* 161: 147-156.

Goodland, R. & Ferri, M.G. 1979. *Ecologia do Cerrado*. Editora da Universidade de São Paulo, São Paulo.

Gottsberger, G. & Silberbauer-Gottsberger, I. 2006. *Life in the cerrado: a South American tropical seasonal vegetation. Origin, structure, dynamics and plant use*. Reta Verlag, Ulm.

Grime, J.P. 2001. *Plant strategies, vegetation processes, and ecosystems properties.* John Wiley, Chichester.

Grishin, S., del Moral, R., Krestov, P. & Verkholat, V. 1996. Succession following the catastrophic eruption of Ksudach volcano (Kamchatka, 1907). *Vegetatio* 127: 129-153.

Grote, R.d., Lehmann, E., BrÃ¼mmer, C., BrÃ¼ggemann, N., Szarzynski, J.r. & Kunstmann, H. 2009. Modelling and observation of biosphereâ€“atmosphere interactions in natural savannah in Burkina Faso, West Africa. *Physics and Chemistry of the Earth, Parts A/B/C* 34: 251-260.

Guenni, O., Marín, D. & Baruch, Z. 2002. Responses to drought of five *Brachiaria* species. I. Biomass production, leaf growth, root distribution, water use and forage quality. *Plant and Soil* 243: 229-241.

Haase, R. 1990. Community composition and soil properties in northern Bolivian savanna vegetation. *Journal of Vegetation Science* 1: 345-352.

Hartnett, D.C., Wilson, G.W.T., Ott, J.P. & Setshogo, M. 2013. Variation in root system traits among African semi-arid savanna grasses: Implications for drought tolerance. *Austral Ecology* 38: 383-392.

Hierro, J.L., Maron, J.L. & Callaway, R.M. 2005. A biogeographical approach to plant invasions: the importance of studying exotics in their introduced and native range. *Journal of Ecology* 93: 5-15.

Hodgkins, E.J. 1958. Effects of Fire on Undergrowth Vegetation in Upland Southern Pine Forests. *Ecology* 39: 36-46.

Hoffmann, W.A. & Haridasan, M. 2008. The invasive grass, Melinis minutiflora, inhibits tree regeneration in a Neotropical savanna. *Austral Ecology* 33: 29-36.

Hoffmann, W.A., Lucatelli, V.M.P.C., Silva, F.J., Azeuedo, I.N.C., Marinho, M.d.S., Albuquerque, A.M.S., Lopes, A.d.O. & Moreira, S.P. 2004. Impact of the invasive alien grass Melinis minutiflora at the savanna-forest ecotone in the Brazilian Cerrado. *Diversity and Distributions* 10: 99-103.

Hughes, F. & Vitousek, P. 1993. Barriers to shrub reestablishment following fire in the seasonal submontane zone of Hawai'i. *Oecologia* 93: 557-563.

Hughes, F., Vitousek, P.M. & Tunison, T. 1991. Alien Grass Invasion and Fire In the Seasonal Submontane Zone of Hawai'i. *Ecology* 72: 743-747.

Jackson, R. & Bartolome, J. 2002. A state-transition approach to understanding nonequilibrium plant community dynamics in Californian grasslands. *Plant Ecology* 162: 49-65.

Jentsch, A., Kreyling, J., Boettcher-Treschkow, J. & Beierkuhnlein, C. 2009. Beyond gradual warming: extreme weather events alter flower phenology of European grassland and heath species. *Global Change Biology* 15: 837-849.

Jolly, D., Harrison, S.P., Damnati, B. & Bonnefille, R. 1998. Simulated climate and biomes of Africa during the late quaternary: comparison with pollen and lake status data. *Quaternary Science Reviews* 17: 629-657.

Katahira, L.K. & Stone, C.P. 1982. Status of management of feral goats in Hawaii Volcanoes National Park. In. Western Region, National Park Service.

Klink, C.A. 1994. Effects of Clipping on Size and Tillering of Native and African Grasses of the Brazilian Savannas (The Cerrado). *Oikos* 70: 365-376.

Klink, C.A. & Joly, C.A. 1989. Identification and Distribution of C3 and C4 Grasses in Open and Shaded Habitats in Sao Paulo State, Brazil. *Biotropica* 21: 30-34.

Končalová, H. 1990. Anatomical adaptations to waterlogging in roots of wetland graminoids: limitations and drawbacks. *Aquatic Botany* 38: 127-134.

Kovach 2004. Oriana for Windows. In. Kovach Computing Services. , Wales.

Larcher, W. 2003. *Physiological Plant Ecology*.4 ed. Springer, Berlin.

Ledru, M.P. 2002. Late Quaternary history and evolution of the cerradões as revealed by palynological record. In: OLIVEIRA, P.S. & MARQUIS, R.J. (eds.) *The cerrado of Brazil: ecology and natural history of a Neotropical savanna*. Columbia University Press, New York.

Lemos-Filho, J., Barros, C., Dantas, G., Dias, L. & Mendes, R. 2010. Spatial and temporal variability of canopy cover and understory light in a Cerrado of Southern Brazil. *Brazilian Journal of Biology* 70: 19-24.

Lenoir, J., Gégout, J.C., Marquet, P.A., Ruffray, P.d. & Brisse, H. 2008. A significant upward shift in plant species optimum elevation during the 20th century. *Sience* 320: 1768-1771.

Lichtenthaler, H.K. 1996. Vegetation Stress: an Introduction to the Stress Concept in Plants. *Journal of Plant Physiology* 148: 4-14.

Lonsdale, W.M. 1994. Inviting trouble: Introduced pasture species in northern Australia. *Australian Journal of Ecology* 19: 345-354.

Ludlow, M.M. 1980. Stress physiology of Trpical Pasture Plants. *Tropical Grasslands* 14: 136-145.

MacDougall, A.S., Boucher, J., Turkington, R., Bradfield, G.E. & Pillar, V.D. 2006. Patterns of plant invasion along an environmental stress gradient. *Journal of Vegetation Science* 17: 47-56.

Malin, M.C., Dzurisin, D. & Sharp, R.P. 1983. Stripping of Keanakakoi tephra on Kilauea Volcano, Hawaii. *Geological Society of America Bulletin* 94: 1148-1158.

Matesanz, S., Escudero, A. & Valladares, F. 2008. Additive effects of a potentially invasive grass and water stress on the performance of seedlings of gypsum specialists. *Applied Vegetation Science* 11: 287-296.

Matson, P. 1990. Plant-soil interactions in primary succession at Hawaii Volcanoes National Park. *Oecologia* 85: 241-246.

Michelan, T.S., Thomaz, S.M., Mormul, R.P. & Carvalho, P. 2010. Effects of an exotic invasive macrophyte (tropical signalgrass) on native plant community composition, species richness and functional diversity. *Freshwater Biology* 55: 1315-1326.

Nicholson, S.E. 2000. The nature of rainfall variability over Africa on time scales of decades to millenia. *Global and Planetary Change* 26: 137-158.

Ning, J. & Cumming, J.R. 2001. Arbuscular mycorrhizal fungi alter phosphorus relations of broomsedge (Andropogon virginicus L.) plants. *Journal of Experimental Botany* 52: 1883-1891.

Novak, S.J. 2007. The role of evolution in the invasion process. *Proceedings of the National Academy of Sciences* 104: 3671-3672.

Ogle, S.M., Reiners, W.A. & Gerow, K.G. 2003. Impacts of Exotic Annual Brome Grasses (Bromus spp.) on Ecosystem Properties of Northern Mixed Grass Prairie. *The American Midland Naturalist* 149: 46-58.

Ollerton, J. & Lack, A.J. 1992. Flowering phenology: An example of relaxation of natural selection? *Trends in Ecology & Evolution* 7: 274-276.

Otte, M.L. 2001. What is stress to a wetland plant? *Environmental and Experimental Botany* 46: 195-202.

Owen-Smith, N. 2013. Contrasts in the large herbivore faunas of the southern continents in the late Pleistocene and the ecological implications for human origins. *Journal of Biogeography* 40: 1215-1224.

Parmentier, I. 2003. Study of the vegetation composition in three inselbergs from Continental Equatorial Guinea (Western Central Africa): effects pf site, soil factor and position relative to forest fringe. *Belgian Journal of Botany* 136: 63-72.

Parsons, J.J. 1972. Spread of African Pasture Grasses to the American Tropics. *Journal of Range Management* 25: 12-17.

Patterson, B. & Pascual, R. 1968. The Fossil Mammal Fauna of South America. *The Quarterly Review of Biology* 43: 409-451.

Pau, S. & Still, C.J. 2014. Phenology and Productivity of C<sub>3</sub> and C<sub>4</sub> Grasslands in Hawaii. *Plos One* 9: e107396.

Pearcy, R.W. & Ehleringer, J. 1984. Comparative ecophysiology of C3 and C4 plants. *Plant, Cell & Environment* 7: 1-13.

Peichoto, M., Mazza, S. & Neffa, V. 2008. Morphometric analysis of Schizachyrium condensatum (Poaceae) and related species. *Plant Systematics and Evolution* 276: 177-189.

Peichoto, M.C. 2010. Revisión taxonómica de las especies del género Schizachyrium (Poaceae: Andropogoneae) de Sudamérica. *Condollea* 65: 301-346.

Pérez, F.L. 2000. The influence of surface volcaniclastic layers from Haleakala (Maui, Hawaii) on soil water conservation. *CATENA* 38: 301-332.

Peters, E.J. & Lowance, S.A. 1974. Fertility and Management Treatments to Control Broomsedge in Pastures. *Weed Science* 22: 201-205.

Pinheiro, J., Bates, D., DebRoy, S. & Deepayan, S. 2014. nlme: Linear and Nonlinear Mixed Effects Models

In. R package version 3.1-118.

Pinheiro, J.C. & Bates, D.M. 2000. *Mixed-Effects Models in S and S-PLUS*. Springer

Pinheiro, M. & Monteiro, R. 2010. Contribution to the discussions on the origin of the cerrado biome: Brazilian savanna. *Brazilian Journal of Biology* 70: 95-102.

Pivello, V.G., Shida, C.N. & Meirelles, S.T. 1999a. Alien grasses in Brazilian savannas: a threat to the biodiversity. *Biodiversity and Conservation* 8: 1281-1294.

Pivello, V.R., Carvalho, V.M.C., Lopes, P.F., Peccinini, A.A. & Rosso, S. 1999b. Abundance and Distribution of Native and Alien Grasses in a “Cerrado” (Brazilian Savanna) Biological Reserve1. *Biotropica* 31: 71-82.

Possley, J. & Maschinski, J. 2006. Competitive Effects of the Invasive Grass Rhynchelytrum repens (Willd.) C.E. Hubb. on Pine Rockland Vegetation. *Natural Areas Journal* 26: 391-395.

Pratt, C. & Lottermoser, B. 2007. Trace metal uptake by the grass Melinis repens from roadside soils and sediments, tropical Australia. *Environmental Geology* 52: 1651-1662.

Price, J.P. 2004. Floristic biogeography of the Hawaiian Islands: influences of area, environment and paleogeography. *Journal of Biogeography* 31: 487-500.

R.Development.Core.Team 2014. R: A language and environment for statistical computing. . In. R Foundation for Statistical Computing, Vienna.

Ramos, D., Diniz, P. & Valls, J.M. 2014. Habitat filtering and interspecific competition influence phenological diversity in an assemblage of Neotropical savanna grasses. *Brazilian Journal of Botany* 37: 29-36.

Ratter, J.A., Ribeiro, J.F. & Bridgewater, S. 1997. The Brazilian Cerrado Vegetation and Threats to its Biodiversity. *Annals of Botany* 80: 223-230.

Rejmánek, M. & Richardson, D.M. 1996. What Attributes Make Some Plant Species More Invasive? *Ecology* 77: 1655-1661.

Rice, E.L. 1972. Allelopathic Effects of Andropogon virginicus and its Persistence in Old Fields. *American Journal of Botany* 59: 752-755.

Rice, K.J. & Nagy, E.S. 2000. Oak canopy effects on the distribution patterns of two annual grasses: the role of competition and soil nutrients. *American Journal of Botany* 87: 1699-1706.

Richards, C.L., Bossdorf, O., Muth, N.Z., Gurevitch, J. & Pigliucci, M. 2006. Jack of all trades, master of some? On the role of phenotypic plasticity in plant invasions. *Ecology Letters* 9: 981-993.

Richardson, A.D., Keenan, T.F., Migliavacca, M., Ryu, Y., Sonnentag, O. & Toomey, M. 2013. Climate change, phenology, and phenological control of vegetation feedbacks to the climate system. *Agricultural and Forest Meteorology* 169: 156-173.

Rossiter, N.A., Setterfield, S.A., Douglas, M.M. & Hutley, L.B. 2003. Testing the grass-fire cycle: alien grass invasion in the tropical savannas of northern Australia. *Diversity and Distributions* 9: 169-176.

Roux, X.L. & Bariac, T. 1998. Seasonal variations in soil, grass and shrub water status in a West African humid savanna. *Oecologia* 113: 456-466.

Rubio, G., Casasola, G. & Lavado, R.S. 1995. Adaptations and biomass production of two grasses in response to waterlogging and soil nutrient enrichment. *Oecologia* 102: 102-105.

Rundel, P.W. 1980. The ecological distribution of C4 and C3 grasses in the Hawaiian Islands. *Oecologia* 45: 354-359.

Salazar, L.F., Nobre, C.A. & Oyama, M.D. 2007. Climate change consequences on the biome distribution in tropical South America. *Geophysical Research Letters* 34: L09708.

Sarmiento, G. 1992. Adaptive strategies of perennial grasses in South American savannas. *Journal of Vegetation Science* 3: 325-336.

Sarmiento, G. 1984. *The Ecology of Neotropical Savannas*. Harvard University Press, Cambridge.

Sarmiento, G. & Pinillos, M. 2001. Patterns and processes in a seasonally flooded tropical plain: the Apure Llanos, Venezuela. *Journal of Biogeography* 28: 985-996.

Sarmiento, G., Pinillos, M., Silva, M.P.d. & Acevedo, D. 2004. Effects of soil water regime and grazing on vegetation diversity and production in a hyperseasonal savanna in the Apure Llanos, Venezuela. *Journal of Tropical Ecology* 20: 209-220.

Sarwatt, S. & Mollel, E. 2006. Contry Pasture/Forage Resources Profiles - United Republic of Tanzania. In. FAO, Rome.

Schlichting, C.D. & Levin, D.A. 1986. Phenotypic plasticity: an evolving plant character. *Biological Journal of the Linnean Society* 29: 37-47.

Silva, D.A. 2005. Levantamento do meio físico das estações Ecológica e Experimental de Itirapina, São Paulo, Brasil. *Revisto do Instituto Florestal* 17: 113-128.

Silva, D.A.D. & Klink, C.A. 2001. Dinâmica de foliação e perfilhamento de duas gramíneas C4 e uma C3 nativas do Cerrado. *Brazilian Journal of Botany* 24: 441-446.

Silva, J.F. & Ataroff, M. 1985. Phenology, seed crop and germination of coexisting grass species from a tropical savanna in western Venezuela. *Acta Oecologia* 6: 41-51.

Silva, J.S.O. & Haridasan, M. 2007. Acúmulo de biomassa aérea e concentração de nutrientes em Melinis minutiflora P. Beauv. e gramíneas nativas do cerrado. *Brazilian Journal of Botany* 30: 337-344.

Souza, A., de Paula, A.C.F. & Figueiredo-Ribeiro, R.C.L. 2004. Effects of irradiance on non-structural carbohydrates, growth, and hypoglycemic activity of *Rhynchelytrum repens* (Willd.) C.E. Hubb. (Poaceae). . *Brazilian Journal of Biology* 64: 9.

Spaans, E.A. & Baker, J. 1992. Calibration of Watermark soil moisture sensors for soil matric potential and temperature. *Plant and Soil* 143: 213-217.

Tannus, J.L.S. & Assis, M.A. 2004. Composição de espécies vasculares de campo sujo e campo úmido em área de cerrado, Itirapina - SP, Brasil. *Brazilian Journal of Botany* 27: 489-506.

Tirado, R. & Pugnaire, F.I. 2005. Community structure and positive interactions in constraining environments. *Oikos* 111: 437-444.

Tunison, J.T., D’Antonio, C.M. & Loh, R. 2001. Fire, grass invasions and revegetation of burned areas in Hawai‘i Volcanoes National Park. In: Galley, K.E. & Wilson, T.P. (eds.) *Proceedings of the Invasive Species Workshop: the role of fire in the controls and spread of invasive species.* Allen Press, Kansas.

Turpe, A.M. 1984. REVISION OF THE SOUTH AMERICAN SPECIES OF SCHIZACHYRIUM GRAMINEAE. *Kew Bulletin* 39: 169-178.

van der Hammen, T. 1974. The Pleistocene Changes of Vegetation and Climate in Tropical South America. *Journal of Biogeography* 1: 3-26.

van Kleunen, M., Dawson, W., Schlaepfer, D., Jeschke, J.M. & Fischer, M. 2010. Are invaders different? A conceptual framework of comparative approaches for assessing determinants of invasiveness. *Ecology Letters* 13: 947-958.

Van Kleunen, M., Weber, E. & Fischer, M. 2009. A meta-analysis of trait differences between invasive and non-invasive plant species. *Ecology Letters* 13: 235-245.

Vervoort, R.W. & van der Zee, S.E.A.T.M. 2008. Simulating the effect of capillary flux on the soil water balance in a stochastic ecohydrological framework. *Water Resources Research* 44: W08425.

Vilà, M., Espinar, J.L., Hejda, M., Hulme, P.E., Jarošík, V., Maron, J.L., Pergl, J., Schaffner, U., Sun, Y. & Pyšek, P. 2011. Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. *Ecology Letters* 14: 702-708.

Vitousek, P., Walker, L., Whiteaker, L. & Matson, P. 1993. Nutrient limitations to plant growth during primary succession in Hawaii Volcanoes National Park. *Biogeochemistry* 23: 197-215.

Vitousek, P.M. 2004. *Nutrient Cycling and Limitation: Hawai'i as a Model System*. Princeton University Press, Princeton.

Vitousek, P.M., Loope, L.L. & Stone, C.P. 1987a. Introduced species in Hawaii. biological effects and opportunities for ecological research. *Trends in Ecology & Evolution* 2: 224-227.

Vitousek, P.M., Walker, L.R., Whiteaker, L.D., Mueller-Dombois, D. & Matson, P.A. 1987b. Biological Invasion by Myrica faya Alters Ecosystem Development in Hawaii. *Science* 238: 802-804.

Volis, S. 2007. Correlated patterns of variation in phenology and seed production in populations of two annual grasses along an aridity gradient. *Evolutionary Ecology* 21: 381-393.

Wagner, W.L., Herbst, D.R., Khan, N. & Flynn, T. 2012. Hawaiian vascular plants updates: a supplement to the Manual of the flowering plants of hawai'i and Hawai'i's ferns and fern allies. In. Smithsonian Institute/ National Tropical Botanical Garden.

Wan, C. & Sosebee, R.E. 1998. Tillering responses to red:far-red light ratio during different phenological stages in Eragrostis curvula. *Environmental and Experimental Botany* 40: 247-254.

Wegner, L.H. 2010. Oxigen trasport in waterlogged plants. In: Mancuso, S.C., S. (ed.) *Waterlogging Signalling and tolerance in Plants*. Springer, Berlin.

Whitcraft, C., Talley, D., Crooks, J., Boland, J. & Gaskin, J. 2007. Invasion of tamarisk (Tamarix spp.) in a southern California salt marsh. *Biological Invasions* 9: 875-879.

Whittaker, R.H. 1956. Vegetation of the Great Smoky Mountains. *Ecological Monographs* 26: 1-80.

Williams, D. & Baruch, Z. 2000. African Grass Invasion in the Americas: Ecosystem Consequences and the Role of Ecophysiology. *Biological Invasions* 2: 123-140.

Williams, D.G. & Black, R.A. 1994. Drought response of a native and introduced Hawaiian grass. *Oecologia* 97: 512-519.

Winemiller, K.O. & Pianka, E.R. 1990. Organization in Natural Assemblages of Desert Lizards and Tropical Fishes. *Ecological Monographs* 60: 27-55.

Wolkovich, E.M. & Cleland, E.E. 2010. The phenology of plant invasions: a community ecology perspective. *Frontiers in Ecology and the Environment* 9: 287-294.

Wood, S.N. 2011. Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. *Journal of the Royal Statistical Society: Series B (Statistical Methodology)* 73: 3-36.

Zenni, R., Lamy, J.-B., Lamarque, L. & Porté, A. 2014. Adaptive evolution and phenotypic plasticity during naturalization and spread of invasive species: implications for tree invasion biology. *Biological Invasions* 16: 635-644.

Zhang, J. 2013. Spaa: SPecies Association Analysis. R package version 0.2.1. http://CRAN.R-project.org/package=spaa. In.

Zimmermann, J., Higgins, S.I., Grimm, V., Hoffmann, J. & Linstadter, A. 2010. Grass mortality in semi-arid savanna: The role of fire, competition and self-shading. *Perspectives in Plant Ecology Evolution and Systematics* 12: 1-8.

Zuur, A., Ieno, E.N., Walker, N., Saveliev, A.A. & Smith, G.M. 2009. *Mixed effects models and extensions in ecology with R*. Springer, Berlin.