UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

FACULDADE DE AGRONOMIA

PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOTECNIA

IMPACTO DE PRÁTICAS DE INTENSIFICAÇÃO EM ATRIBUTOS ECOSSISTÊMICOS DO CAMPO NATIVO

MARTÍN ALEJANDRO JAURENA BARRIOS

Engenheiro Agrônomo/UdelaR
Mestre em Ciencias Agrárias/UdelaR

Tese apresentada como um dos requisitos à obtenção do Grau de Doutor em Zootecnia

Área de Concentração Plantas Forrageiras

Porto Alegre (RS), Brasil

Outubro, 2016.

CIP - Catalogação na Publicação

Jaurena Barrios, Martín Alejandro
Impacto de práticas de intensificação em atributos ecossistêmicos do campo nativo / Martín Alejandro Jaurena Barrios. -- 2016.
94 f.

Orientador: Carlos Nabinger.

Tese (Doutorado) -- Universidade Federal do Rio Grande do Sul, Faculdade de Agronomia, Programa de Pós-Graduação em Zootecnia, Porto Alegre, BR-RS, 2016.

1. diversidade de espécies. 2. estabilidade temporal. 3. irrigação. 4. adubação. 5. produtividade. I. Nabinger, Carlos, orient. II. Título.

Elaborada pelo Sistema de Geração Automática de Ficha Catalográfica da UFRGS com os dados fornecidos pelo(a) autor(a).

MARTIN ALEJANDRO JAURENA BARRIOS
Engenheiro Agrônomo e Mestre em Ciências Agrárias

TESE

Submetida como parte dos requisitos
para obtenção do Grau de

DOUTOR EM ZOOTECNIA

Programa de Pós-Graduação em Zootecnia
Faculdade de Agronomia
Universidade Federal do Rio Grande do Sul
Porto Alegre (RS), Brasil

Aprovada em: 07/10/2016
Pela Banca Examinadora

Homologado em: 23.11.2016
Por

CARLOS NABINGER
PPG Zootecnia/UFRGS
Orientador

PAULO CÉSAR DE FACCIO CARVALHO
Coordenador do Programa de
Pós-Graduação em Zootecnia

Carolina Bremm
CAROLINA BREMM
PPG Zootecnia - UFRGS

OLIVIER JEAN FRANÇOIS BONNET
UFPel

GERHARD ERNEST OVERBECK
PPG Bôtanica - UFRGS

Selbach
PEDRO ALBERTO SELBACH
Diretor da Faculdade de Agronomia

DEDICATÓRIA

A mis amores March, Toto e Inesita

"Lo intentaste. Fracasaste. No importa. Inténtalo de nuevo.
Fracasa otra vez. Fracasa mejor"

(Samuel Beckett)

AGRADECIMENTOS

Ao Instituto Nacional de Investigación Agropecuaria de Uruguay (INIA) pelo estimulo para minha formação e liberação para realizar o doutorado.

À Agencia Nacional de Investigación e Innovación de Uruguay (ANNI) pela concessão da bolsa de estudos.

À UFRGS e ao Programa de Pós Graduação em Zootecnia da pela oportunidade de participar do seu sistema de formação.

Ao Professor orientador Carlos Nabinger, exemplo de ser humano, professor e pesquisador com proximidade ao produtor pecuário. Agradeço pela oportunidade, pela dedicação à orientação e pela experiência transmitida.

Ao Professores da banca: Carolina Bremm, Olivier Bonnet e Gerhard Overbeck pelas sugestões para a melhoria do manuscrito.

Ao pesquisador Fernando Lattanzi, pelos conselhos para focalizar o trabalho e pelos tantos incentivos e sugestões para melhorar os manuscritos.

Aos pesquisadores e professores Pablo Cruz, Gilles Lemaire, Homero Bergamaschi, Paulo Carvalho e Gerard Overbeck pelas sugestões para melhorar o projeto de pesquisa.

Ao pessoal de INIA que foram meus braços no campo, especialmente a Saulo, Juan, “torito” y “vasquito” com os quais é um prazer trabalhar juntos no campo.

Aos colegas do programa Pasturas y Forrajes de INIA, especialmente Diego Giorello, Gerónimo Cardozo e Rafael Reyno por suas sugestões para melhorar o trabalho de campo e os manuscritos.

Ao casal de amigos e companheiros da República Diamantina Jean e Patrícia, obrigado pela amizade, e pela ajuda para melhorar o “portunhol” da tese.

Aos companheiros da Pós-Graduação: Jean, Patrícia, Pablo, João, Lucas, Juliana, Marlon, Geraldo, Marcelo Fett, Marcelo Tischler, Éder, Laion e muitos outros com quem compartilhei discussões em meio de mates e asados.

Aos colegas de INIA: Fernando Lattanzi, Federigo Condón, Claudia Marchesi, Gonzalo Carracelas, Virginia Porcile e Fiorella Cazzulli pelas sugestões para melhorar o inglês dos artigos.

Também agradeço aos estagiários e passantes: Rodrigo, William, André, Eduardo, Luis Fernando, Sylvio, Denise, Carolina, Fabricio, Alejandro, Romina e Cyntia que muito colaboraram com a execução do trabalho de campo.

A todos os que de alguma forma contribuíram para que eu pudesse chegar a esta importante realização. Obrigado y muchas gracias.

Impacto de praticas de intensificação em atributos ecossistêmicos do campo nativo [1]

Autor: Martín Alejandro Jaurena Barrios
Orientador: Carlos Nabinger

Resumo – O manejo de campo nativo apresenta o desafio de desenvolver estratégias de intensificação sustentável para superar o dilema produção-conservação. Embora a intensificação da produção através da agregação de insumos (fertilizantes e/ou sementes) seja possível, também pode causar mudanças negativas na sustentabilidade. Apesar das quantificações preliminares, existe uma lacuna no conhecimento acerca de como e quando são alteradas a diversidade, a estabilidade e a resiliência em processos de intensificação. Neste sentido, esta tese se concentra em identificar, através de dois experimentos: i) os principais fatores de controle da estabilidade temporal da produtividade de pastagens enriquecidas com nitrogênio (N), fósforo (P) e água; e ii) determinar os efeitos da fertilização fosfatada na diversidade de espécies e no estado da vegetação em campos nativos sobressemeados com leguminosas exóticas. O primeiro experimento apresenta o efeito de quatro anos de adição de N, P e água na produtividade e estabilidade temporal da produção. A adição de N e N + P diminuiu a estabilidade, processo associado à redução da constância da produção das espécies mais dominantes, enquanto que as adições de P e água incrementaram a estabilidade devido ao aumento da assincronia das espécies. Estas duas formas de controle da estabilidade são alternativas e a expressão está regulada pela limitação de N. Contrariamente ao proposto na teoria predominante "diversidade-estabilidade", os resultados deste trabalho indicam que a estabilidade da produção depende principalmente do comportamento das espécies dominantes e não da diversidade de espécies. O segundo experimento expõe o efeito de mais de 10 anos de fertilização fosfatada na diversidade de espécies de um campo nativo sobressemeado com *Trifolium repens* e *Lotus corniculatus.* As respostas em riqueza e diversidade de espécies se relacionaram negativamente com a concentração de P do solo. Foram identificados limiares de concentração de P a partir dos quais se alcançaram as perdas máximas de diversidade e também foi desenvolvido um modelo de estados e transições da vegetação. Em síntese, esta tese contribui para ampliar o conhecimento do impacto de práticas de melhoramento em atributos de sustentabilidade (produtividade, diversidade, estabilidade, e resiliência) dos pastos nativos do bioma Pampa. A continuação destes esforços permitirá avanços na definição dos limites da intensificação baseados em critérios ecológicos, e assim contribuirá com o desafio de desenvolver estratégias de manejo sustentável.

Palavras chave: diversidade de espécies, estabilidade temporal, irrigação, fertilização, fósforo, nitrogênio, pastos nativos.

---

[1] Tese de Doutorado em Zootecnia – Plantas forrageiras, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil (94 p.), Outubro, 2016.

Impact of management intensification on native grassland ecosystems attributes [1]

Author: Martín Alejandro Jaurena Barrios
Advisor: Carlos Nabinger

Abstract - Native grassland management faces the challenge to develop sustainable intensification strategies to overcome the dilemma between production and conservation. Although the intensification of the production through addition of inputs (fertilizers and/or seeds) to be possible, it can potentially induces negative changes in the sustainability of the ecosystems. There is a gap in the knowledge about how and when diversity, stability and resilience are altered in the intensification process. The main body of this thesis consist of two experiments focused on: i) identifying the main factors that control the temporal stability of productivity of a native grassland enriched with nitrogen (N), phosphorus (P) and water; and ii) determining the effects of phosphorus fertilization in the diversity and the state of the vegetation of a native grassland overseeded with exotic legumes. The first experiment analyzed the effect of four years of N, P and water addition on the productivity and the temporal stability of a native grassland production. The single addition of N or combined with P decreased the temporal stability by reducing the constancy of the production of the most dominant species, whereas the additions of P and water increased the stability by increasing species asynchrony. Both controls of temporal stability appear to be alternative mechanisms, and the specific expression of each one would be regulated by the limitation of N. In the second experiment, the long term effects of two P fertilization doses on species richness and diversity of a native grassland overseeded with *Trifolium repens* and *Lotus corniculatus* were examinated. Extractable soil P was negatively related with species richness and diversity. Additionally, P concentration thresholds related to the maximum loss of species richness and diversity were identified, and a model of the states and transitions of the vegetation was developed. In summary, this thesis contributes to the understanding of the impact of intensification practices on sustainability attributes (productivity, diversity, stability and resilience) of native grasslands. The continuation of this research will focus on a detailed definition of the thresholds of the intensification based on ecological criteria, and thus contribute to the challenge to develop sustainable systems.

Key words: fertilization, native grassland, nitrogen, phosphorus, species diversity, temporal stability.

---

[1] Doctoral thesis in Forage Science – Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil. (94 p.), October, 2016.

SUMÁRIO

RELAÇÃO DE TABELAS

CAPÍTULO II pag

CAPÍTULO III

RELAÇÃO DE FIGURAS

CAPÍTULO II pag

CAPÍTULO III

RELAÇÃO DE APÊNDICES

CAPÍTULO II

CAPÍTULO IV

LISTA DE ABREVIATURAS

ANPP	Above-ground Net Primary Production
CEC	Cationic Exchange Capacity
CYDA	*Cynodon dactylon*
DM	Dry Matter
HP	High Phosphorus fertilization
G&Leg	Grasses and legumes
LOMU	*Lolium multiflorum*
MLR	Multiple Linear Regression
N	Nitrogênio / Nitrogen
NG	Native Grassland
NCSPG	Native Cool Season Perennial Grasses
NWSPG	Native Warm Season Perennial Grasses
MP	Moderate Phosphorus fertilization
P	Fósforo / Phosphorus
PPAL	Produção Primária Aérea Líquida
RF	Rainfed
RMSE	Root Mean Square Error
SD	Standard Deviation
SI	Supplementary Irrigation
SW	Shannon Weaver Index
VSWC	Volumetric Soil Water Content
W	Water

CAPÍTULO I

1. INTRODUÇÃO GERAL

1.1 Marco Conceitual

As comunidades de plantas de pastagens são frequentemente utilizadas como ecossistemas modelo para o estudo dos efeitos das mudanças globais (Craine et al., 2013; Hautier et al., 2015; Shi et al., 2016). Esta situação se relaciona com a facilidade e precisão com que produtividade, diversidade, estabilidade, resistência e resiliência podem ser avaliadas. A produtividade primária aérea líquida (PPAL) é a função mais estudada dos ecossistemas de pastagens devido à possibilidade de integrar processos de múltiplos níveis tróficos e diferentes escalas espaço-temporais (Mc Naughton et al., 1989). A diversidade de espécies é também funcionalmente importante porque, teoricamente, incrementa as categorias de atributos funcionais das plantas, e desta maneira aumenta a variedade de condições em que os ecossistemas podem funcionar. A teoria dominante sugere que mudanças na diversidade levam a subsequentes alterações na estabilidade da PPAL das pastagens (Tilman et al., 2006; Hector et al., 2010; Hautier et al., 2015). No entanto, esta teoria tem sido muito polemizada quanto à falta de mecanismos consistentes que expliquem os efeitos da diversidade, limitando, assim, as possibilidades de entender os processos dos ecossistemas.

As intervenções antrópicas induzem a mudanças em escala global, as quais influenciam a diversidade, produtividade, estabilidade e resiliência dos ecossistemas a nível regional e local (Chapin et al., 2000; Oliver et al., 2015). Especificamente, mudanças no uso do solo, enriquecimento de nutrientes e alterações nos padrões de temperatura e regimes pluviométricos são exemplos de transformações globais mais comuns (Sala et al., 2000; Chapin et al., 2000). Os ecossistemas de campo nativo, caracterizados pela sua alta diversidade espacial e temporal, estão altamente expostos aos efeitos das mudanças globais. Neste sentido, a resposta das pastagens à adição de nutrientes interage com as variações climáticas. Portanto, o ambiente hídrico deve ser considerado no desenvolvimento de estratégias de melhoramento da eficiência do uso de N nas pastagens (Gastal & Lemaire, 2002).

Apesar disso, o entendimento dos processos mediante os quais estas mudanças globais influenciam a diversidade, estabilidade da PPLA e resiliência das pastagens naturais ainda é limitado. Este desconhecimento dificulta a capacidade de predizer futuras alterações nos ecossistemas e há, portanto, necessidade de estudos para entender os mecanismos que regulam a estabilidade e diversidade das pastagens.

1.2 Problema de estudo

Para atender aos futuros desafios climáticos, econômicos e sociais, a produção de alimentos deverá ser mais produtiva, estável e resiliente, minimizando os impactos ambientais (Foley et al., 2005). Neste contexto, um dos desafios mais importantes da pesquisa atual é a articulação da demanda crescente de alimentos com a proteção do meio ambiente (Henle et al., 2008). Para enfrentar esse desafio, o recente paradigma da intensificação ecológica visa promover o uso sustentável da biodiversidade e dos serviços ecossistêmicos associados para desenvolver sistemas eficientes de produção

(Bommarco et al., 2013; Geertsema et al., 2016). Especificamente, os produtores que utilizam os campos nativos do bioma Pampa, na América do Sul, apresentam o desafio de superar o dilema produção-conservação mediante o desenvolvimento de estratégias de intensificação sustentável. Por conseguinte, é necessário o desenvolvimento de práticas específicas de manejo que permitam atingir metas produtivas sem comprometimento ambiental (Lemaire, 2012).

Existem possibilidades de intensificação da produção através da agregação de insumos (fertilizantes e/ou sementes). Embora os ecossistemas de pastagens estejam adaptados às mudanças regulares do ambiente e, até certo ponto, aos distúrbios antrópicos, a aplicação persistente de distúrbios (ex. adição de fertilizantes e introdução de espécies) pode alterar simultaneamente a composição, a diversidade, a estabilidade e a resiliência das comunidades (Tilman & Downing, 1994; MacDougall et al., 2013). Estas mudanças, no médio e longo prazo, podem modificar importantes atributos da estrutura e função das comunidades (Gonzalez & Loreau, 2009), tais como a resistência à invasão de espécies exóticas, e o efeito estabilizador da diversidade de espécies frente às mudanças do clima (MacDougall et al., 2013). A teoria dominante prediz que a combinação dos efeitos das mudanças ambientais e da perda de diversidade aumenta o risco de um colapso abrupto e potencialmente irreversível do ecossistema (Ives & Carpenter, 2007).

Existem evidências de degradação em praticamente todos os experimentos de longo prazo de melhoramento do campo nativo da região, tanto naqueles baseados em fertilização com nitrogênio (N) e fósforo (P), como nos estudos que utilizaram a fertilização juntamente com introdução de espécies exóticas cultivadas. Contudo, os resultados não são conclusivos com relação às principais causas deste fenômeno. Neste contexto, torna-se de grande relevância identificar limiares de fertilização que permitam gerar estratégias de manejo de insumos que evitem a deterioração irreversível do ecossistema. Em outras regiões do mundo, vários antecedentes de pesquisa destacam que a intensificação do ecossistema pastoril via adição de fertilizante nitrogenado diminui tanto a diversidade de espécies como a estabilidade da produção (Tilman et al., 2006; Hector et al., 2010; Hautier et al., 2015). Apesar de haver uma quantificação preliminar da perda de diversidade da vegetação em processos de intensificação em pastagens do bioma Pampa (Nabinger & Carvalho, 2009; De Avila, 2012; Brambilla, 2014), existe um vazio no conhecimento de como e quando são alteradas a diversidade, estabilidade e resiliência em processos de intensificação.

A partir destes antecedentes, os trabalhos científicos da presente tese tiveram a intenção de responder às seguintes questões:

- Quais são os principais fatores e mecanismos que regulam a produtividade e a estabilidade temporal da produção de forragem de pastagens nativas fertilizadas com nitrogênio e fósforo em dois ambientes hídricos contrastantes?

- A dose de fertilizante fosfatado afeta a diversidade de espécies e a dinâmica da vegetação de pastagens nativas sobressemeadas com leguminosas exóticas cultivadas?

1.3 Estrutura da tese

O capitulo I apresenta uma revisão bibliográfica dos diferentes efeitos de alternativas de melhoramento das pastagens nativas do bioma Pampa. Especificamente, se discutem os efeitos de longo prazo da adição de nutrientes e espécies exóticas na dinâmica da vegetação do campo nativo no âmbito de indicadores de sustentabilidade (diversidade, resistência, resiliência e estabilidade) e modelos de estados e transições.

O capitulo II consiste em um artigo científico que reporta os efeitos de quatro anos de adição de nitrogênio, fósforo e água na produtividade, diversidade e estabilidade temporal da produção de um campo nativo do bioma Pampa. Este artigo revela a existência de vias alternativas de controle da estabilidade, demonstrando que a constância da produção das espécies dominantes e as interações das espécies da comunidade regulam a estabilidade temporal da produção de forragem.

O capitulo III é um artigo que descreve os efeitos de longo prazo da dose de fertilização fosfatada na diversidade e riqueza de espécies de um melhoramento de campo nativo sobressemeado com *Trifolium repens* e *Lotus corniculatus*. Neste trabalho se identificaram teores de fósforo disponível no solo que estariam relacionados com a perda de resiliência e mudança de estado da vegetação destas comunidades. Finalmente, o capitulo IV apresenta as conclusões gerais e implicações práticas dos trabalhos, e se propõe futuras linhas de ação.

2. REVISÃO BIBLIOGRÁFICA

2.1 Contexto de uso do campo nativo no bioma Pampa

A pecuária baseada em campo nativo é uma das principais atividades agropecuárias tanto no Uruguai como no sul de Brasil. Esta atividade apresenta uma grande importância ambiental, econômica, social e territorial. No entanto, durante um longo período de tempo, as pastagens do campo nativo têm sido negligenciadas (Overbeck et al., 2007) e foram consideradas como um fator limitante para o desenvolvimento de sistemas eficientes de produção pecuária. No entanto, há um crescente interesse em entender o impacto de diferentes práticas de manejo sobre os serviços ecossistêmicos prestados pelos campos nativos (Carvalho & Batello, 2009; Paruelo & Vallejos, 2013). Estas pastagens formam o ecossistema mais extenso da região (Soriano, 1991) e constituem a base alimentar para a produção pecuária (Nabinger & Carvalho, 2009). Além disso, aportam outros valiosos serviços ecossistêmicos como o sequestro de carbono, a preservação do solo contra a erosão (Sala & Paruelo, 1997) e valores únicos de biodiversidade com mais de três mil espécies vegetais (Burkart, 1975; Boldrini, 2009).

Estes sistemas, que apresentam elevado potencial de aumento da produção (Nabinger & Carvalho, 2009), porém, exibem uma acelerada degradação dos recursos naturais e perda de habitat. Neste contexto, a integridade dos ecossistemas de campo nativo está criticamente ameaçada devido a mudanças no uso da terra (Laterra & Rivas, 2005; Overbeck et al., 2015). A conversão de campos nativos do bioma Pampa em culturas agrícolas e florestais tem levado a uma rápida perda de habitat assim como da biodiversidade e seus serviços ecossistêmicos associados. Consequentemente, os ecossistemas de pastagem natural do bioma Pampa apresentam os índices de risco de conservação mais altos do Brasil (Overbeck et al., 2015).

Apesar da importância econômica da pecuária no sul de Brasil e Uruguai, nos últimos anos está sendo pressionada por outras atividades que competem pelo uso do solo. Neste sentido, a ampliação das áreas agrícolas e florestais ocasionaram nas ultimas décadas perdas de anuais de áreas de campos naturais estimadas em 137 mil hectares no Rio Grande do Sul (Carasaw et al. 2007) e 110 mil hectares no Uruguai (Jaurena et al. 2013). Esta expansão da agricultura desafia aos sistemas tradicionais de produção pecuária baseados em campo nativo a produzir com maior eficiência e se intensificar. Porém, as áreas remanescentes de campo nativo apresentam reduções de vigor (Wagner et al. 2013), tornando-se mais vulneráveis em processos de intensificação. É necessário, portanto, entender os efeitos das práticas de intensificação em um conjunto de atributos que definem a sustentabilidade dos ecossistemas de campo nativo.

2.2 Alternativas de intensificação da produção pecuária baseada em campo nativo

Embora o campo nativo seja um recurso relativamente estável e persistente, o resultado produtivo e seu estado de conservação dependem da intensidade de utilização. Neste sentido, o ajuste da carga animal em função da disponibilidade de forragem é considerado uma prática básica e fundamental para aumentar a produção animal a custo zero (Nabinger & Carvalho, 2009). Após este ajuste, existem outras tecnologias que permitem a intensificação da produção através da agregação de insumos (fertilizantes e/ou sementes). A disponibilidade de nutrientes, especialmente de nitrogênio, é um fator limitante para a produção de forragem do campo nativo nas condições do bioma Pampa (Pallares et al., 2005). Estas respostas estão baseadas no fato de que a maioria das áreas remanescentes de campos nativos se concentram sobre solos com limitações crônicas de nitrogênio (N) e fósforo (P) (Morón, 1994), os quais também apresentam baixa capacidade de armazenamento de água.

Na região do bioma Pampa, várias tecnologias de melhoramento das pastagens têm sido validadas, como por exemplo, a sobressemeadura de leguminosas associada à aplicação de fertilizantes fosfatados (Risso & Berretta, 1997; Soca et al., 2002), a sobressemeadura de leguminosas e gramíneas hibernais combinada com a adição fertilizantes nitrogenados e fosfatados (Ferreira et al., 2011) e a simples fertilização do campo nativo com nitrogênio e fósforo (Bemhaja et al., 1998; Gomes, 2000; Zanoniani et al., 2011). A adubação nitrogenada geralmente incrementa a produção de forragem e a produção animal do campo nativo (Berretta et al 1998; Gomes, 2000; Boggiano et al., 2005; Rodriguez Palma & Rodriguez, 2010). Semelhantemente, a adubação fosfatada incrementa a produção dos melhoramentos com introdução de leguminosas (Ayala & Carámbula, 1996). No entanto, a resposta dos campos nativos à fertilização unicamente fosfatada é muito limitada ou nula, devido principalmente ao escasso aporte das leguminosas nativas (Berretta et al., 1998). Contudo, a contribuição deste elemento poderia ser complementar à fertilização com N, visto que a fertilização nitrogenada incrementa a produção de forragem e, portanto, a demanda de P, ocorrendo consequentemente resposta à adubação conjunta com N e P (Rubio et al., 1997).

Tradicionalmente, considera-se que os déficits individuais de nitrogênio (N), fósforo (P) ou água diminuem o crescimento das plantas forrageiras. Não obstante, teorias emergentes e evidências indicam que estes recursos geralmente operam em forma simultânea e sinérgica, co-limitando à produção das pastagens (Harpole et al., 2011). Neste contexto, Sadras (2005) destaca a importância da limitação conjunta de nitrogênio e água, enquanto Harpole et al. (2011) enfatiza as limitações da produção induzidas por um conjunto de nutrientes. Quando uma limitação é removida, também é relevante identificar os efeitos dessa remoção na dinâmica de outras limitações, já que, por exemplo, a fertilização nitrogenada induziria a uma transição da limitação por N, a uma co-limitação N-P (Vitousek et al., 2010) ou a uma maior limitação por água (Snyman, 2002). Alem disso, Fay et al. (2015) e Eskelinen & Harrison (2015) verificaram a existência de interações ainda mais complexas entre N, P e água que regulam a produtividade das pastagens. Neste sentido, na medida em que o aumento da disponibilidade de água melhora o crescimento das pastagens, espera-se que a contribuição do solo não supra a crescente demanda de N e outros nutrientes, e sejam necessárias aplicações de fertilizantes.

A limitação ou co-limitação hídrica é extremamente importante na região do bioma Pampa, devido à alta probabilidade de ocorrência de déficits hídrico nos períodos de verão (Betolli et al., 2010), o que afeta negativamente a resposta produtiva das pastagens à adubação nitrogenada. Neste contexto, os padrões espaciais e temporais da precipitação apresentam, portanto, importantes efeitos diretos na determinação da produtividade (Sala et al., 1988; Knapp et al., 2001) e indiretos por meio de alterações nas respostas nutricionais. Além dos efeitos dos nutrientes na produção, a composição de espécies das pastagens também é determinada pela disponibilidade de água (Vila & Sardans, 1999; Lezama, 2005). Neste sentido, cada espécie, individualmente, pode responder diferentemente à aplicação isolada ou combinada de N, P e água no solo, podendo ocasionar mudanças na dominância, perda de espécies e consequentemente diminuições na diversidade (Bai et al., 2009). Por outro lado, a fertilização nitrogenada também pode atuar como um filtro ambiental, selecionando espécies e genótipos, especialmente de gramíneas que são mais sensíveis à deficiência hídrica (Snyman, 2002), o que pode incrementar a vulnerabilidade do ecossistema.

2.3 Efeitos de longo prazo da adição de nutrientes e espécies na dinâmica da composição botânica do campo nativo

A dinâmica das comunidades de plantas em geral e do campo nativo em particular, é o resultado das interações interespecíficas. Estas interações são reguladas por fatores do ambiente, tais como as condições climáticas ou o nível nutricional do solo. A forma em que estes processos influenciam a dinâmica da comunidade é de extrema importância para a compreensão das respostas dos ecossistemas de pastagens, tanto em composição (Avolio et al., 2014) e produtividade (Mouquet et al., 2002) como em estabilidade (Hector et al., 2010) e resiliência (Oliver et al., 2015). A dinâmica temporal destas comunidades é o padrão de ocupação no tempo e espaço dos indivíduos e genótipos, o qual determina a distribuição espaço-temporal das funções das plantas, como por exemplo, da produtividade. O conhecimento desta dinâmica ajuda a entender as respostas das comunidades às mudanças existentes no ambiente. No contexto de comunidades diversas, a vegetação é muito mais do que a interface com o ambiente, portanto deve-se considerar como um conjunto de indivíduos, ecótipos, espécies e grupos funcionais compartilhando recursos comuns.

Para analisar a dinâmica da formação do rendimento e a qualidade das pastagens em comunidades diversas, como é o caso do campo nativo, é necessário considerar a vegetação como um conjunto agregado de indivíduos e espécies. Além disso, o estudo do conjunto da comunidade também é importante para compreender como as mudanças na abundância de espécies afetam a competição por recursos e o funcionamento do ecossistema. Nesta circunstância, a competição é definida como as interações entre plantas induzidas pela necessidade de compartilhar recursos, o que leva à diminuição do crescimento e até o desaparecimento de alguns indivíduos (Begon et al., 1996). As comunidades de campo nativo apresentam respostas complexas à fertilização devido à convivência de espécies com diferentes estratégias de competição por luz e recursos do solo. A adição de nutrientes ou água geralmente intensifica a competição, o que pode provocar a perda de espécies em processos de exclusão competitiva.

Para predizer os efeitos da adição crônica de nutrientes, Smith et al. (2009) propuseram um esquema hierárquico, pois a fertilização poderia ter diferentes efeitos no ecossistema em função das mudanças temporais que ocorrem nas comunidades. Inicialmente, a adição de nutrientes, especialmente de nitrogênio, pode estimular diferencialmente o crescimento de algumas espécies e dessa forma incrementar a produtividade. No entanto, a adição de nutrientes também favorece a algumas espécies em detrimento de outras, resultando em mudanças na composição e em uma reestruturação das espécies dominantes (Avolio et al., 2014). De fato, vários estudos têm reportado alterações na composição das comunidades em resposta às adições crônicas de nutrientes no longo prazo (Hejcman et al., 2007; Isbell et al., 2013). Nesta situação, a reestruturação da comunidade pode ter efeitos positivos ou negativos no funcionamento do ecossistema dependendo do modo em que a composição de espécies foi impactada (Smith et al., 2009).

Um dos objetivos do melhoramento de campo nativo é a promoção de um novo equilíbrio da comunidade através da adição de sementes e fertilizantes. A meta principal desta prática de manejo é incrementar o desempenho animal e a produtividade pecuária por meio do aumento da contribuição das espécies nativas e introduzidas de alta produtividade e qualidade nutricional. Apesar dos agro-ecossistemas de pastagens naturais estarem adaptados à variabilidade regular do ambiente e de manejo, o enriquecimento crônico de nutrientes pode transformar ao mesmo tempo tanto a biodiversidade como a abundância relativa das espécies. Estas mudanças, no médio e longo prazo, por sua vez podem causar transformações ainda maiores em outros importantes atributos relacionados com o funcionamento das comunidades (Gonzalez & Loreau, 2009), tais como a estabilidade temporal da produção, resistência e resiliência às flutuações do clima, e a resistência à invasão de espécies exóticas (MacDougall et al., 2013).

A teoria geral da invasibilidade proposta por Davis et al. (2000) prediz que a rápida flutuação dos recursos dos ecossistemas (por exemplo, o rápido enriquecimento de nutrientes em solos com altas limitações) incrementa a probabilidade de invasão por espécies não nativas. Nos casos de melhoramentos de campos nativos, a introdução de espécies e a fertilização persistente, no longo prazo estariam provocando importantes flutuações nos recursos do solo e facilitando as invasões de espécies exóticas. Especificamente, a elevada abundância de nitrogênio em melhoramentos de campo nativo – via direta por fertilização ou indireta através da fixação biológica das leguminosas - promoveria mudanças no estado da vegetação.

O mecanismo que regula a reorganização da composição botânica em situações de adição persistente de nitrogênio não está totalmente claro, já que a adição deste nutriente estaria reduzindo a dimensão dos nichos ecológicos particulares das diferentes espécies (Harpole et al., 2016) e ao mesmo tempo estaria modificando a competição por luz e recursos do solo (Hautier et al., 2009). Por outro lado, também ocorrem mudanças nas interações das gramíneas C4 com fungos micorrízicos, já que quando N e P são abundantes prevê-se que os efeitos da simbiose podem mudar de mutualismo a parasitismo (Johnson, 2010). Portanto, as respostas de curto e longo prazo à fertilização podem ser totalmente diferentes, inclusive opostas, devido à alteração da composição da comunidade ocorrida ao longo do tempo.

Existem evidências de estados degradados da vegetação nas comunidades da maioria dos experimentos de longo prazo da região, tanto naqueles de fertilização com N e P, como nos de adubação combinada com introdução de espécies exóticas (De Avila, 2012; Brambilla, 2014). Apesar disso, ainda não foi possível identificar os principais mecanismos causais da degradação nem gerar medidas de gestão para evitá-lo. Trabalhos desenvolvidos por Willems & van Nieuwstadt (1996) e Isbell et al. (2013) demonstraram que os estados degradados, caracterizados pela baixa diversidade de espécies e dominância de exóticas, podem persistir depois de várias décadas de concluída a fertilização. Neste sentido Tognetti & Chaneton (2012) demonstraram que as espécies exóticas dominantes nos estados degradados restringem o estabelecimento das gramíneas perenes nativas a partir do banco de sementes. Em síntese, estes trabalhos destacam que a adição persistente de fertilizantes afeta negativamente a capacidade de resiliência do campo nativo causando transições difíceis de reverter no médio e longo prazo. Em sistemas melhorados sustentáveis, este colapso da vegetação do campo nativo deveria ser evitado, portanto é necessário desenvolver indicadores precisos que auxiliem no manejo da fertilização e introdução de espécies.

2.4 Modelos de estados e transições aplicados à dinâmica da vegetação em processos de intensificação do campo nativo

Nas situações de enriquecimento crônico de nutrientes, as novas espécies dominantes (geralmente exóticas) apresentam um rol de espécies transformadoras, já que modificam o funcionamento (Richardson et al., 2000) e induzem uma mudança de estado das comunidades. Vários estudos têm demonstrado que a evolução da estrutura e função dos ecossistemas de pastagens não é linear, consequentemente o funcionamento da vegetação poderia ser descrito em modelos de estados e transições (Westoby et al., 1989). Estes modelos descrevem a dinâmica de estados alternativos da vegetação, os quais são separados por limiares, descritos por Westoby et al. (1989) como o limite máximo de distúrbio tolerado pela comunidade precedente a uma mudança de estado. Esta modificação de estado tem importantes implicações do ponto de vista econômico, produtivo e ambiental, já que pode diminuir a resposta a novas alternativas de manejo, causar importantes perdas de diversidade e funções ecológicas, e dessa forma a reversão pode ser muito difícil e custosa (Suding & Hobbs, 2009).

Especificamente, estes modelos constituem um método para organizar informações das complexas relações entre vegetação, solos, animais em pastejo, ciclos hidrológicos e de nutrientes, assim como os efeitos de distúrbios e ações de manejo (Caudle et al., 2013). Os esquemas resultantes destes modelos representam as possíveis condições da vegetação resumidas em estados e fases de uma comunidade determinada. Um estado é caracterizado por um conjunto de espécies que apresentam determinados atributos de resistência e resiliência em relação a eventos de clima e manejo

(Caudle et al., 2013). A maioria dos distúrbios e estresses provocam alterações relativamente reversíveis dentro dos estados (por exemplo, fogo, secas, inundações, hervivoría e outros), enquanto a dinâmica de sucessão das comunidades acontece como fases dentro de um estado. Os diferentes estados são separados por limiares, que representam as condições necessárias para superar a resiliência ecológica e promover a formação de estados alternativos com diferente estrutura e funções ecossistêmicas (Briske et al., 2008).

Nestes modelos, os mecanismos que impedem ou facilitam as trocas de estados estáveis são categorizados (por exemplo, mudanças: demográficas, no banco de sementes e estabelecimento de espécies, na competição por luz, e nas condições dos solos tanto físicas como químicas). Em um contexto de intensificação no manejo do campo nativo, estes modelos seriam muito úteis para reconhecer os estados alternativos da vegetação e para gerar indicadores que contribuiriam a evitar o colapso abrupto e irreversível da vegetação.

2.5 Efeitos da adição de recursos em atributos ecossistêmicos do campo nativo

Um ecossistema pastoril de campo nativo está composto por um conjunto de organismos vivendo na comunidade, interligados uns com outros e com seu ambiente tanto químico como físico. O elevado nível de complexidade organizacional destas comunidades determina impactos diferenciais das práticas de manejo nos diferentes atributos ecossistêmicos (produtividade, estabilidade, diversidade, resiliência e resistência). Portanto, estes ecossistemas apresentam necessidades específicas de manejo para preservar o balanço dos atributos ecossistêmicos. Deste modo, qualquer alternativa de manejo que procure incrementar a produtividade deverá ser ponderada considerando o seu impacto sobre os outros atributos ecossistêmicos. Para atingir este objetivo é necessário considerar um enfoque sistêmico que permita avaliar diferentes alternativas de intensificação mediante indicadores dos diferentes atributos ecossistêmicos.

2.5.1 Efeitos da adição de recursos na diversidade de espécies e grupos funcionais

A diversidade de espécies depende principalmente do número de espécies presentes, de sua abundância relativa e das variações espaciais e temporais destes componentes (Chapin et al., 2002). Se todas as espécies fossem funcionalmente diferentes, contribuiriam da mesma forma aos processos dos ecossistemas, portanto, as taxas dos processos deveriam aumentar linearmente com o número de espécies. No entanto, na prática a relação entre o número de espécies e os processos ecossistêmicos tende a se saturar. Isto acontece porque nas comunidades, por um lado, há espécies ecologicamente similares ou redundantes (Tilman et al., 1996) e, por outro lado, há espécies dominantes que controlam o fluxo de nutriente e energia dos ecossistemas (Grime, 1998). Em consequência, a perda das espécies dominantes afeta significativamente os processos ecossistêmicos.

Além da diversidade de espécies, a especialização fenológica e funcional também pode incrementar a captura de recursos, como é o caso de espécies coexistentes que diferem no momento de máximo crescimento, ou na adaptação a diferentes estresses. Exemplo desta especialização é a mistura de tipos fotossintéticos C3 e C4 característica dos campos nativos do bioma Pampa, a qual permite otimizar o crescimento em diferentes períodos do ano. Adicionalmente, a alternância no tempo e espaço de grupos de espécies com estratégias de captura e conservação de recursos é outra forma de complementaridade funcional que acontece em decorrência das variações nas condições ambientais (por exemplo, no clima e na oferta de forragem) (Cruz et al., 2010).

Frequentemente é reportado que o enriquecimento de nitrogênio (Gough et al., 2000; Suding et al., 2005) de fósforo (Lambers et al., 2010; Ceulemans et al., 2011) ou de múltiplos nutrientes combinados (Harpole et al., 2016) diminui a diversidade de espécies das pastagens naturais. Um dos mecanismos mais reportados para explicar esta diminuição na riqueza de espécies é que a adição de nutrientes incrementa a produtividade das pastagens e ao mesmo tempo aumenta a competição por luz e diminui a concorrência por recursos do solo (Hautier et al., 2009). Na medida em que muda a oferta de recursos no solo, e especialmente quando diminui a limitação de nitrogênio, acontece um incremento na dominância das espécies mais produtivas em detrimento das espécies subordinadas, em um processo comumente reportado como exclusão competitiva. Não obstante, o mecanismo que provoca estas perdas de espécies não está claramente determinado, já que Harpole et al. (2016) reportaram que o número de nutrientes adicionados se relacionou com as perdas de diversidade independentemente dos níveis de produtividade das pastagens.

2.5.2 Efeitos da adição de recursos na resistência e resiliência dos ecossistemas

O modo como as comunidades preservam a sua estrutura e função no tempo é um ponto central das pesquisas ecológicas (Tilman & Downing, 1994). Os processos dos ecossistemas estão em constante variação em resposta as mudanças no ambiente (Wu & Loucks, 1995). Consequentemente, o comportamento dos ecossistemas é sempre influenciado por condições ambientais atuais e por eventos do passado. Neste contexto, um distúrbio é um evento discreto no tempo e espaço que modifica a estrutura da disponibilidade de recursos e ou o ambiente físico (Pickett et al., 1999). A resposta de um ecossistema a um distúrbio pode ser descrita com base em várias propriedades gerais, também chamadas indicadores ou atributos ecossistêmicos. Estas propriedades permitem avaliar a sensibilidade dos ecossistemas às mudanças de estado.

A resistência de um sistema descreve a sua tendência a permanecer no mesmo estado de referência sob um estresse ambiental, o que representa a capacidade para resistir à mudança ante um determinado estresse (Hoover et al., 20014). No contexto da ecologia, a resistência é a capacidade do sistema de manter determinados atributos funcionais e estruturais apesar da perturbação (Chapin et al., 2002). Por exemplo, uma pastagem que apresenta uma baixa diminuição da produção de forragem em uma situação de seca é mais resistente do que outra com alta redução do crescimento quando é submetida a essa mesma perturbação. Neste sentido, ecossistemas que não tem experiência prévia de um distúrbio são muito mais vulneráveis que os que têm um histórico de exposição, já que estas comunidades estão relativamente mais adaptadas às alterações que ocorreram com maior frequência no ambiente.

A resiliência é definida como a capacidade dos ecossistemas de retornar a um estado de referência prévio ao distúrbio (Pimm, 1984). Os sistemas pouco resilientes são aqueles que não recuperam o estado original e rapidamente mudam a outro estado estável, enquanto os sistemas mais resilientes são os que rapidamente voltam ao estado inicial. Neste sentido, os ecossistemas resistentes e resilientes têm propriedades mais estáveis e previsíveis (Holling, 1986). A resiliência não é uma característica própria dos ecossistemas, portanto deve ser definida em relação a distúrbios específicos que afetam potencialmente os sistemas, já que os ecossistemas são altamente resilientes a distúrbios sazonais ou leves. O conhecimento da resiliência sob diferentes distúrbios e estresses severos é considerado um pré-requisito para o delineamento de estratégias de manejo adaptativas, especialmente em face da ameaça das mudanças climáticas (Hulme, 2005).

2.5.3 Efeitos da adição de recursos na produção e estabilidade temporal

Os padrões regionais e globais de produção primária aérea líquida (PPAL) dos ecossistemas de pastagens e suas determinantes têm sido de grande interesse no campo da ecologia e da agronomia. Recentemente, este interesse tem sido intensificado devido às preocupações pelas ameaças globais das mudanças climáticas, deposição de nitrogênio e alterações no uso da terra que modificam os fluxos de carbono e energia dos ecossistemas (Sala et al., 2000; Steffen et al., 2015). Outros fatores como o manejo do pastoreio e a adição de nutrientes via fertilizante, assim como a interação destes com a variabilidade climática também influenciam a PPAL e sua estabilidade temporal. Estas alterações na PPAL poderiam afetar potencialmente a todos os processos ecossistêmicos, portanto detectar as mudanças na produtividade e sua estabilidade temporal tem grande importância para o delineamento de estratégias de manejo e conservação de ecossistemas de pastagens nativas.

Diversos trabalhos realizados em dierentes regiões do mundo reportam uma relação positiva entre a diversidade de espécies e a estabilidade produtiva dos ecossistemas (Tilman et al., 2006; Hector et al., 2010; Hautier et al., 2015). A partir destas evidencias, estes autores propõem uma teoria em que as mudanças na diversidade de espécies seriam a causa principal das alterações na estabilidade da produção e sugerem uma série de mecanismos para explicar esta relação. Um dos mecanismos mais referenciados para explicar a relação diversidade-produtividade é o efeito da assincronia na produção das espécies da comunidade (Yachi & Loreau, 1999; Loreau & De Mazancourt, 2013). Em comunidades com alta assincronia, o declínio na produção em algumas espécies é compensado por aumentos nas outras espécies e, dessa forma, se reduz a variabilidade temporal da produtividade. No entanto, esta teoria tem sido largamente criticada, devido a que as alterações na diversidade e na estabilidade seriam efeitos paralelos, provocadas, por exemplo, pela fertilização com nitrogênio (Huston, 1997). Além disso, outros trabalhos têm evidenciado relações neutras ou negativas entre diversidade e estabilidade (Grman et al., 2010; Yang et al., 2011). A maioria dos trabalhos da teoria “diversidade-estabilidade” têm sido realizados em base a experimentos manipulativos de biodiversidade (ecossistemas artificiais) que não necessariamente predizem influência das espécies em comunidades reais (Wardle, 2016).

Em síntese, considerando a necessidade de preservar a singularidade da diversidade de espécies do bioma Pampa e a oportunidade para desenvolver estratégias de intensificação sustentável, existe uma oportunidade para avançar na pesquisa dos impactos de práticas de melhoramento nos atributos ecossistêmicos do campo nativo.

HIPÓTESES DO TRABALHO

Baseado nas evidências de degradação de pastagens ocorridas no longo prazo em experimentos de melhoramento de campo nativo são apresentadas as seguintes hipóteses:

- A adição de água e nutrientes tem efeitos divergentes na estabilidade da produção do campo nativo (a aplicação de água estabiliza, enquanto a de nutrientes desestabiliza).

- As alterações na diversidade de espécies se relacionam com as mudanças na estabilidade temporal da produção do campo nativo.

- A dose de fertilizante fosfatado acelera, direta ou indiretamente, o declínio da diversidade e incrementa a dominância de espécies exóticas em melhoramentos de campo nativo com leguminosas.

- A introdução de leguminosas, acompanhada da aplicação de fertilizantes fosfatados, provoca mudanças no estado da comunidade de campo nativo (menor diversidade e dominância de espécies exóticas).

OBJETIVOS

- Identificar os principais fatores de controle da produtividade e da estabilidade temporal de campos nativos enriquecidos com água e nutrientes.

- Quantificar em que medida os atributos da comunidade (grupos funcionais, diversidade, dominância e assincronia) influenciam na estabilidade temporal da produção do campo nativo.

- Determinar o efeito do aumento da fertilização fosfatada na diversidade de espécies e invasão de exóticas em campos nativos melhorados com leguminosas.

- Explicar as mudanças de estado da vegetação de campos nativos melhorados com leguminosas num modelo de estados e transições.

CAPÍTULO II

Dominant species and community asynchrony modulate grassland stability responses to management intensification[1]

---

[1] Paper format based on guidelines of Ecology Letters (Apêndice 1)

Dominant species and community asynchrony modulate grassland stability response to management intensification

ABSTRACT

Changes in nutrient and water availability are important drivers of ecosystem stability. Many reports suggest positive effects of diversity on ecosystem productivity. Nevertheless, dominant species responses and community asynchrony were also proposed as factors that regulate stability of productivity. Here, in a species-rich grassland exposed to four years of nitrogen (N), phosphorus (P) and water addition, we found evidence that these management intensification alter stability through dominant species and community asynchrony rather than diversity. Both N and N+P addition reduced stability by increased production variability of the most dominant species, while P and water addition increased stability by increasing species asynchrony. The prevalence of one or another pathway depended on N-limitation, since the control for N effect change the factor regulating stability from dominant species to asynchrony. These findings emphasize that dominant species and temporal complementarities among species can play a key role to improve the predictions of grassland stability under management intensification.

Key words: grassland, nitrogen, phosphorus, species richness, temporal stability, water.

INTRODUCTION

Intensification of grassland management by nutrient **enrichment** and irrigation are important drivers of global change, which are rapidly modifying ecosystem functioning. Increased nitrogen (N) and phosphorus (P) fertilization, and their interactions with alterations in precipitation patterns are expected to influence biodiversity and stability of grasslands ecosystems. Thus, understanding how these drivers of global change affect grassland composition, diversity and stability is critical to improve ecosystem management and biodiversity conservation. A recent synthesis of Hautier et al. (2015) suggest that changes in plant diversity caused by drivers of environmental changes, such as N and water addition among others, would be major factors influencing the stability of above-ground net primary production (ANPP). Most of the research has been focused on the positive relationship between species diversity and ecosystem temporal stability (Tilman et al. 2006; Isbell et al. 2009; Hector et al. 2010; Hautier et al. 2015). In these reports, species richness is positively related to temporal stability: the ratio between the mean and the standard deviation of the productivity (Lehman & Tilman 2000). Several mechanisms have been proposed to explain the diversity-stability relationship. Among them, the effect of species richness on asynchrony (a measure of the compensation capacity in the production of biomass among species) is commonly reported (Yachi & Loreau 1999; Loreau and de Mazancourt 2008).

Although there is substantial evidence that species loss impairs several ecosystem functions and services (Balvanera et al. 2006; Cardinalle et al. 2006), the role of diversity in regulating stability has generated a large debate (Huston 1997; Loreau 1998). On one hand, the underlying mechanisms of these effects are poorly understood (Huston 1997; Ives & Carpenter 2007; Loreau & de Mazancourt et al. 2013). On the other hand, controversial results have been reported. In particular, Grman et al. (2010) and Yang et al. (2011) found that communities maintained or increased stability despite losses in species richness. Much past research on diversity-stability relationships in N fertilization experiments (e.g. Tilman & Downing 1994; Hautier et al. 2015) did not separate the particular effect of species richness on ANPP temporal stability from the hidden effects of N fertilization rate on species richness and stability (Huston 1997; Loreau 1998; Wardle et al. 2000). Testing the effect of diversity on the residuals of ANPP temporal stability after controlling the effects of N doses is an accurate procedure to discover the diversity effects on stability (Zhang et al., 2016).

Alternatively, the ANPP stability of dominant species has been proposed to control temporal stability of ANPP. Smith & Knapp (2003), Polley et al. (2007) and Sasaki & Lauenroth (2011) reported that only few species are needed to sustain ANPP, suggesting that ANPP variations depend more on the stabilizing effect of dominant species rather than on biodiversity. These reports are in accordance with Grime´s (1998) mass-ratio hypothesis which proposes that ecosystem processes are proportional to species abundance. On the other hand, ANPP stability could be highest if species fluctuations were completely asynchronous, e.g. when the decreases in the ANPP of some species were compensated by increases in the ANPP of other species. Theoretically, a larger species pool can enhance the asynchrony of species, due to the increased probability of containing species with divergent responses to environmental conditions (Yachi & Loreau 1999). Nevertheless, Hautier et al. (2014) and Zang et al. (2016) reported, for N fertilized grasslands, that community asynchrony could mediate ANPP stability regardless of species richness, revealing that environmental factors effects on community dynamics are stronger than those of biodiversity.

The addition of nitrogen (N) and phosphorus (P) on grasslands is one of the main management tools to influence forage yield and quality of these pastures, and at the same time a serious threat to biodiversity (Bobbink et al. 2010; Ceulemans et al. 2013). Results from N (Stevens et al. 2004; Bobbink et al. 2010) and combined N and P (Hejcman et al. 2007) fertilization experiments suggest that nutrient addition regularly increases ANPP, drive changes in species composition and simultaneously reduces species diversity. However, the effect of N addition on ANPP stability of natural grasslands has been difficult to generalize, since some N fertilization experiments did not show the predicted destabilizing effects of diversity loss (Yang et al. 2011; Hautier et al. 2014;

Zhang et al. 2016). Nevertheless, results from global change experiments indicate that several factors may be acting simultaneously to control ANPP (Elser et al. 2007; Harpole et al. 2011). Based on this findings, when N limitation is removed, a shift to ANPP limitated by water (Huston 1997), or by multiple limitations such as N and water (Yahdjian et al. 2011) or N and P (Fay et al. 2015) can be expected.

The change in the factors controlling ANPP could also imply in a greater year-to-year variability in ANPP by a higher dependence on climatic conditions. However, to date, there is scarce evidence from global change experiments to support the role of biodiversity, dominant species and community asynchrony responses in regulating ANPP temporal stability. Understanding these roles is especially interesting when multiple resources are considered in species-rich natural grasslands communities. In a four-year experiment, we used factorial additions of N and P under either rainfed and irrigated conditions to test how nutrients and water addition affect ANPP temporal stability in a natural species-rich community. We aim to answer three questions: first, what are the main drivers of grassland ANPP and their temporal stability under nutrients and water addition?, second, to what extend do species richness, dominant species and community asynchrony influence ANPP temporal stability?, and third, what are the main pathways influencing ANPP stability?

MATERIALS AND METHODS

Site description

The experiment was carried out at an experimental farm of INIA Tacuarembó, in northern Uruguay (31.53´ S, 56.14´ W; 245 m above sea level). Climate is temperate to subtropical, with mean monthly temperature of 12°C in winter and 25°C in summer, and average annual rainfall of 1500 mm. with monthly coefficients of variation above 50% (Cruz et al. 2014). Soils are basaltic Vertisols with an average rooting depth of 70 cm. The detailed soil properties are presented in table S1. The vegetation –referred to as “Campos grasslands“– is part of the “Río de la Plata” grasslands, a biome that includes

East Central Argentina, Southern Brazil and Uruguay (Soriano 1991). These grasslands communities are dominated by C4 and C3 grasses (Lezama 2006; Burkart et al. 2011) and have a very high fine-scale diversity with an average of aproximately 30 species by m^2 (Overbeck et al. 2005).

Water and nutrient addition experiment

In the spring of 2011, we established a factorial experiment (2 x 2 x 4) that combined two water managements, two phosphorus levels and four nitrogen levels. The experimental design was a split plot design with three replications in randomized blocks. On the main plots (24 x 16 m) two water supply levels were located: supplementary irrigation (SI) and rainfed (RF) treatments. On the subplots (8 x 6 m), we assigned eight fertilization treatments consisting of P rates of 0 and 35 kg P ha^{-1} $year^{-1}$ combined with 0, 50, 100 and 200 kg N ha^{-1} $year^{-1}$. P (superphosphate) was added in one application in early spring, while N fertilization (urea) was done from early spring to mid-summer by using 1, 2 and 4 applications of 50 kg ha^{-1} for 50, 100 and 200 kg N ha^{-1} $year^{-1}$ treatments, respectively. SI was performed from October to April, after depletion of 50% of available soil water (monitored on a weekly basis using a TDR probe), whereas RF plots received only natural precipitation. The annual rainfall of the four-year experimental period ranged from 1250 mm to 1860 mm, and the annual supplementary water applied ranged from 70 to 385 mm. The detailed water balance of the experimental period is presented in figure S2.

Plant Community Measurements

Between October 2011 and September 2015, plots were clipped every 40-50 days in spring and summer, and every 80-120 days during autumn and winter, by clipping 6 m^2 per subplot at 5 cm height (which represent a removal of about 40-60% of total standing biomass when sward reach 95 % light interception in the high-growth periods of spring and summer). At each harvest, a forage sample of each subplot was oven-dried at 60 ºC for 72 hours and then weighed. Prior to each cut, we visually estimated the aerial cover percentage of each dominant species in five permanent 0.5-m^2 quadrats per subplot. All vegetation surveys were done by the same trained team, considering all species with a minimum score of 5% of cover. Species richness was measured recording all vascular plant species, independent of their cover, present in a 20 m^2 quadrat per subplot after four years of treatments.

Community Calculations

We estimated the individual contribution of species to ANPP assuming a direct relationship between species cover and ANPP of the 12 most dominant species (mean cover >1%; all of them were native grasses). For each subplot and sampling period, the ANPP of every dominant species was calculated weighting the species cover by the total ANPP. This methodology was calibrated by testing the relationship between the visual species cover percentage estimation and the real biomass proportion of each species by destructive samplings (R^2 = 0.78, $P < 0.0001$) (Fig. S3). Then, the ANPP of the community was divided into three groups: i) C4 dominants grasses (*Axonopus fissifolius, Paspalum notatum, Paspalum dilatatum, Bothriochloa laguroide*s, *Paspalum plicatulum, Mnesithea selloana and Steinchisma hians*), ii) C3 dominant grasses (*Bromus auleticus*, *Nassella neesiana, Piptochaetium montevidense, Poa lanigera* and *Piptochaetium stipoides*), and iii) the rest of the species grouped as rare species (from 40 to 46 species depending on treatments, which were mostly dicots). Annual ANPP was calculated by adding the production of the sampling periods corresponding to each year. Temporal stability for each subplot was determined as μ/σ (Lehman & Tilman 2000), where μ is the mean ANPP from 2011 to 2015 and σ is the standard deviation of the ANPP over the 4 years. Additionally, to compare treatment effects on stability and richness, as well as with other research, we calculated the ANPP temporal stability effects in each treatment subplot. A description of the estimation of effects is detailed in Appendix 1. Community species asynchrony (Loreau & de Mazancourt 2008) was calculated for each subplot as:

$$1 - \varphi = \frac{\sigma^2}{\left(\sum_{i=1}^{s} \sigma_i\right)^2} \qquad (1)$$

This statistic ranges between 0 (perfect synchrony) and 1 (perfect asynchrony), where σ^2 is the variance of community ANPP and σ_i is the standard deviation of

ANPP in a subplot with S species over the years 2011–2015. Because the biomass of each individual rare species could not be estimated, we consider it as group in addition to the dominants. A significant increase in ANPP temporal stability related to an increase on community asynchrony would suggest that the asynchronous species responses play a role in increasing stability (Loreau & de Mazancourt 2013).

Statistical Analyses

A general linear mixed model analysis of variance was performed for ANPP, ANPP stability, species richness, community asynchrony, and mean and standard deviation (SD) of dominant species ANPP. The analyses were done considering water management (nested within blocks), fertilization and all interactions as fixed effects, while blocks were treated as random effect. All interactions were included in a preliminary model, but non-significant interactions were removed according to Engqvist (2005), leaving only the significant nitrogen–phosphorus interaction in the final model. The Shapiro–Wilk test was used to check the assumption of normally distributed residuals for all response variables. To meet assumptions of normality, data of ANPP temporal stability and species richness was natural log transformed before analyses (species richness and ANPP stability in one case were then retro-transformed from the averages obtained in the statistical analyses to show the original values in the figures). Several models combining different functions for correlations and variance of errors were tested, and then the best-fitted models were selected based on the Akaike Information Criterion (Akaike 1974). Means for the significant fixed effects were compared using Fisher’s LSD test ($p < 0.05$).

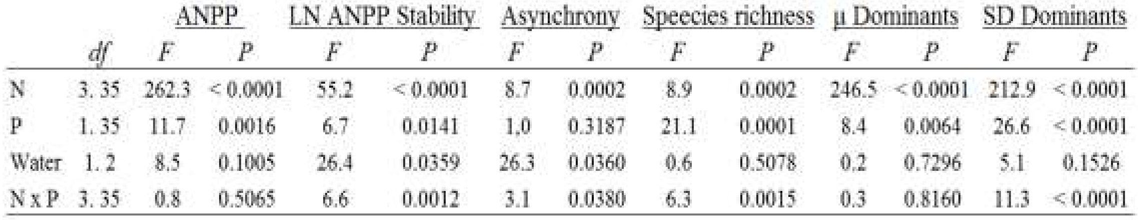
Residuals from regressions of ANPP temporal stability (natural log transformed) with N dose were used to explore the remaining effects of N addition. We used simple and stepwise multiple linear regression (MLR) analysis to examine the association between mean and standard deviation of ANPP of dominant grasses, species richness and community asynchrony with ANPP temporal stability (natural log transformed) and their residuals after controlling for N addition. We specified *α* = 0.10 as the significance level for the variables for entry and stay in the MLRs. Finally, were used to explore the remaining effects of N addition. All of our selected variables had variance inflation factors < 2, which were much lower than the critical value of 10 suggested by Gujarati & Porter (2009), indicating that multicollinearity was not a problem. All statistical analyses were performed using Infostat (Di Rienzo et al. 2015) and JMP® (SAS Institute, Cary, NC) software packages.

RESULTS

Treatment effects on ANPP

ANPP increased with N dose (*F* = 262, *P* < 0.0001) 27%, 51% and 93% with 50, 100 and 200 kg N ha^{-1} year^{-1} respectively, meanwhile ANPP increased only 6% in P fertilized plots (*F* = 11.7, *P* = 0.0016). However, water and N x P interaction did not have significant effects on ANPP (Fig. 1A, table 1).

Table 1. Results of analysis of variance (ANOVA) for the effects of Nitrogen (N), phosphorus (P), water (W) addition and N x P interaction on the community aboveground net primary productivity (ANPP), ANPP temporal stability, species asynchrony, species richness, dominant species mean (μ) ANPP and standard deviation (SD). Numerator and denominator degrees of freedom (*df*), F-test and P-values are given.



		ANPP		LN ANPP Stability		Asynchrony		Speecies richness		μ Dominants		SD Dominants	
	df	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
N	3. 35	262.3	< 0.0001	55.2	< 0.0001	8.7	0.0002	8.9	0.0002	246.5	< 0.0001	212.9	< 0.0001
P	1. 35	11.7	0.0016	6.7	0.0141	1,0	0.3187	21.1	0.0001	8.4	0.0064	26.6	< 0.0001
Water	1. 2	8.5	0.1005	26.4	0.0359	26.3	0.0360	0.6	0.5078	0.2	0.7296	5.1	0.1526
N x P	3. 35	0.8	0.5065	6.6	0.0012	3.1	0.0380	6.3	0.0015	0.3	0.8160	11.3	< 0.0001

Treatment effects on temporal stability of ANPP

ANPP temporal stability was affected by water (*F* = 26.4, P = 0.0359), N (*F* = 55.2, *P* < 0.0001), P (*F* = 6.7, *P* = 0.0141), and N x P interaction (*F* = 6.6, P = 0.0012) (Table 1). Water addition increased the year-to-year stability by decreasing the SD of ANPP (Fig. 1B, table 1, S4 and S5A). Meanwhile, the N, P and N x P influence on ANPP stability was related to three main effects. First, in the absence of N addition, P fertilization had a slight positive effect on ANPP stability. Second, ANPP stability decreased incrementally with N doses. And, third, the combined addition of N and P further decreased ANPP stability at the extremes doses of N (50 and 200 kg N ha^{-1} $year^{-1}$) compared to the single N application (Fig. 1B and S5B).

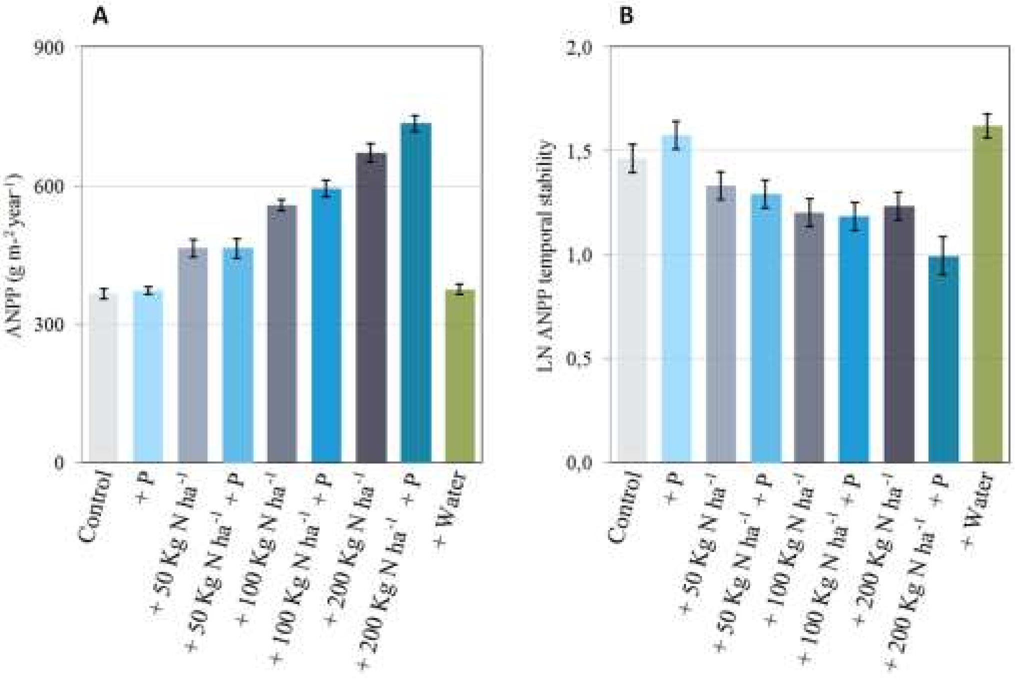


Figure 1. Effects of nitrogen (N), phosphorus (P) and water addition on: A) average annual aboveground net primary productivity (ANPP), and B) temporal stability of ANPP through time calculated as temporal mean/temporal standard deviation; (data was natural log transformed for analysis). Bars represent mean ± SE (n = 3).

Treatment effects on plant community

A total of 119 different species were recorded in the experimental area, where controls averaged 58 species per 20 m^2 subplots. Species richness was affected by N (F = 8.9., P = 0.0002), P (F = 21.1 P < 0.0001) and N x P interaction (F = 6.3, P = 0.0015), whereas it was not affected by water (Fig. 2A, S5C). The N, P and N x P interaction impacts on species richness were explained by the lack of effect at the lowest dose of N (50 Kg N ha^{-1} $year^{-1}$), meanwhile the number of species declined approximately 10% in the rest of N and P fertilized treatments (5-7 rare species were lost per 20 m^2 subplots). Surprisingly, except for this treatment, we did not find any significant differences among N and P fertilized treatments (Table 1, Fig. 2A, S5D).

Community asynchrony was affected by water (F = 26.3, P = 0.0360), N (F = 8.7, P = 0.0002) and N x P interaction (F = 3.1, P = 0.0380), whereas it was not affected by P (Fig. 2B). Species asynchrony increased with water addition, and decreased with the intermediate N dose (100 Kg N ha^{-1} $year^{-1}$), whereas the N x P interaction was explained by a further decreased when N was combined with P fertilization at the highest doses of N (200 kg N ha^{-1} $year^{-1}$) compared to the single N application (Fig. 2B, table 1).

Treatment effects on species groups

The average contribution of the 12 most dominant species (mean cover >1%; all of them were grasses) to community ANPP was 83.9% (65.9% and 18.0% for C4 and C3 dominant grasses, respectively). The remaining 16.1% corresponded to rare species (mostly dicots). The main groups were only slightly influenced by N treatments: cover of dominant C3 grasses increased in all N fertilized treatments, while cover of dominant C4 grasses decreased at the lower N dose, whereas cover of rare species decreased at the higher doses of N (Fig. S6A). These changes imply a decreased ratio of dominant C4/C3 grasses in all N fertilized treatments compared to control (Fig. S6B). The mean ANPP of dominant species was only positively influenced by N (F = 246, P < 0.0001) and P (F = 8.4, P = 0.0064).

Similar to the results of productivity at community level, dominant species mean ANPP increased 25%, 55% and 100% with 50, 100 and 200 kg N ha^{-1} $year^{-1}$ respectively (Table 1, S5G). On the other hand, dominant species mean ANPP only increased 5.6% in P fertilized plots, whereas water and N x P interaction were not significant (Table 1, S5H). However, in a sharp contrast with the effects on mean ANPP, the standard deviation (SD) of the ANPP of these species was affected by N (F = 212.9., P < 0.0001), P (F = 26.6 P <

0.0001) (Table 1, S5I) and N x P interaction (*F* 11.2, *P* < 0.0001), whereas water addition was not significant (Table 1, S5J).

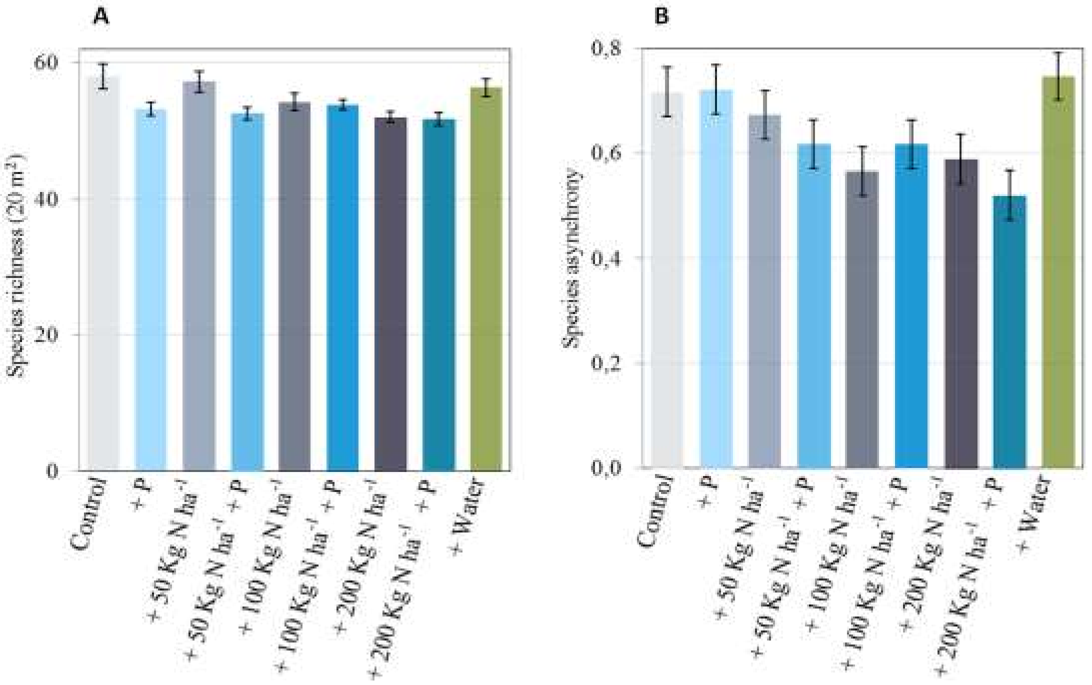


Figure 2. Effects of nitrogen (N), phosphorus (P) and water addition on: A) average species richness (number of species/20m^2), and B) species asynchrony (ranged from 0 “perfect synchrony” to 1 “perfect asynchrony”). Bars represent mean ± SE (n = 3).

The N, P and N x P interaction effects were related to three main effects. First, in absence of N fertilization, P addition reduced the SD of dominant species ANPP. Second, the SD of dominant species ANPP increased with N dose (50%, 125% and 230% with 50, 100 and 200 kg N ha^{-1} year^{-1} respectively). Third, the SD of dominant species ANPP was further increased when the highest dose of N (200 kg N ha^{-1} year^{-1}) was combined with P compared to the single N addition (Table 1).

Relationships between plant community and temporal stability of ANPP

Dominant species ANPP stability evidenced a strong association with community ANPP stability (R^2 = 0.80, *P* < 0.0001), whereas rare species ANPP stability showed a weak relationship (R^2 = 0.22, *P* < 0.0005) (Fig. S7). This role of dominant species was subsequently confirmed by the coefficient of determination of the relation between the SD of dominant species ANPP and the community ANPP temporal stability, both with the cumulative number ($R^2 = 0.988$, $P < 0.0001$) and cover of dominant species ($R^2 = 0.996$, $P < 0.0001$) (Fig. S8A and S8B). This analysis revealed that most of the reduction in ANPP stability caused by N and N+P addition occurred through weakening the stabilizing effect of the six most abundant species (which included five C4 grasses) (Fig. 3 A). Overall, these results indicated that ANPP of the six most dominant species might be more sensitive to environmental fluctuations than the subsequent dominant species under N and N+P addition. Otherwise, both water and P addition increased ANPP stability by reinforcing the stabilizer effect of the subsequent dominant species (Fig. 3 A and 3 B).

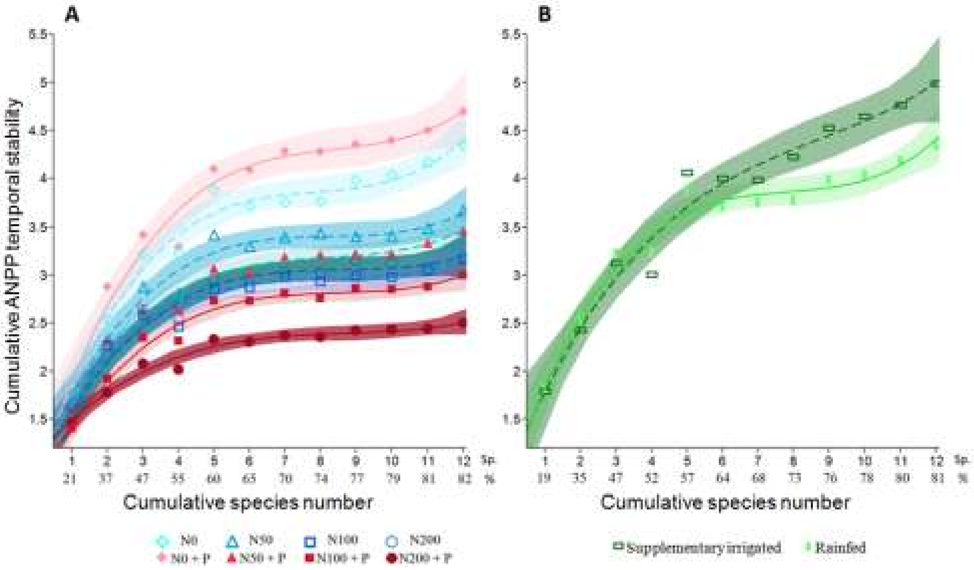


Figure 3. Cumulative ANPP temporal stability (data was natural log retro-transformed) of dominant species in: a) nitrogen and phosphorus treatments; and b) water management treatments. Dots represent the average accumulated cover (%) of the 12 most dominant species, ordered from highest to lowest abundance, and their relative ANPP stability. Shaded areas represent 95% confidence intervals.

The temporal stability of ANPP was negatively associated with the SD of dominant species ANPP ($R^2 = 0.80$, $P < 0.0001$) (Fig. 4A) and positively related with asynchrony (R^2 = 0.64, P < 0.0001) (Fig. 4C). However, ANPP stability was only slightly positively linked to species richness (R^2 = 0.12, P = 0.0059) (Fig. 4E).

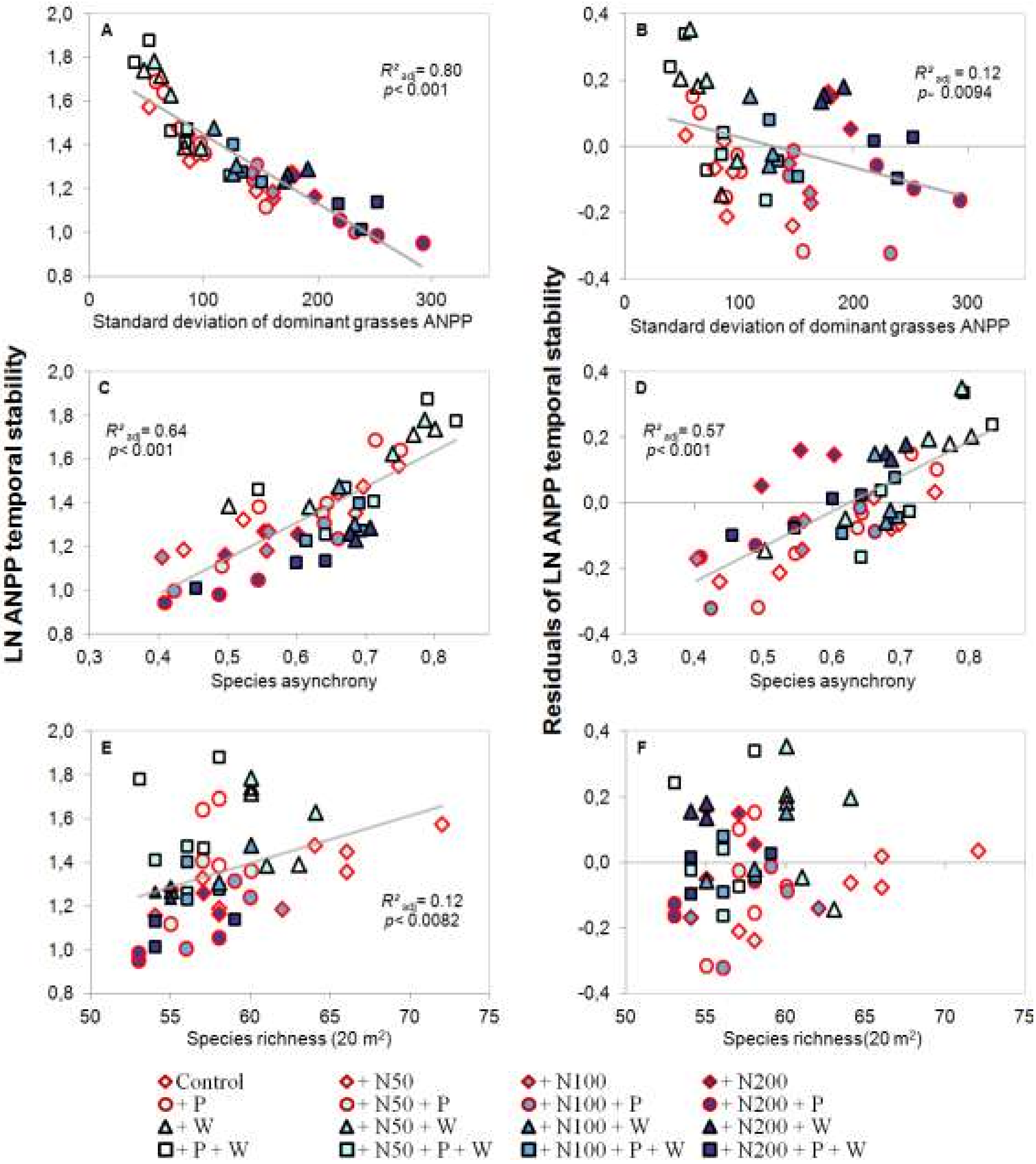
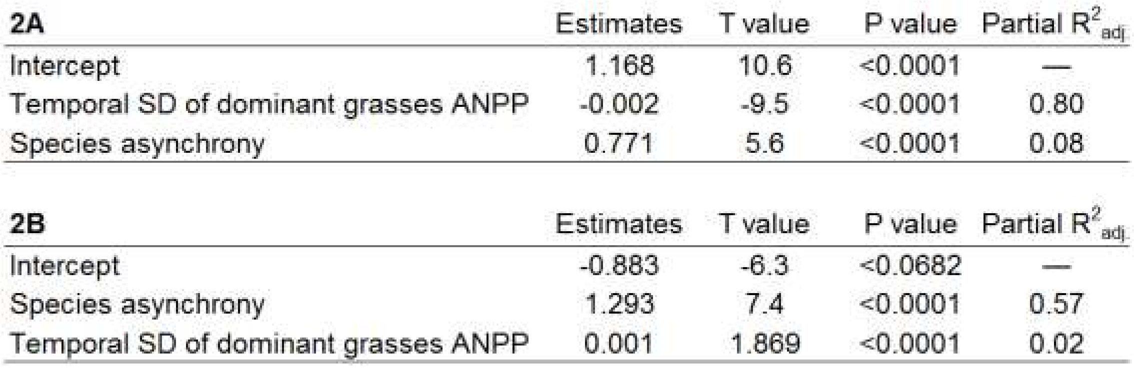


Figure 4. Relationship of the standard deviation of dominant grasses ANPP, species asynchrony and species richness with ANPP stability (natural log transformed): A, C and E) before and B, D and F) after controlling the effects of N dose. Dots represent each subplot.

Multiple regression model (MRM) confirmed the main role of the SD of dominant species ANPP complemented by asynchrony, explaining together 88% of the variation in ANPP temporal stability (Table 2). After controlling the effects of N dose the importance of both factors was inverted. ANPP stability was positively related with asynchrony ($R^{2\ \mathrm{adj.}}$= 0.57, P < 0.0001) but only slightly negatively related with the SD of dominant species ANPP (R^2 = 0.12, P < 0.0094) (Fig. 4B and 4D). In absence of N addition effects, the MRM evidenced the main role of species asynchrony, that was scarcely complemented by the SD of dominant species ANPP, in explaining the variation in ANPP temporal stability (R^2 = 0.61, P < 0.0001) (Table 2). Meanwhile, species richness was not significant in both single and multiple regression models.

Table 2. Results of multiple linear regression for the effects of mean and SD of dominant species ANPP, asynchrony and species richness on ANPP temporal stability (natural log transformed). 2A) before (R R^2_{adj}. = 0.88, P < 0.0001); and 2B) after controlling for the effects of N dose on stability (R^2_{adj}. = 0.59, P < 0.0001).



2A	Estimates	T value	P value	Partial R^2_{adj}
Intercept	1.168	10.6	<0.0001	—
Temporal SD of dominant grasses ANPP	-0.002	-9.5	<0.0001	0.80
Species asynchrony	0.771	5.6	<0.0001	0.08

2B	Estimates	T value	P value	Partial R^2_{adj}
Intercept	-0.883	-6.3	<0.0682	—
Species asynchrony	1.293	7.4	<0.0001	0.57
Temporal SD of dominant grasses ANPP	0.001	1.869	<0.0001	0.02

DISCUSSION

To our knowledge, this report is the first to test the simultaneous effects of the rate of N addition combined with P and water addition on temporal stability of ANPP of a native grassland community. Our findings revealed the influence of the temporal variation of most dominant species productivity and species asynchrony as the main pathways that regulate the short-term temporal stability of grassland ANPP under nutrient and water addition. Our results also revealed that the importance of the mechanisms regulating ANPP temporal stability depends on whether the driver changes productivity. Decreased ANPP stability caused by N and N+P addition was mainly related to an increased ANPP variation of the six most dominant species, while the increased ANPP stability caused by P or water addition was associated to an increased species asynchrony.

Despite the fact that the SD of dominant species ANPP and species asynchrony had opposite trends, the selection of both variables in the MRM evidenced possible complementary roles of them in explaining the variations in ANPP stability. However, after controlling N effect, the species asynchrony influence on ANPP stability was almost exclusive, indicating that it was quite independent of N addition. The prevalence of one pathway over the other appears to be regulated by N limitation, since N enrichment promotes the competition among species (Hillebrand et al. 2008) and, at same time, decrease the chances of temporary tradeoffs among species (Hautier et al. 2014). To date, different works reported the role of dominant species (Smith & Knapp 2003; Polley et al. 2007; Sasaki & Lauenroth, 2011), species asynchrony (Hautier et al. 2014), dominant species and species asynchrony (Zhang et al. 2016) and multiple mechanisms (Chen et al. 2016). Nevertheless, most of these studies analyzed separately each cause of variation and failed to explain ANPP stability changes under a broad range of resources addition.

In our study, N proved consistently to be the single most important factor constraining ANPP, in line with the studies of Vitousek & Howarth (1991) and LeBauer (2008). ANPP only becomes N-P co-limited at the highest N fertilization rates and we found no relevant effects of the single addition neither of water nor P. The lack of effect of water addition on ANPP reflected that only sporadic water shortages occurred in the 4 years. In spite of this, water and their interactions effects could be relevant in a more variable or stronger seasonal climatic context, eg, as projected by climate change scenarios (Betolli et al. 2010). Otherwise, the lack of effect of P addition may be related to the Mycorrhizal–plant interactions which play a key role in perennial grasses nutrient uptake in P-limited systems (Johnson et al. 2015).

As was expected, we found that N addition decreased while water increased ANPP stability, but strikingly, we found strong positive or negative effects of P addition on annual ANPP stability depending on the N dose. These changes in ANPP stability changes were produced by larger variations in the standard deviation (SD) relative to the mean (Fig. S4). It is commonly suggested that N additions induce a directional change in the drivers of ANPP from N limitation to N and P co-limitation (Vitousek et al. 2010; Fay et al. 2015). Specifically, we detected only sporadic high ANPP responses to N and N+P addition (mainly in spring and at highest N fertilization rates), which increased more the SD than mean ANPP in a process that appears to be regulated by temperature (S9 and table S10). Therefore, the shift from chronic N limitation to more irregular drivers such as N-P co-limitation would increase the SD of dominant grasses ANPP, in consistence with Huston (1997). Given the importance of the SD of productivity in defining ANPP stability, one subject to be explored in future studies are the mechanisms which led to the larger variations in the SD of dominant species.

Contrary to the prevailing theory for long term data (Tilman et al. 2006; Hector et al. 2010; Hautier et al. 2015), our results evidence a weak relationship of species richness with ANPP stability, which also became insignificant after controlling the effects of N enrichment. Even though we did not sample the complete community composition during the entire experiment, the data from the last year give the net effect after four years, and it becomes clear that there were no pronounced effects of treatments on species richness. Instead, the strength of the relationship between the SD of dominant species ANPP and temporal stability of community ANPP allow us to confirm Grime (1998) mass-ratio hypothesis (Fig. S8). In other words, ANPP stability decreased mainly by the increased variation in the productivity of dominant grasses under N and N+P addition and not by the negligible production of the rare species lost (< 1% of ground cover). Greater ANPP stability has also been commonly associated with an increased asynchrony of the species (Hautier et al. 2014). In this sense, our results also support the stabilizing effect of species asynchrony on ecosystem stability, showing that, despite slight losses of rare species, species-rich grasslands could maintain complementary interactions among species.

The argument of nutrient and water supply driving stability trough changes in the SD of dominants and species complementary interactions, at least in the short term, appears to be relevant for species-rich communities with few highly dominant species. Despite this evidence, our findings do not necessarily suggest that diversity is not relevant to ANPP temporal stability. For instance, the importance of diversity mediating ANPP stability could be increased at larger time-scales (Cardinale et al. 2007; Reich et al. 2012; Isbell et al. 2013). Specifically, rare species might control ANPP stability in a longer term by limiting the recruitment of dominant species (Grime 1998) or by the turnover of dominant species (Allan et al. 2011).

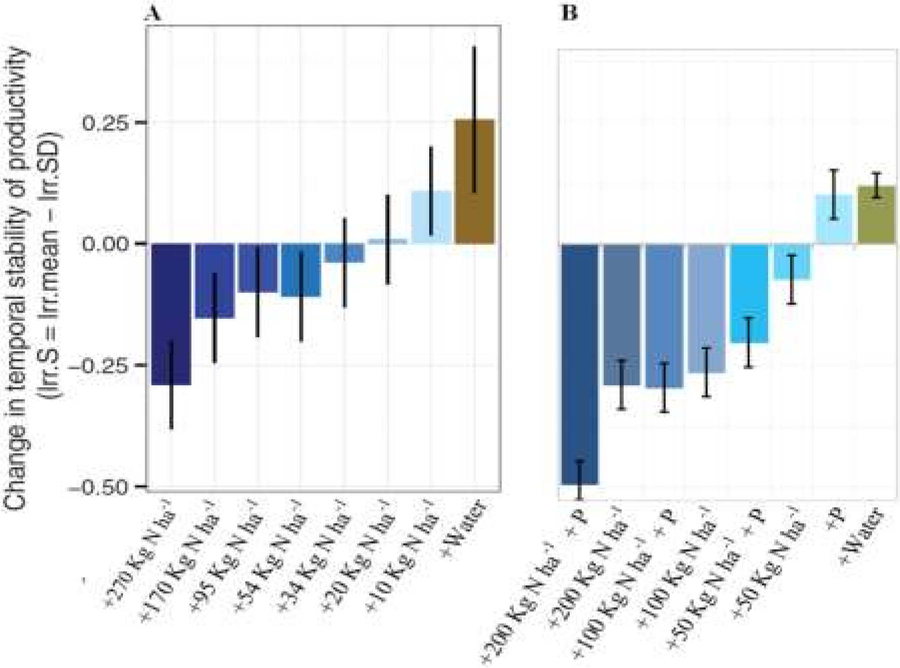


Figure 5. Effects of nitrogen (N), phosphorus (P) and water on changes of ANPP temporal stability relative to reference plots (natural grasslands): A) long term stability data of Hautier et al. (2015), and B) short term stability data of our experiment. LRR.S = the natural logarithm of the ratio of the mean and standard deviation within each treatment plot divided by the average mean and standard deviation across all reference plots for a treatment. Bars represent mean ± SE (n = 3).

Despite the lack of association between species richness and ANPP stability, we found a short-term pattern of water and nutrients addition effects on ANPP stability that was very similar to the long term trends (4-28 years) reported by Hautier et al. (2015) who worked with different levels of N addition and water addition, but no P addition (Fig. 5 A and B). This pattern suggests that short term trends of the ANPP stability could be relevant to predict long term impacts. Overall, despite short term of the research, our findings contribute to the knowledge integrating the role of dominant species and asynchrony pathways regulating ANPP stability. Therefore, a key challenge for future grassland management intensification research is to determine, in the medium to long term, the thresholds for species losses and turnover of dominant species which significantly affect ANPP stability in natural communities.

ACKNOWLEDGEMENTS

The field work was supported by the Instituto Nacional de Investigación Agropecuaria (INIA) of Uruguay, and by a doctoral scholarship provided by ANII-Uruguay. We gratefully acknowledge the forage staff of INIA Tacuarembó and the colleagues of UFRGS-Brazil for their assistance with laboratory and field work. We especially acknowledge to Fiorella Cazzulli and Claudia Marchesi for the English corrections, to Jean Fedrigo for his collaboration in the analysis of the data and the preparation of the figures, and to Fernando Lattanzi for the critical review of the manuscript and for English corrections.

REFERENCES

Allan, E., Weisser, W., Weigelt, A., Roscher, C., Fischer, M. & Hillebrand, H. 2011. More diverse plant communities have higher functioning over time due to turnover in complementary dominant species. *Proc. Natl Acad. Sci. USA,* 108(41), 17034-17039.

Akaike, H., 1974. A new look at the statistical model identification. *IEEE transactions on automatic control*, 19(6), pp.716-723.

Balvanera, P., Siddique, I., Dee, L., Paquette, A., Isbell, F., Gonzalez, A. *et al*. 2014. Linking biodiversity and ecosystem services: current uncertainties and the necessary next steps. *BioScience*, 64(1), 49-57.

Bettolli, M. L., M. A. Altamirano, G. Cruz, F. Rudorff, A. Martínez, J. Arroyo, and J. Armoa. 2010. Pastura natural de Salto (Uruguay): relación con la variabilidad climática y análisis de contextos futuros de cambio climático. *Revista Brasileira de Meteorologia* 25(2): 248-259.

Bobbink, R., Hicks, K., Galloway, J., Spranger, T., Alkemade, R., Ashmore, M. *et al*. 2010. Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. *Ecol. Appl.* 20(1), 30-59.

Burkart, S. E., León, R. J., Conde, M. C. & Perelman, S. B. 2011. Plant species diversity in remnant grasslands on arable soils in the cropping Pampa. *Plant Ecol. 212*(6), 1009-1024.

Cardinale, B. J., Srivastava, D. S., Duffy, J. E., Wright, J. P., Downing, A. L., Sankaran, M. *et al*. 2006. Effects of biodiversity on the functioning of trophic groups and ecosystems. *Nature*, 443(7114), 989-992.

Cardinale, B.J., Wright, J.P., Cadotte, M.W., Carroll, I.T., Hector, A., Srivastava, D.S. *et al*. 2007. Impacts of plant diversity on biomass production increase through time because of species complementarity. *Proc. Natl Acad. Sci. USA,* 104(46), 18123-18128.

Ceulemans, T., R. Merckx, Hens, M. & Honnay, O. 2013. Plant species loss from European semi natural grasslands following nutrient enrichment–is it nitrogen or is it phosphorus? *Glob. Ecol. Biogeogr.* 22(1), 73-82.

Chen, W., Zhang, Y., Mai, X. & Shen, Y. 2016. Multiple mechanisms contributed to the reduced stability of Inner Mongolia grassland ecosystem following nitrogen enrichment. *Plant Soil*, 1-14.

Cruz, G., Baethgen, W., Picasso, V. & Terra, R. 2014. Análisis de sequías agronómicas en dos regiones ganaderas de Uruguay. *Agrociencia Uruguay*, *18*(1), 126-132.

Di Rienzo, J. A., Casanoves, F., Balzarini, M. G., Gonzalez, L., Tablada, M. & Robledo, C.W. InfoStat versión 2015. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina.

Elser, J.J., Bracken, M.E., Cleland, E.E., Gruner, D.S., Harpole, W.S., Hillebrand, H. *et al*. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecol. Lett.* 10(12), 1135-1142.

Fay, P.A., Prober, S.M., Harpole, W.S., Knops, J.M., Bakker, J.D., Borer, E.T. *et al*. 2015. Grassland productivity limited by multiple nutrients. Nat. plants, 1(7).

Grman, E., Lau, J.A., Schoolmaster, D.R. & Gross, K.L. 2010. Mechanisms contributing to stability in ecosystem function depend on the environmental context. *Ecol. Lett.* 13, 1400-1410.

Grime, J. P. 1998. Benefits of plant diversity to ecosystems: immediate, filter and founder effects. *J. Ecol.* 86(6), 902-910.

Gujarati, D. N., & Porter, D. C. 2009. Basic Econometrics. McGraw-Hill Irwin, New York.

Harpole, W.S., Ngai, J.T., Cleland, E.E., Seabloom, E.W., Borer, E.T., Bracken, M.E. *et al*. 2011. Nutrient co-limitation of primary producer communities. *Ecol. Lett.* 14(9), 852-862.

Hautier, Y., Seabloom, E.W., Borer, E.T., Adler, P.B., Harpole, W.S., Hillebrand, H. *et al*. 2014. Eutrophication weakens stabilizing effects of diversity in natural grasslands. *Nature*, *508*(7497), 521-525.

Hautier, Y., Tilman, D., Isbell, F., Seabloom, E.W., Borer, E.T. & Reich, P.B. 2015. Anthropogenic environmental changes affect ecosystem stability via biodiversity. *Science*, 348, 336-340

Hejcman, M., Klaudisová, M., Schellberg, J. & Honsová, D. 2007. The Rengen Grassland Experiment: plant species composition after 64 years of fertilizer application. *Agric. Ecosyst. Environ.* 122(2), 259-266.

Hector, A., Hautier, Y., Saner, P., Wacker, L., Bagchi, R., Joshi, J. *et al*. 2010. General stabilizing effects of plant diversity on grassland productivity through population asynchrony and overyielding. *Ecology*, 91(8), 2213-2220.

Hillebrand, H., Bennett, D. M. & Cadotte, M. W. 2008. Consequences of dominance: a review of evenness effects on local and regional ecosystem processes. *Ecology*, 89(6), 1510-1520.

Huston MA. 1997. Hidden treatments in ecological experiments: re-evaluating the ecosystem function of biodiversity. *Oecologia* 110:449-60.

Isbell, F.I., Polley, H.W. & Wilsey, B.J. 2009. Biodiversity, productivity and the temporal stability of productivity: patterns and processes. *Ecol. Lett.* 12(5), 443-451.

Isbell, F., Reich, P. B., Tilman, D., Hobbie, S. E., Polasky, S. & Binder, S. 2013. Nutrient enrichment, biodiversity loss, and consequent declines in ecosystem productivity. *Proc. Natl Acad. Sci. USA,* 110(29), 11911-11916.

Ives, A. R. & Carpenter, S. R. 2007. Stability and diversity of ecosystems. *Science*, 317(5834), 58-62.

Johnson, N.C., Wilson, G.W., Wilson, J.A., Miller, R.M. & Bowker, M.A. 2015. Mycorrhizal phenotypes and the Law of the Minimum. *New Phytol.* 205(4), 1473-1484.

Lehman, C.L. & Tilman, D. 2000. Biodiversity, stability, and productivity in competitive communities. *Am. Nat.* 156, 534-552.

Loreau, M. & de Mazancourt, C. 2008. Species synchrony and its drivers: neutral and nonneutral community dynamics in fluctuating environments. *Am. Nat.* 172(2), E48-E66.

Loreau, M. & de Mazancourt, C. 2013. Biodiversity and ecosystem stability: a synthesis of underlying mechanisms. *Ecol. Lett.* 16, 106-115.

Overbeck, G. E., Müller, S. C., Pillar, V. D. & Pfadenhauer, J. 2005. Fine-scale post-fire dynamics in southern Brazilian subtropical grassland. *J. Veg. Sci.* 16(6), 655-664.

Polley, H.W., Wilsey, B.J. & Derner, J.D. 2007. Dominant species constrain effects of species diversity on temporal variability in biomass production of tallgrass prairie. *Oikos*, 116, 2044-2052.

Reich, P. B., Tilman, D., Isbell, F., Mueller, K., Hobbie, S. E., Flynn, D. F. *et al*. 2012. Impacts of biodiversity loss escalate through time as redundancy fades. *Science*, 336(6081), 589-592.

SAS Institute Inc. 2010. JMP Software, Cary, NC: SAS Institute Inc. 145 p.

Sasaki, T. & Lauenroth, W. 2011. Dominant species, rather than diversity, regulates temporal stability of plant communities. *Oecologia*, 166, 761-768.

Smith, M.D. & Knapp, A.K. 2003. Dominant species maintain ecosystem function with non-random species loss. *Ecol. Lett.* 6, 509-517

Soriano, A. 1991. Río de la Plata grasslands. In: Coupland, R.T (ed.) Natural grasslands: Introduction and Western Hemisphere. Elsevier, Amsterdam, The Netherlands. p. 367-407.

Stevens, C.J., Dise, N.B., Mountford, J.O. & Gowing, D.J. 2004. Impact of nitrogen deposition on the species richness of grasslands. *Science*, 303, 1876-1879.

Tilman, D., Reich, P.B. & Knops, J.M.H. 2006. Biodiversity and ecosystem stability in a decade-long grassland experiment. *Nature*, 441, 629-632.

Vitousek, P. M., Porder, S., Houlton, B. Z. & Chadwick, O. A. 2010. Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen–phosphorus interactions. *Ecol. Appl.* 20(1), 5-15.

Yachi, S. & Loreau, M. 1999. Biodiversity and ecosystem productivity in a fluctuating environment: the insurance hypothesis. *Proc. Natl Acad. Sci. USA,* 96, 1463-1468

Yang, Z.L., Van Ruijven, J. & Du, G.Z. 2011. The effects of long-term fertilization on the temporal stability of alpine meadow communities. *Plant Soil*, 345, 315-324.

Wardle, D.A., Huston, M.A., Grime, J.P., Berendse, F., Garnier, E., Lauenroth, W.K. et al. 2000. Biodiversity and ecosystem function: an issue in ecology. *Bull. Ecol. Soc. Am. 81*(3) 235-239.

Yahdjian, L., Gherardi, L. & Sala, O. E. 2011. Nitrogen limitation in arid-subhumid ecosystems: a meta-analysis of fertilization studies. J. Arid Environ. 75(8), 675-680.

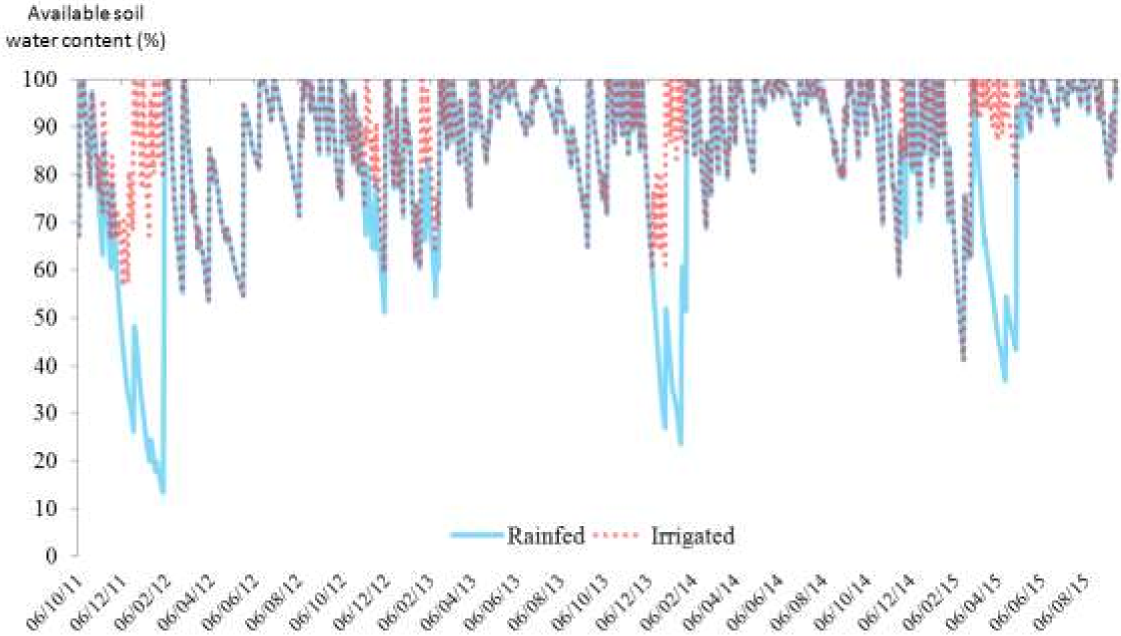
Zhang, Y., Loreau, M., Lü, X., He, N., Zhang, G. & Han, X. 2016. Nitrogen enrichment weakens ecosystem stability through decreased species asynchrony and population stability in a temperate grassland. *Glob. chang. Boil.* 22(4), 1445-1455.

SUPPORTING INFORMATION

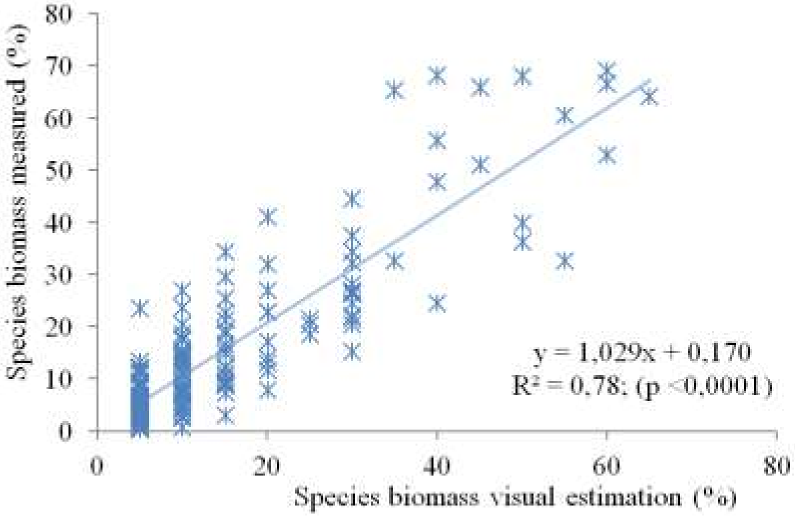
S1. Soil characteristics (0.15 cm soil layer) of the native grassland at the experimental site.

Ph (H_2O)	Organic C (%)	P Citric acid (µg	K (meq/100g)	Ca (meq/100g)	Mg (meq/100g)	Na (meq/100g)	CEC*
5.6	4.2	5.7	0.5	20.3	11.9	0.2	40.4

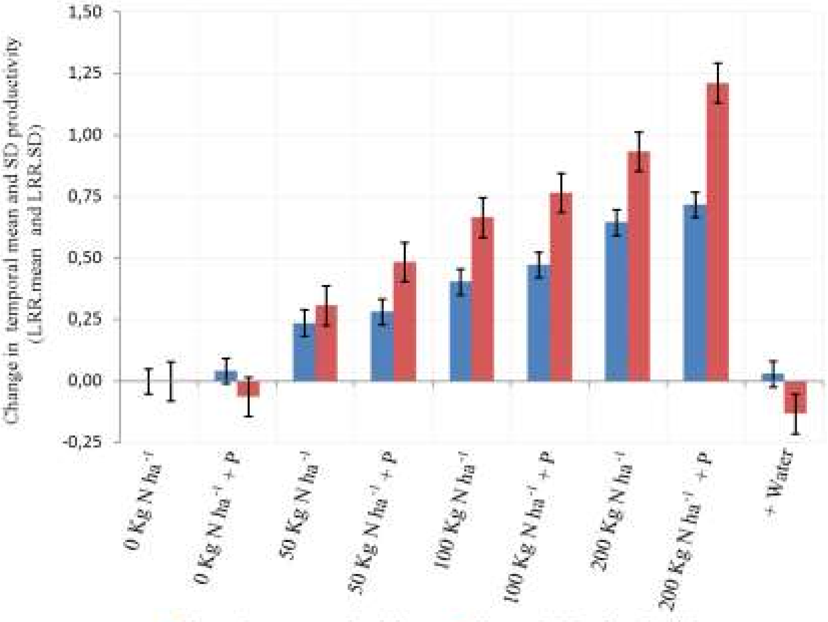
*CEC = Cationic Exchange Capacity



S2. Soil water balance of the experimental period. Each line represents the temporal evolution of the average volumetric soil water content for rainfed an irrigated subplots.



S3. Calibration of the relationship between visual estimation of species cover and the measured proportion of the biomass of each species obtained in 15 destructive samplings of 0.1 m^2 (R^2 = 0.78, P < 0.0001).



S4. Effects of nitrogen (N), phosphorus (P), N+P and water addition on relative changes in ANPP: A) mean, and B) standard deviation (SD). Bars represent mean ± SE (n = 3).

S5. Summary of ANPP stability (A and B), species richness (C and D), asynchrony (E and F), ANPP-mean (G and H) and ANPP-SD of dominant species (G and H) treatment means. ANPP stability and species richness data was natural log transformed for analysis and then retrotransformed to be presented in the table. The left and right letters between brackets indicate water, N and P main effects, and N x P interaction effects, respectively. Different letters within each effect (columns between lines) indicate significant differences $p < 0.05$.

A

Rainfed	3,7 B
Irrigated	4,2 A
N 0	5,0 A
N 50	4,1 B
N 100	3,6 C
N 200	3,2 D
P 0	4,1 A
P 35	3,9 B

B

	P 0	P 35
N 0	4,7 B	5,2 A
N 50	4,4 B	3,8 C
N 100	3,6 C	3,5 C
N 200	3,5 C	2,9 D

G

Rainfed	456 A
Irrigated	452 A
N 0	313 D
N 50	393 C
N 100	484 B
N 200	627 A
P 0	442 B
P 35	467 A

H

	P 0	P 35
N 0	306 E	319 E
N 50	381 D	405 D
N 100	471 C	498 C
N 200	609 B	645 A

C

Rainfed	58,7 A
Irrigated	57,3 A
N 0	59,8 A
N 50	59,4 A
N 100	57,4 B
N 200	55,4 C
P 0	59,5 A
P 35	56,5 B

D

	P 0	P 35
N 0	62,7 A	56,8 B
N 50	62,5 A	56,3 B
N 100	57,3 B	57,5 B
N 200	55,7 B	55,2 B

I

Rainfed	120 A
Irrigated	142 A
N 0	65 D
N 50	99 C
N 100	146 B
N 200	213 A
P 0	142 A
P 35	119 B

J

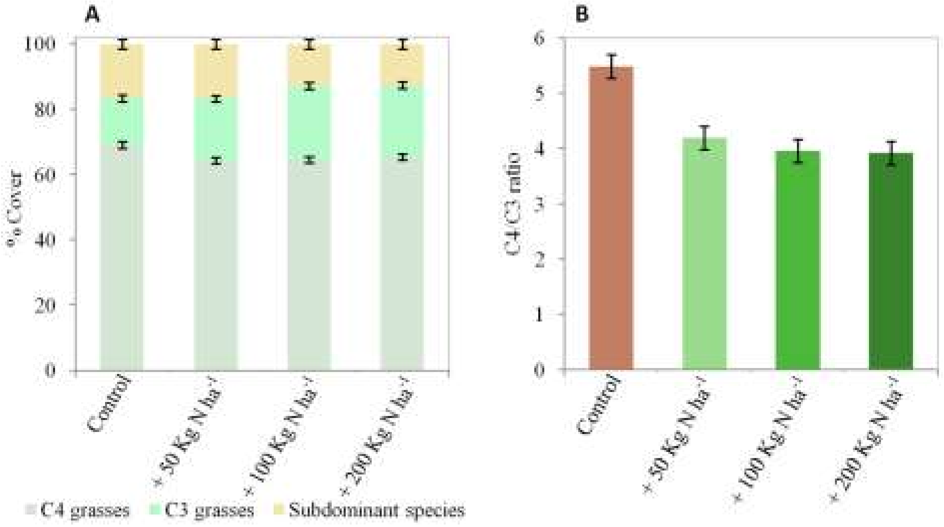
	P 0	P 35
N 0	68 E	61 E
N 50	91 D	108 D
N 100	137 C	154 C
N 200	181 B	245 A

E

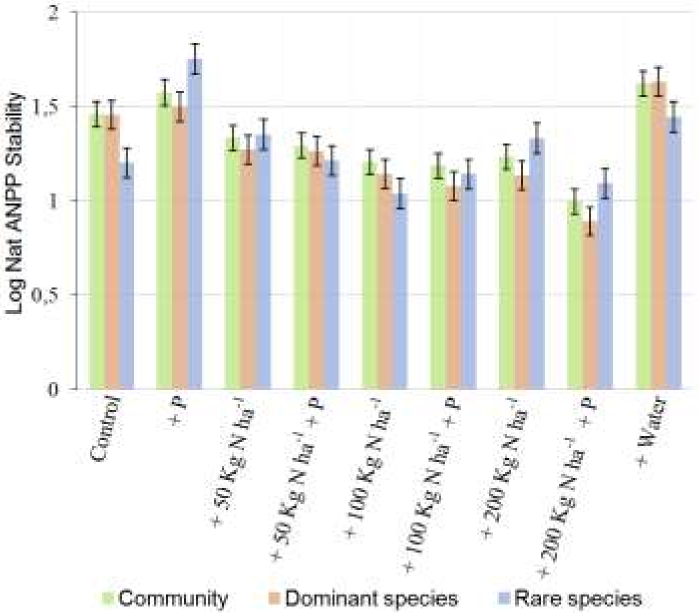
Rainfed	0,58 B
Irrigated	0,67 A
N 0	0,67 AB
N 50	0,65 ABC
N 100	0,59 C
N 200	0,62 BC
P 0	0,63 A
P 35	0,62 A

F

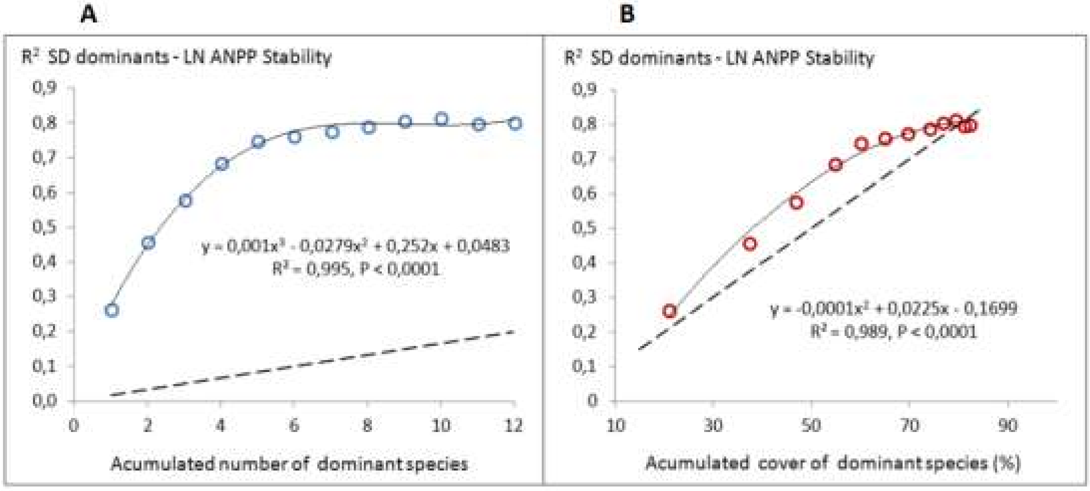
	P 0	P 35
N 0	0,67 AB	0,70 A
N 50	0,65 ABC	0,63 ABC
N 100	0,59 C	0,62 BC
N 200	0,62 BC	0,52 D



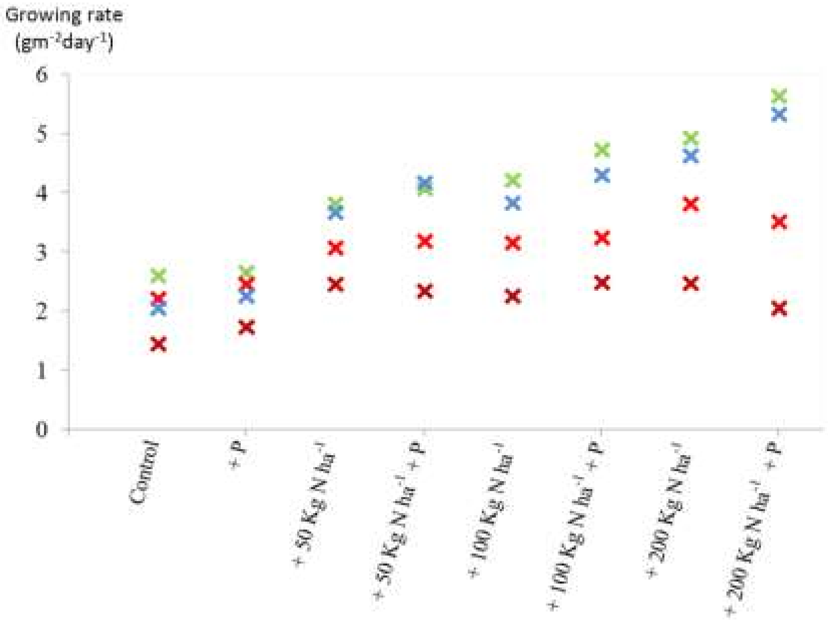
S6. Effects of nitrogen (N) treatments on plant community composition: A) dominant (C4 and C3 grasses) and subdominant species cover, and B) ratio of dominant grasses cover (C4/ C3 grasses). Bars represent mean ± SE (n = 12).



S7. Effects of nitrogen (N), phosphorus (P), N+P and water addition on community, dominant and subdominant species ANPP stability (natural log transformed). Dominant species ANPP stability was highly related with community ANPP stability (R^2 = 0.96, P < 0.0001), whereas subdominant species ANPP stability showed a weak relationship (R^2 = 0.23, P < 0.0003). Bars represent mean ± SE (n = 3).



S8. Evolution of the relationship (R^2) between the SD of dominant species ANPP and the community ANPP temporal stability, with: a) the cumulative number of species ordered by ranking of abundance (r^2 = 0.988, p < 0,0001), and b) cumulative cover of dominant species ordered by ranking of abundance (r^2 = 0.996, p < 0,0001). The dashed lines imply: a) an equal contribution to ANPP stability of each species; b) an equal contribution of each species to ANPP stability weighted by their abundance. If all species were functionally different and contribute equally to ANPP stability, the relationship should be analogous to the dashed lines.



S9. Effects of nitrogen (N), phosphorus (P), N+P addition on average grassland growing rate in the spring of each year. The response to N and N+P addition appears to be positively correlated with the average spring temperature detailed in table S8.

S10. Summary of spring temperature conditions at the experimental site.

Mean temperature	Mean degree-days	Accumulated degree-days	
17,8	9,8	442	×2011
18,2	10,2	459	×2012
17,4	9,4	451	×2013
20,1	12,1	484	×2014

Appendix. Estimation of ANPP temporal stability effect

To compare our results with the synthesis of 12 multiyear manipulate experiments of Hautier et al. (2015). we calculated the mean and standard deviation effects sizes as natural-log response ratios (LRR) comparing each treatment subplot with the control subplot (rainfed-without fertilizers) in each block. ANPP temporal stability effect (LRR.S) was then calculated as the difference between the log response ratio of the mean (LRR.mean) and the standard deviation (LRR.SD).

$$\text{LRR.S} = \text{LRR.mean} - \text{LRR.SD}$$

Similarly, species richness effect (LRR.richness) was calculated for each subplot as the natural log of the ratio of treatment subplot species richness divided by the species richness of the control subplots.

CAPÍTULO III

The Dilemma of Improving Native Grasslands by Overseeding Legumes: Production Intensification or Diversity Conservation [2]

---

[2] A paper published in Rangeland Ecology and Management 2016. 69(1) 35-42
Jaurena, M., Lezama, F., Salvo, L., Cardozo, G., Ayala, W., Terra, J. and Nabinger, C.

The Dilemma of Improving Native Grasslands by Overseeding Legumes: Production Intensification or Diversity Conservation

Abstract

In native campos of Uruguay, overseeding legumes coupled with phosphorus (P) fertilization is a technology used to increase animal production. Short-term improvements in both forage productivity and quality are repeatedly reported. But some evidence suggests that this management may at times lead to the collapse of the native community and invasions by exotic species. Indeed, it is yet unclear to what extent overseeding legumes into native grasslands affects its long-term integrity. This study uses data from a long-term experiment to assess whether increased P fertilizer rates – typically used to encourage legume establishment and growth– are associated with reduced species diversity. In 1996 a grazed native grassland in eastern Uruguay was either left untouched (control) or overseeded with a mix of *Trifolium repens* and *Lotus corniculatus* and then fertilized at either a moderate or high rate of phosphorus (197 or 394 kg · ha^{-1} over 13 years, respectively). The three treatments were arranged in a randomized block design with 4 replicates of 2 hectares each. In 2005 the experiment was exhaustively sampled: 11 georeferenced sampling points per replicate, each encompassing ~20 m^2. Extractable P was measured in the 0 to 5 and 5 to 15 cm soil layers. In 2009, species presence and cover was measured at the same points. Across treatments, wherever legumes were introduced, extractable soil P was negatively related to species richness and diversity ($P<0.01$) and native grass cover was reduced. This effect became asymptotic once soil P exceeded 27 and 36 mg · kg^{-1} of P (0-5 cm), respectively. Therefore, the documented reduction in species richness and diversity suggests a trade-off between increased pasture production and decreased vegetation stability may be operating in response to P fertilization of overseeded grasslands. The underlying ecophysiological mechanisms, and grazing management options to mitigate species diversity decline, should be further studied.

Key words: Campos, improvement, legumes, trade-off, short-term production, long-term integrity

INTRODUCTION

Overseeding legumes coupled with phosphorus (P) fertilization is an effective procedure to establish high quality forage species into native grasslands, and has been adopted in many agro-ecological regions of the world (Cook 1980; Coates et al. 1990; Risso and Berretta 1997; Tiecher et al. 2014). In the short term, this technology improves the quantity of forage, prevents protein deficiency in the diet of animals, and thus increases livestock productivity (Risso and Berretta 1997; Soca et al. 2002; Ferreira et al. 2011). This practice also replaces the need for nitrogen (N) fertilization (Bryan 1985). However, the limited persistence of legumes (Muir et al. 2011) and frequent invasions of exotic species (Tognetti and Chaneton 2012) are a relevant long-term concern, from both agronomic management and ecological conservation points of view. Low legume persistence in overseeded grasslands emphasizes the need to identify management thresholds that prevent alternative vegetation states dominated by exotic invaders. At the same time, it is important to know how different species and communities respond to soil nutrient enrichment if we are to conserve grassland biodiversity (Isbell et al. 2013).

There is a growing interest to understand the impact of management practices on ecosystem services of native grasslands (Carvalho and Batello 2009; Paruelo and Vallejos 2013). For example, in the campos biome region of South America, native grasslands represent the largest agro-ecosystem and provide valuable economic and ecosystem services. These ecosystems are part of the Río de la Plata grasslands, representing a unique biodiversity reserve with over three thousand species of temperate and subtropical plants (Bilenca and Miñarro 2004). However, they are critically threatened by changes in land use (Overbeck et al. 2007). Native grasslands are in general very stable and persistent, but livestock production with traditional management is economically limited, driving changes in land use to forestry or crop production. Therefore, sustainable management of these grasslands requires reconciling environmental conservation and production priorities (Bullock et al. 2001; Lemaire 2012).

Variability of climatic and soil conditions in the Río de la Plata grasslands are the main drivers that favor the existence of highly diverse plant communities with differing growth strategies. Species diversity can stabilize productivity and prevent exotic species invasions through multiple strategies to acquire resources (Dukes 2001; Hooper et al. 2005; Hector et al. 2010), and greater use of limiting factors (Hooper and Dukes 2004). However, it has been found that fertilization weakens the positive effects of diversity on stability (Suding et al. 2005, Hautier et al. 2014), especially at local scales (Di Tommaso and Aarssen 1989), and increases the probability of system collapse and subsequent evolution to low diversity states. The fluctuating resources theory of invasibility postulates that rapid increases in resources supplies makes a plant community more susceptible to exotic species invasion (Davis et al. 2000). In this case, dominant invasive species may act as transformer species, modifying both diversity and function (Richardson et al. 2000) and changing the state of the grassland community.

Production limitations in the campos biome ecosystems are mainly related to climatic variability (Bettolli et al. 2010) and deficiencies of N and P content in soils (Pallares et al. 2005). It has been recognized that soil P controls the growth and persistence of forage legumes in native grasslands (Coates et al. 1990; Pallares et al. 2005). Nevertheless, there are concerns about adverse effects of P enrichment on the diversity of species adapted to N and P limited soils (Ceulemans et al. 2011). The aim of this study was to determine whether increased P fertilizer rates are associated with reduced species richness and diversity in native grasslands in which legumes were overseeded. We hypothesize that increased levels of soil P concentrations from P additions aimed at encouraging legume establishment and growth led to: i) reductions in species richness and diversity, and ii) increases the abundance of exotic invasive species.

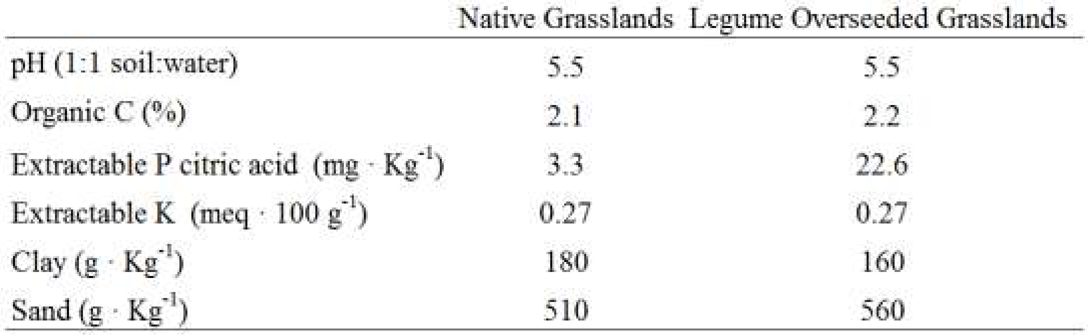
METHODS

Study area

The study was carried out in the “Palo a Pique” experimental farm, located in Eastern Uruguay (33°15'44" S, 54°28´59” W; 50 m above sea level). This region is part of “Rio de la Plata grasslands”, a biome of temperate and subtropical grasslands, dominated by C4 and C3 perennial grasses that extends from East Central Argentina to Southern Brazil and includes most of Uruguay (Soriano 1991). At the experimental site, mean annual rainfall varies between 1000 and 1600 mm, evenly distributed throughout the year, and mean monthly temperature varies from 11°C in winter to 23°C in summer.

The topography is softly hilly (slope 2 to 3%), and soils are a mixture of Abruptic Argiaquolls and Oxyaquic Vertic Argiudolls (fine, smectitic and thermic) (Durán et al. 2005). The most abundant species are C_4 grasses, such as: *Axonopus fissifolius* (Raddi) Kuhlm, *Paspalum notatum* Flüggé, *Aristida murina* Cav., *Andropogon ternatus* (Spreng.) Nees, *Paspalum plicatulum* Michx. This grassland community averaged a dry matter (DM) production of 3400 kg · ha^{-1} · yr^{-1} over a 12-yr period (Bermúdez and Ayala 2005). The main soil characteristics of the study site are presented in table 1.

Table 1. Major soil characteristics at Native and Overseeded Grasslands in the year 2005 (0-15 cm)



	Native Grasslands	Legume Overseeded Grasslands
pH (1:1 soil:water)	5.5	5.5
Organic C (%)	2.1	2.2
Extractable P citric acid (mg · Kg^{-1})	3.3	22.6
Extractable K (meq · 100 g^{-1})	0.27	0.27
Clay (g · Kg^{-1})	180	160
Sand (g · Kg^{-1})	510	560

Experimental design and treatments

The experiment was established in 1996 in a completely randomized block design with four replications. The three treatments consisted of: native grasslands (NG); and native grassland broadcasted with a mixture of 4 kg · ha^{-1} of *Trifolium repens* cv. Zapican and 8 kg · ha^{-1} of *Lotus corniculatus* cv. San Gabriel (each one inoculated with their specific *Rhizobium*) and then fertilized with either a moderate (MP) or a high rate of P (HP). The MP received 19.6 kg P · ha^{-1} · yr^{-1} at sowing and then 13.1 kg P · ha^{-1} · yr^{-1}, while the HP received 39.3 kg P · ha^{-1} · yr^{-1} and then 26.2 kg P · ha^{-1} · yr^{-1}. During the first three years superphosphate was used (21% P_2O_5 soluble - 23% P_2O_5 total), later rock phosphate (0% P_2O_5 soluble - 29% P_2O_5 total) was used. All experimental units (12 paddocks in total, of two hectares each) were rotationally grazed by yearling steers and heifers. Paddocks were grazed with similar moderate grazing intensities among treatments. In a previous study, Palacio (1999) reported forage allowances of 8.3 and 13.0 (kg of DM · 100 kg of animal live weight) for legume overseeded treatments and NG, respectively.

Average above-ground forage mass ± standard error from March 2008 to March 2010 were 2802 ± 194 kg DM $\cdot$ ha^{-1}, 3412 ± 193 kg DM $\cdot$ ha^{-1} and 3578 ± 183 kg DM $\cdot$ ha^{-1}, for NG, MP and HP, respectively.

Soil sampling and analysis

Soils were sampled in the spring of 2005 in 11 georeferenced points regularly distributed within each paddock (132 sampling points in total). At each sampling point, within a 2.5-m radius, eight 2.5 cm diameter sampling cores were taken from the 0 to 5 cm and 5 to 15 cm soil depths. Soil samples were then dried at 45 °C for 72 h, ground and sieved to pass through a 2-mm sieve. Extractable phosphorus content was measured adapting the citric acid method proposed by Thompson (1995). Fifty milliliters of citric acid (0.5%) were added to 5 g of soil and stirred for 30 minutes. The extract was filtered through a Whatman No. 2 filter paper. A 5-ml aliquot of filtered extract was removed and mixed with 8 ml of colorimetric reagent (Murphy and Riley 1962) and then diluted to 50 ml. The blue color development was measured after 45 minutes by absorbance at 882 nm with a UV spectrometer (Thermo Spectronic, Genesys 10S) and compared to known reference concentrations. Soil texture was evaluated by a modification of the Bouyoucos hydrometer method with gravimetric determination of the sand content (Beretta et al. 2014).

Vegetation sampling

Plant species composition was measured in the late spring of 2009 using a modified Braun-Blanquet abundance scale as proposed by Mueller-Dombois and Ellenberg (1974). In the exact same 11 georeferenced points per replicate were soil had been sampled, the name of all vascular plant species present was recorded, and their aerial cover (when greater than 5%) was visually estimated in 1-m^2 quadrats. Species-area curves determined previously (not shown here) confirmed that 11 m^2 of sampling area was adequate to represent the community species richness. Above-ground net primary pasture production was seasonally estimated from March 2008 to March 2010 using two exclusion cages (1 x 0.5 m) per paddock (moved after each cut) according to the method described by Klingman et al. (1943). At the beginning of every season of the year herbage was clipped to ground level. At the end of each season, all accumulated above-ground standing plant material was clipped again in two 0.1 m^2 quadrats per cage to estimate the forage production over the growth period. The sampled biomass was dried at 60°C for at least 72 h and weighed to determine the DM content.

Data analysis

Species cover and concentration of extractable soil P of the 132 sampling points constituted the primary database. Six sampling points were removed from the database because their extractable soil P in the 0 to 5 cm layer was higher than two standard deviations from the mean of the treatment and thus considered outliers. Species richness, native species richness, native grasses species richness and the Shannon Index (Shannon and Weaver 1949) were calculated for every sampling unit. The mean seasonal pasture production –expressed as daily growth rate ($kg\ DM \cdot ha^{-1} \cdot d^{-1}$) to compare different periods– was calculated for every paddock. The Shapiro-Wilks test was used to check the assumption of normally distributed residuals for all response variables and square root transformation was used when necessary. General linear mixed models were performed for each response variable considering block and sampling units nested within treatments as random factors, while treatments were considered a fixed factor. Several models combining different functions for correlations and variance of errors were tested. Then, the best-fitted models for each response variable was selected based on Akaike Criterion. Means for the significant fixed effects were compared using Fisher's LSD test ($p < 0.05$). Orthogonal contrasts (control *vs.* overseeded grasslands, and MP *vs.* HP) were performed to compare species richness and diversity, and cover of the main species and functional groups. To examine changes in species composition a principal component analysis was used. Extractable soil P at both depths and functional group cover (native warm season perennial grasses, native cool season perennial grasses, native species non grasses, exotic legumes, exotic winter annual grasses and exotic warm season perennial grasses) were the studied variables. We analyzed only the first two principal component axes because they explained a large proportion of the variation in species composition. Afterward, to identify the occurrence of changes in vegetation state related to the experimental treatments, a discriminant analysis of the dominant functional groups cover was performed. Segmented regressions (Toms and Lesperance (2003) were used to describe the relationship between richness and diversity *vs.* extractable soil P concentrations:

$$\text{if } x < R,\ \underline{y_{ij} = L + U\,(R - x) + e_{ij}} \qquad [1]$$

and

$$\underline{\text{if } x > R,}\ \underline{y_{ij} = L + e_{ij}} \qquad [2]$$

where yij is the dependent variable, x is the independent variable, eij represent the experimental error, R is the break point, L is the mean value of the zero slope segment, U indicates the magnitude of the non-zero slope segment. R values are referred to as the thresholds of extractable soil P concentration beyond which losses of richness and diversity became negligible. Statistical analyzes were performed using Infostat (Di Rienzo et al. 2015) and JMP$^{®}$ (SAS Institute, Cary, NC) software packages.

RESULTS

Continued applications of P fertilizers from 1996 to 2005 increased extractable soil P by 25 and 50 mg . kg^{-1} in the 0 to 5 cm layer, and by 0.7 and 3 mg . kg^{-1} in the 5 to 15 cm layer, in MP and HP treatments respectively (Fig. 1a). These values reflect both a substantial increase and stratification of the distribution of P availability for plants: clearly, resource supply increased faster than the capacity of the vegetation to use it and the system was becoming progressively enriched in P. Forage production of overseeded and P fertilized grasslands was higher than native grasslands across all growing seasons except in autumn 2009. The average forage production of overseeded and P fertilized grasslands was 60 % higher than native grasslands, while the P fertilization level had no significant effect. The maximum differences between overseeded and P fertilized grasslands with native grasslands were found in the periods of elevated growth (spring 2009, summers 2008-2009 and 2009-2010), with increments in forage production between 69% and 84% (Fig. 2).

A total of 156 different species were recorded in the experimental area. There were significant differences when comparing the native grasslands *vs.* the overseeded and P fertilized grasslands and between fertilization treatments. In the overseeded grasslands, exotic grasses displaced native species and overseeded legumes. Both cool and warm season native perennial grasses cover, as well as native non grasses cover, decreased in overseeded grasslands (Fig. 1b, 1c and 1d).

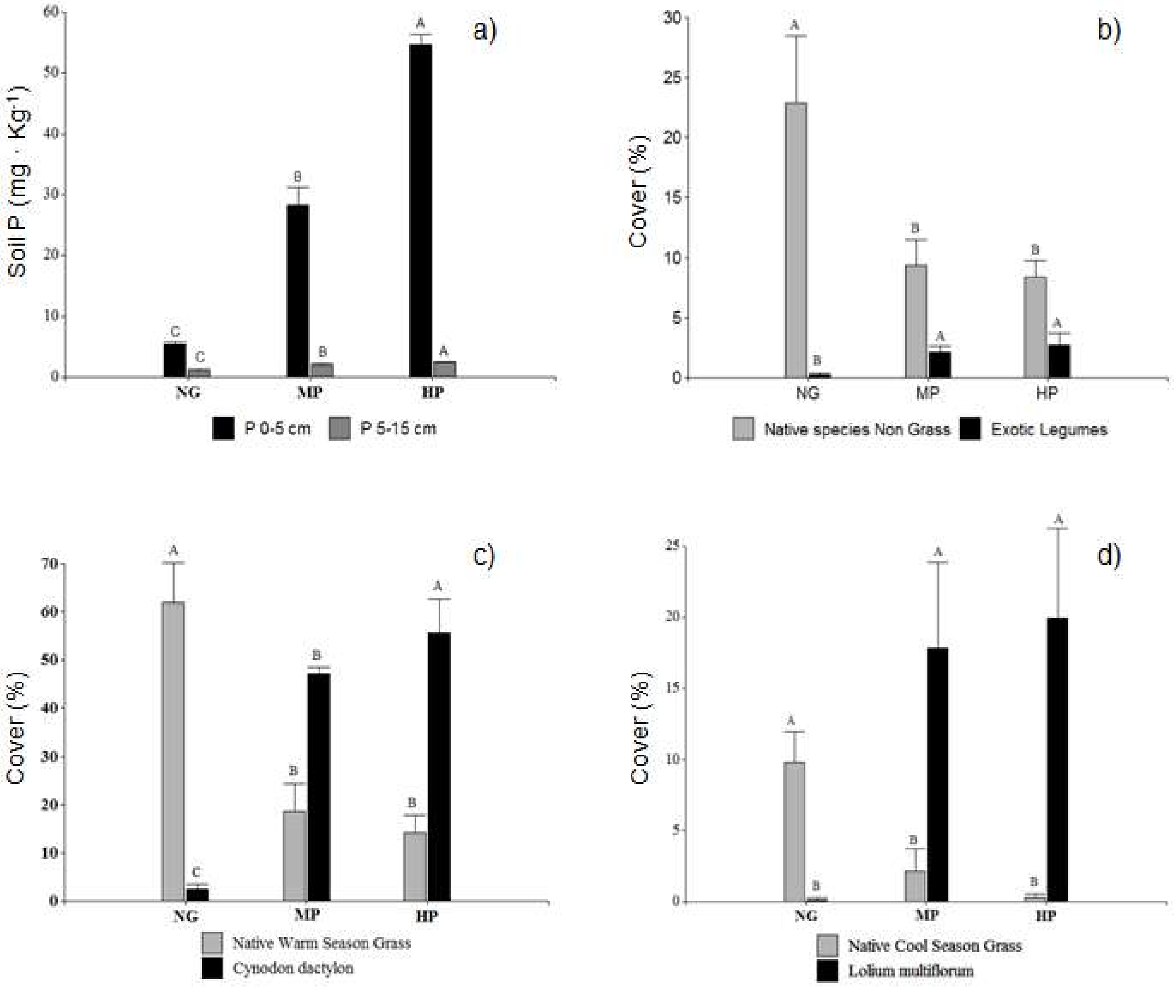


Figure 1. Treatment effects on: a) extractable soil P in the 0 to 5 cm and 5 to 15 cm layer; b) native species non grass and exotic legumes; c) native warm season grass and *Cynodon dactylon*; d) native cool season grass and *Lolium multiflorum*. NG= Natural Grassland; MP= Moderate Phosphate legume overseeded grassland; HP= High Phosphate legume overseeded grassland. Different letters within each parameter (columns within the same color) indicate significant differences $p < 0.05$, and bars represent the standard error.

The legume abundance of the grasslands was generally low, although legume cover was higher in overseeded grasslands than native grasslands (2.5 *vs.* 0.25%). Overseeded grasslands were dominated by two exotics grasses, the C4 perennial *Cynodon dactylon* and the C3 annual *Lolium multiflorum*, which accounted for an average 68% of cover in overseeded grasslands, but only 2.5% in native grasslands. Higher P fertilization resulted in higher cover of exotic species (61% in MP vs 75% in HP). Overseeded grasslands had lower diversity, species richness, native species richness and native grasses species richness than natural grasslands (Fig. 3). All of these variables were further reduced at the higher P fertilization rate. The most negative effects of the high P fertilization treatment were recorded in native species richness and native grasses richness, which had less than one-third and one-fifth of the native grassland species respectively. The overseeded grasslands had higher exotic species richness that partially compensated the reduction in native species.

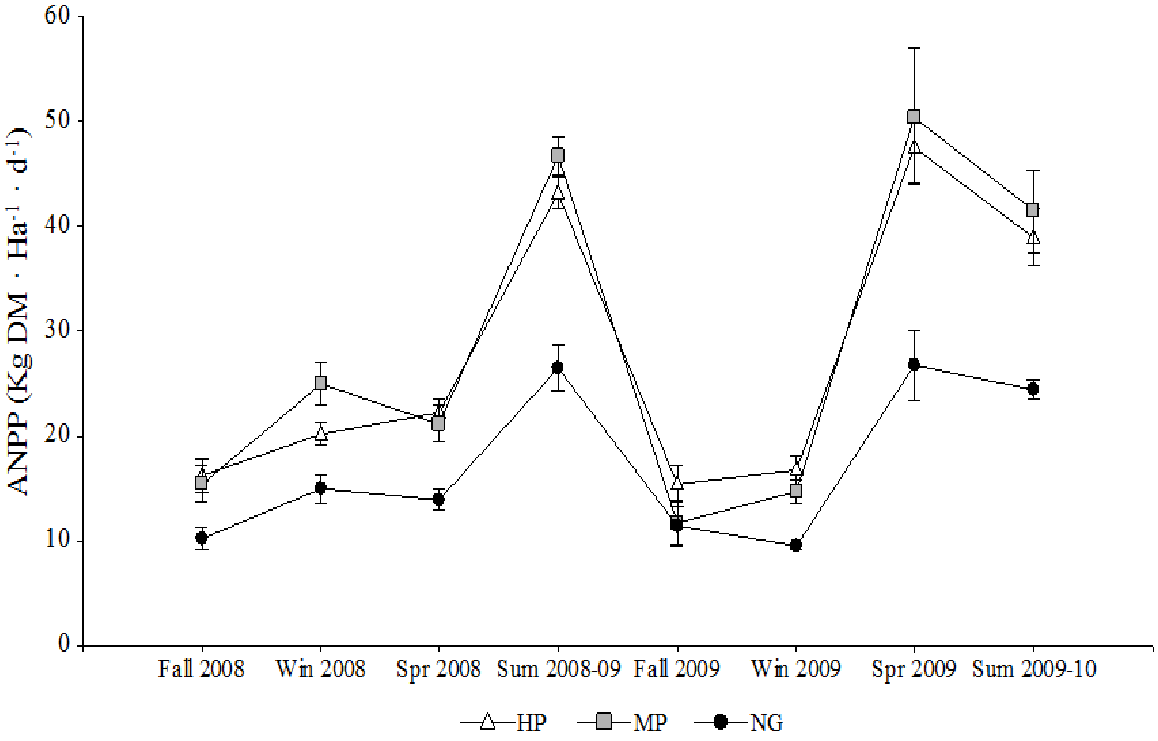


Figure 2. Seasonal variation in Aboveground Net Primary Production (ANPP). Grassland Dry Matter (DM) production from March 2008 to March 2010. Each point represents the daily mean growth (kg DM · ha^{-1}) of the treatment in each season. NG = Native Grassland (circles). MP = Moderate Phosphate legume overseeded grassland (squares). HP = High Phosphate legume overseeded grassland (triangles).

Species diversity and richness had negative relationships with extractable soil P concentration in the 0 to 5 cm layer (Fig. 4a and 4b). Segmented regression analysis indicated P thresholds of 36 and 27 mg · kg^{-1} beyond which the minimum values of 1.1 of the Shannon-Weaver diversity index and 13.9 species per m^{-2} were reached respectively. Species diversity and richness followed the same trend in the 5 to 15 cm layer, but this relationship was weaker than in the 0 to 5 cm depth.

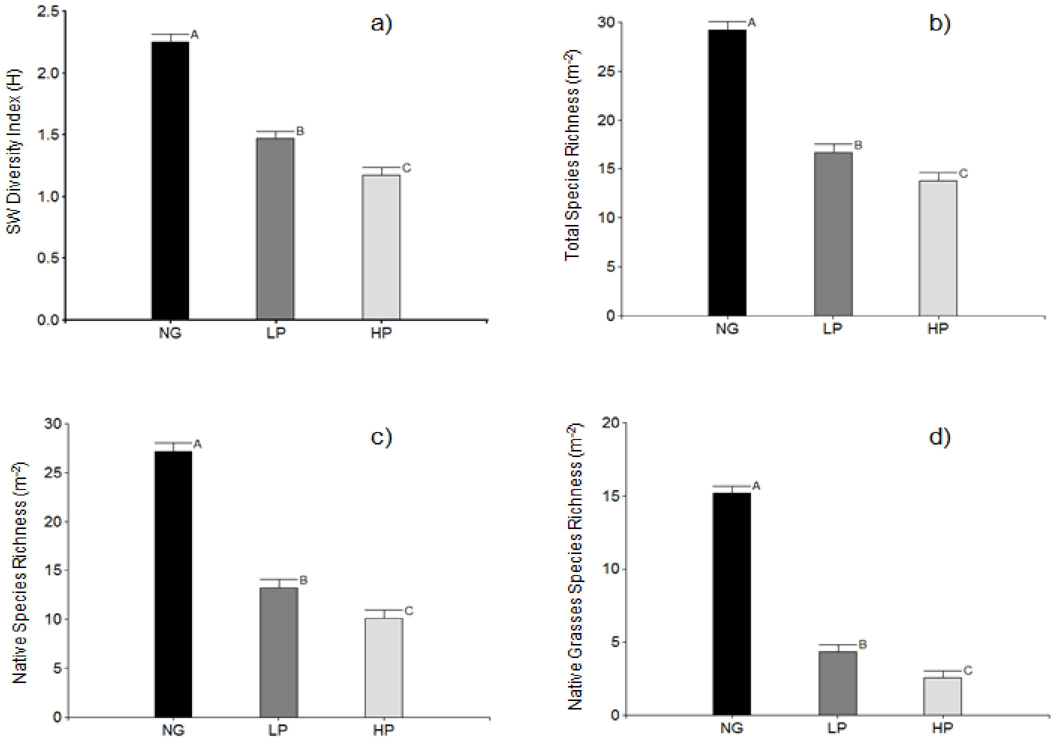


Figure 3. Treatment effects on: a) Shannon-Weaver diversity index; b) total species richness; c) native species richness; d) native grasses species richness. NG= Natural Grassland; MP= Moderate Phosphate legume overseeded grassland; HP= High Phosphate legume overseeded grassland. Different letters between treatments correspond to significant differences $p < 0.05$, and bars represent the standard error.

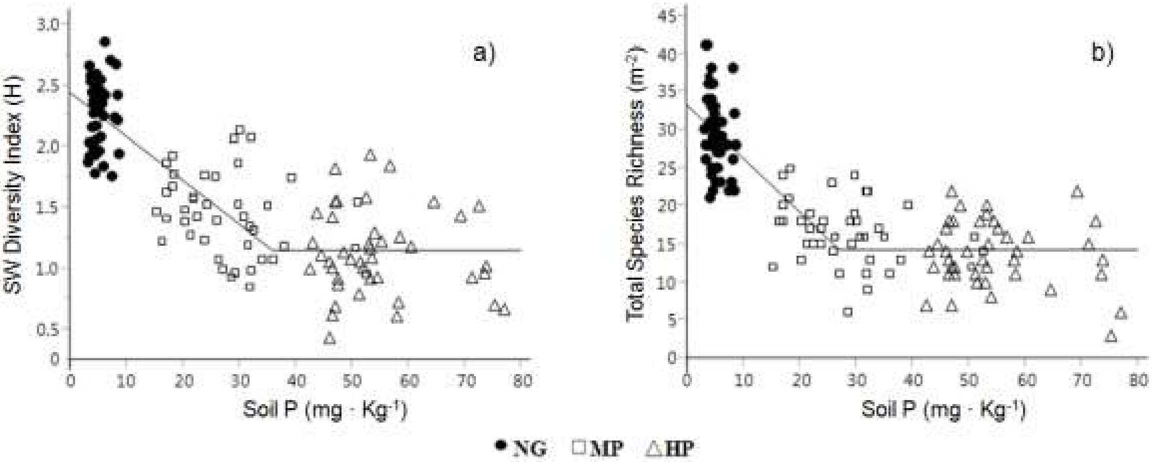


Figure 4. Relationships between extractable soil P concentrations ($mg \cdot kg^{-1}$) with:
a) Shannon Weaver Index $(H) = 1.11 + 0.037 * (35.9 - P)$ if $P < 35.9$ and $H = 1.11$ if $P > 35.9$, $R^2 = 0.67$, $P < 0.0001$, Root Mean Square Error (RMSE) = 0.34; b) Total Species Richness $(R) = 13.6 + 0.73 * (27.0 - P)$ if $P < 27.0$ and $R = 13.6$ if $P > 27.0$, $R^2 = 0.69$, $P < 0,0001$, RMSE = 4.60. The 126 sampling units surveyed consist of native grasslands (circles), moderate P fertilized legume overseeded grassland (squares) and high P fertilized legume overseeded grassland (triangles).

Principal component analysis ordination based on the cover of dominant species, functional groups and extractable soil P concentration also indicates close relationships of extractable soil P with species composition (Fig. 5). The first principal component explained 72% of variation and neatly separated native from overseeded grasslands. The second component explained 11% of the variation, and separated experimental units associated to warm season from cool season species cover, that is *Cynodon dactylon* from *Lolium multiflorum* and the oversown legumes in overseeded grasslands, and warm season from cool season native perennial grasses in native grasslands.

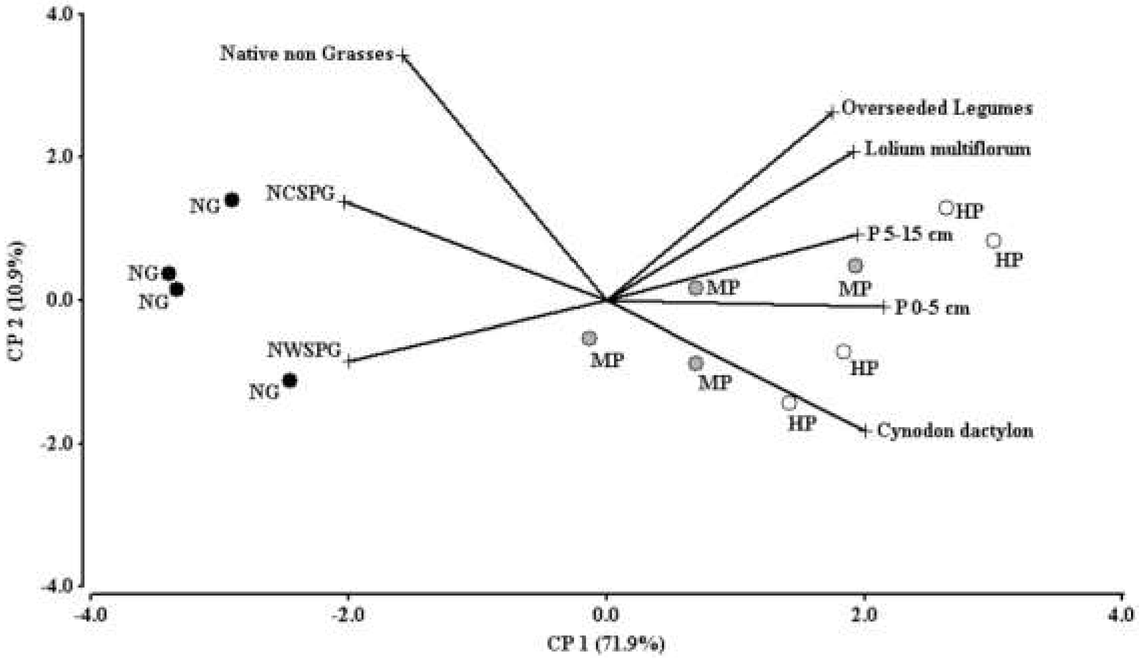


Figure 5. Biplot of Principal Components Analysis considering the principal species or groups of species cover and soil characteristics. The arrows represent the direction of the main effects and the circles summarize each paddock position in the multivariate space. Treatments: NG = Native Grassland; MP = Moderate Phosphate legume overseeded grassland; HP = High Phosphate legume overseeded grassland, the numbers following treatment are the identification of each paddock replication. Cover of: NWSPG = Native Warm Season Perennial Grasses; NCSPG = Native Cool Season Perennial Grasses; Non grasses = Native species non grasses; Legumes = Over seeded Legumes; Lolium = *Lolium multiflorum*; Cynodon = *Cynodon dactylon.* Soil characteristics: P = mg · kg^{-1} in the depths of: 0-5 and 5-15 cm.

The biplot of communities ordination by discriminant analysis summarizes in a simple manner the patterns of variation in species composition. This analysis confirms that native differ from overseeded grasslands, however the fertilization level did not significantly influence the state of overseeded communities (Fig. 6).

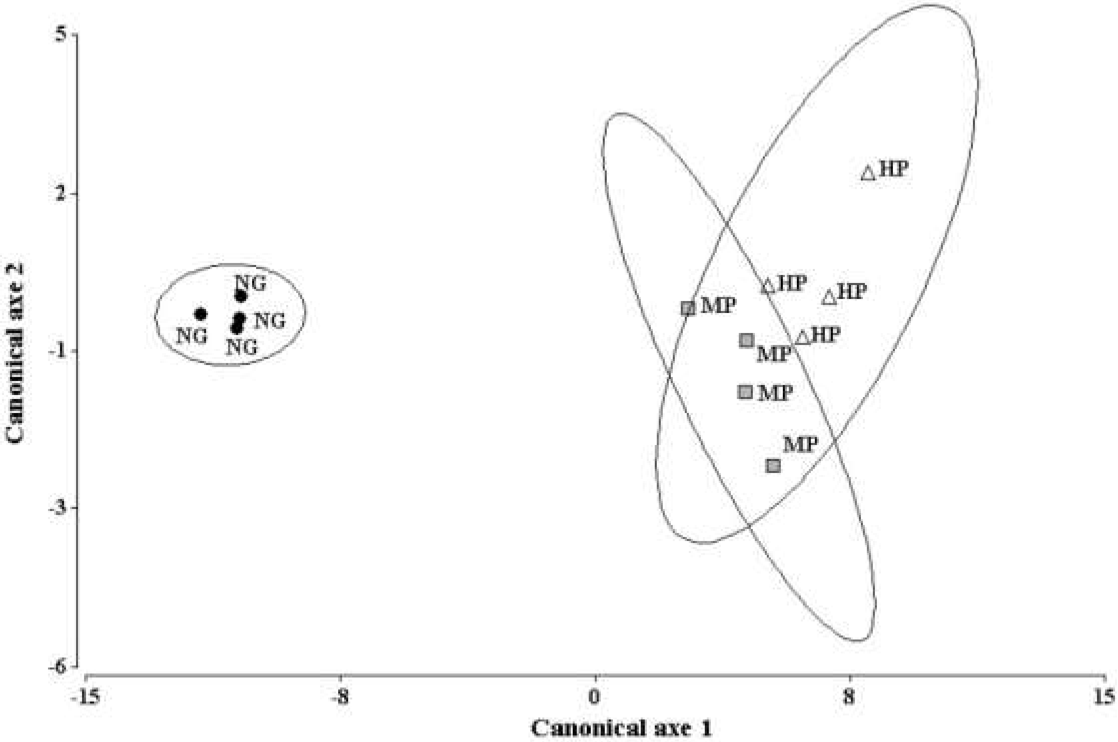


Figure 6. Graphic representation of discriminant analysis considering the principal species or groups of species cover. The symbols summarize each paddock position in the multivariate space. Treatments: NG = Native Grassland (circles). MP = Moderate Phosphate legume overseeded grassland (squares). HP = High Phosphate legume overseeded grassland (triangles), and the ellipses shows the prediction interval with a probability of 95%.

DISCUSSION

This study revealed that higher extractable soil P concentrations in the surface soil of native grasslands overseeded with legumes were associated with lower species richness and diversity. Native perennial grasses were particularly sensitive: after 13 years, their cover decreased by two-thirds. After a decade, this space was not occupied by the overseeded legumes, as legumes were almost absent in all treatments. Instead, two exotic species increased in relative cover: a C3 annual ryegrass (*Lolium multiflorum*) and a C4 stoloniferous perennial grass (*Cynodon dactylon*).

Potential negative effects of P availability on native communities have been described repeatedly, for instance in semi-natural grasslands of Europe (Janssens et al.

1998 and Gilbert et al. 2009) and by Lambers et al. (2010) in ancient landscapes of Western Australia. The latter authors reported that species-rich communities are absents in soils with high P concentrations. Likewise, Ceulemans et al. (2013) identified that soil P is a better predictor of plant species richness than soil N, and concluded that P enrichment is a threat to plant diversity. However, the actual ecophysiological mechanism(s) by which P enrichment affected native species in the overseeded grasslands of the present study are not known. Multiple, non-mutually exclusive, alternatives are possible.

It is well established that legume establishment and growth are encouraged by P fertilization both in native overseeded grasslands (Risso and Berretta 1997) and sown grasslands (Bermudez et al. 2008). The cause, it is believed, is related to the very limiting levels of plant available P and nitrogen that these soils have (Pallares et al. 2005). Legumes, having the P restriction eased by fertilization, become highly competitive due to their ability to fix atmospheric nitrogen. Previous work in our study site reported 31% and 69% of legume cover in MP and HP respectively in the third year of the experiment (Palacio 1999), revealing the dominance of overseeded species at this stage. Some evidence indeed indicates that these legumes were actively fixing N in these grasslands (Mallarino and Wedin 1990).

The reasons for the limited persistence of the legumes are even less certain. It may be that sustained progressive enrichment of soil N due to biological N fixation makes legumes less competitive and thus grasses dominate. A model of self-regulation was proposed by Chapman et al. (1996) to explain the temporal/spatial variations in legume content of ryegrass/white clover swards based on the amount of mineral N in the soil. Nevertheless, this dynamic response of grasses and legumes to N availability also indicates that it is unlikely to maintain high legume contents in native grasslands if soils are simultaneously N and P enriched. Another possibility is that drought events drastically reduced legume persistence, leaving empty gaps in the sward (Acuña et al. 2012). In any case, the grasses that took advantage of the reduced legume cover were not the original native species. However, two exotics: the C3 winter growing *L. multiflorum* which has a high response to N (Vallano et al. 2012) and the C4 summer growing *C. dactylon* which has large ability to explore the soil (Llobet et al. 2012) became dominant.

Several functional mechanisms have been proposed to explain fertilization effects on species richness and diversity. One regularly hypothesized mechanism is a shift from below-ground competition for nutrients to above-ground competition for light as the environment becomes richer in any limiting resource (Goldberg and Miller 1990; Hautier et al. 2009). In the third year of the experiment, the forage production was in average 101% higher in overseeded and P fertilized grasslands than native grasslands (Palacio 1999). In addition, after a decade, the forage production was in average 60% higher in overseeded and P fertilized grasslands than native grasslands. The increased production would be one of the main causes of species losses in overseeded and P fertilized grasslands, but it is unknown if native species richness resulted from the additive or interactive effects of root and shoot competition. Mycorrhizal–plant interactions could be another mechanisms by which P availability affected native species (Van der Heijden et al. 1998). Pezzani et al. (2012) found lower root mycorrhizal colonization in the natives grasses *Steinchisma hians* and *Mnesitea selloana* when present in legume overseeded and P fertilized grasslands than in native grassland. Interestingly, *Cynodon dactylon* showed high levels of mycorrhizal colonization in both situations.

The observed relationship between P availability and species richness and identity in overseeded grasslands lead us propose a graphical state and transition model depicted in Figure 7. State and transition models are a useful tool for grasslands management (Westoby et al. 1989; Laycock 1991; Knapp et al. 2011). The proposed model consists of two alternative steady states (native grassland and exotic grass invaded grassland) and an intermediate unsteady state characterized by (native grasses plus overseeded legumes) “the at-risk community” whose duration would be related to legumes persistence. Then, the return to a “native grassland” state or the transition to a state “dominated by exotics grasses” would depend on soil P concentration and native species abundance. This first approach needs further research to establish state and transition framework recommendations (Briske et al. 2008). Despite the lower diversity, the grasslands invaded by exotics grasses were more productive than native grasslands, particularly in springs and summers with good growing conditions, but it is not known what the response might be in periods of drought.

Willems and van Nieuwstadt (1996) and Isbell et al. (2013) have already shown that low-diversity exotic dominated states may persist decades after N or P enrichment ceases, and Tognetti and Chaneton (2012) showed that re-establishment of native perennial grasses was constrained by species like *Cynodon dactylon* and *Lolium multiflorum* which prevent the recovery from the soil seed bank.

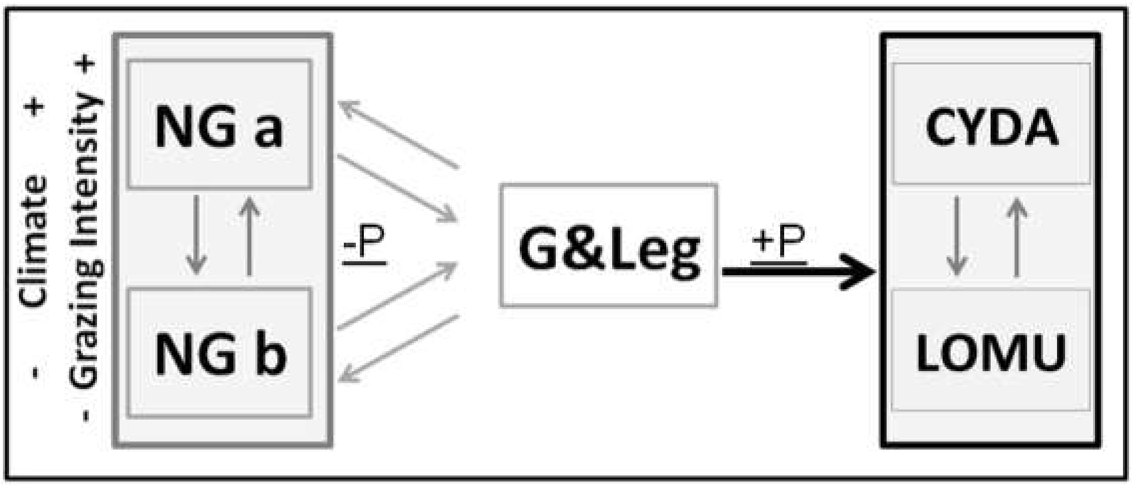


Figure 7. A conceptual state and transition model proposed for long term evolution of over seeded and P fertilized grasslands. Grey arrows are community pathways, the black arrow is the transition, and the grey boxes are the steady states. In the cases of native grasslands, the community phases a or b are related to grazing or climate events. In the exotic invaded grasslands, the *Cynodon dactylon* (CYDA) or *Lolium multiflorum* (LOMU) dominance are related to the season of the year. The at-risk grassland community is the legumes over seeded grassland (G&Leg) and the transition is controlled mainly by soil P concentration.

It is noteworthy that all of HP and half MP sampling units exceed the P threshold beyond which diversity and richness appears to remain at consistent lower levels. While we do not know whether the condition observed at every sampling point was in a steady state or evolving towards another condition, the asymptotic shape of the relationship – virtually no change in species composition above extractable soil P of ~ 30 mg · kg^{-1} – suggest a new vegetation state may have been reached after the 13 years of the experiment. The validity of this hypothesis should be the aim of subsequent studies. Specifically, because this study did not included soils within the range between 6 and 18 mg · kg^{-1} of extractable P: therefore, the possibility of long-term coexistence of a diverse array of native species with overseeded legumes cannot be ruled out. Especially, when the likelihood of strong interactions between P availability and grazing management is high (Schwinning and Parsons 1996).

IMPLICATIONS

The negative relationship documented by the present study between species diversity and the concentration of extractable soil P in native grasslands overseeded with legumes suggests that a trade-off between increased forage production and quality *vs.* reduced integrity of the plant community may be operating. Neither the underlying ecophysiological mechanisms, nor whether this response is modified by grazing management, is known, and should therefore be the subjects of further investigation. Our findings do indicate that, first, a precautionary approach is warranted when considering the level of P fertilization, and second, soil testing to quantify extractable soil P must be a standard part of the agronomic management to avoid unnecessary inputs and to prevent diversity losses. Therefore, the trade-off between short term economic response and long term sustainability should be taken into account considering the ecological limits of the intensification.

ACKNOWLEDGEMENTS

The work was supported by the Instituto Nacional de Investigación Agropecuaria (INIA), Uruguay. The authors gratefully acknowledge the forage staff of INIA Treinta y Tres and Tacuarembó for their assistance with laboratory and field work, and especially to Raúl Bermúdez and Graciela Quintans for their vision to install and maintain the Palo a Pique long-term experiment. We also thank Federico Condón, Rafael Reyno and Claudia Marchesi for the English corrections, and especially to Fernando Lattanzi for critical review of the manuscript and English corrections.

LITERATURE CITED

Acuña, H., Inostroza, L., and G. Tapia. 2012. Strategies for selecting drought tolerant germplasm in forage legume species. *In:* I. Rahman and H. Hasegawa [eds.]. *Water Stress*, Rijeka, Croatia. P. 277-300.

Beretta, A. N., Silbermann, A. V., Paladino, L., Torres, D., Bassahun, D., Musselli, R., and A. García-Lamohte. 2014. Soil texture analyses using a hydrometer:

modification of the Bouyoucos method. *Ciencia e Investigación Agraria*, *41*(2):263-271.

Bermudez, R., and W. Ayala. 2005. Producción de forraje de un campo natural de la zona de lomadas del este. *In*: Seminario de actualización técnica en manejo de campo natural. Montevideo, Uruguay: INIA. p. 33-40. (Serie Técnica 151)

Bermúdez, R., W. Ayala, M. Carámbula, and A. Morón. 2008. Fuentes fosfatadas en la rotación soja-pasturas en lomadas del este. *In*: Seminario de actualización técnica en fertilización fosfatada de pasturas en la región este. INIA, p. 111-118 (Seríe Técnica, 172).

Bettolli, M. L., M. A. Altamirano, G. Cruz, F. Rudorff, A. Martínez, J. Arroyo, and J. Armoa. 2010. Pastura natural de Salto (Uruguay): relación con la variabilidad climática y análisis de contextos futuros de cambio climático. *Revista Brasileira de Meteorologia* 25(2):248-259.

Bilenca, D., and F. Miñarro. 2004. Identificación de Áreas Valiosas de Pastizal (AVPs) en las Pampas y Campos de Argentina, Uruguay y sur de Brasil. Buenos Aires: Fundación Vida Silvestre. 353 p.

Briske, D. D., B. T. Bestelmeyer, T. K. Stringham, and P. L. Shaver. 2008. Recommendations for development of resilience-based state-and-transition models. *Rangeland Ecology and Management* 61(4):359-367.

Bryan, W. B. 1985. Effects of sod-seeding legumes on hill land pasture productivity and composition. *Agronomy Journal*, *77*(6), 901-905.

Bullock, J. M., J. Franklin, M. J. Stevenson, J. Silvertown, S. J. Coulson, S. J. Gregory, and R. Tofts. 2001. A 12-year grazing experiment on species-poor grassland: vegetation responses and correlation with plant traits. *Journal of Applied Ecology*, 38:253–267.

Carvalho, P. C., and C. Batello. 2009. Access to land, livestock production and ecosystem conservation in the Brazilian Campos biome: the natural grasslands dilemma. *Livestock Science*, 120(1):158-162.

Ceulemans, T., R. Merckx, M. Hens, and O. Honnay. 2011. A trait based analysis of the role of phosphorus vs nitrogen enrichment in plant species loss across North-west European grasslands. *Journal of Applied Ecology* 48:1145-1163.

Ceulemans, T., R. Merckx, M. Hens, and O. Honnay. 2013. Plant species loss from

European semi natural grasslands following nutrient enrichment–is it nitrogen or is it phosphorus?. Global Ecology and Biogeography, 22(1):73-82.

Chapman, D. F., A. J. Parsons, and S. Schwinning. 1996. Management of clover in grazed pastures: expectations, limitations and opportunities. Special Publication *Agronomy Society Of New Zealand*, 55-64.

Coates, D. B., P. C. Kerridge, C. P. Miller, and W. H. Winter. 1990. Phosphorus and beef production in northern Australia. 7. The effect of phosphorus on the composition, yield and quality of legume-based pasture and their relation to animal production. *Tropical Grasslands*, *24*(3):209-220.

Cook, S. J. 1980. Establishing pasture species in existing swards: a review. *Tropical grasslands*, 14(3):181-187.

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

Di Rienzo J. A., F. Casanoves, M. G. Balzarini, L. Gonzalez, M. Tablada, and C.W. Robledo. InfoStat versión 2015. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina.

Di Tommaso A., and L. W. Aarssen. 1989. Resource manipulation in natural vegetation: a review. *Vegetatio* 84:9-29.

Dukes, J. S. 2001. Biodiversity and invasibility in grassland microcosms. *Oecologia*, 126(4), 563-568.

Durán, A., A. Califra, J.H. Molfino, and W. Lynn. Keys to Soil Taxonomy for Uruguay. United States Department of Agriculture, Natural Resources Conservation Service. 77 pp.

Ferreira, E. T., C. Nabinger, D. A. G. Elejalde, A. K. D. Freitas, I. J. Carassai, and F. Schmitt. 2011. Fertilization and oversowing on natural grassland: effects on pasture characteristics and yearling steers performance. *Revista Brasileira de Zootecnia* 40(9):2039-2047.

Gilbert, J., D. Gowing, and H. Wallace. 2009. Available soil phosphorus in semi-natural grassland: assessment methods and community tolerances. *Biological Conservation* 142:1074-1083.

Goldberg, D. E., and T. E. Miller. 1990. Effects of different resource additions of species diversity in an annual plant community. *Ecology*, *71*(1):213-225.

Hautier, Y., P. A. Niklaus, and A. Hector. 2009. Competition for light causes plant biodiversity loss after eutrophication. *Science* 324.5927:636-638.

Hautier, Y., E. W. Seabloom, E. T. Borer, P. B. Adler, W. S. Harpole, … and A. Hector. 2014. Eutrophication weakens stabilizing effects of diversity in natural grasslands. *Nature* 508:521–525

Hector, A., Y. Hautier, P. Saner, L. Wacker, R. Bagchi, J. Joshi, and M. Loreau. 2010. General stabilizing effects of plant diversity on grassland productivity through population asynchrony and overyielding. *Ecology* 91(8):2213-2220.

Hooper, D. U., and J. S. Dukes. 2004. Overyielding among plant functional groups in a long-term experiment. *Ecology Letters* 7:95-105.

Hooper, D.U., F.S. Chapin, J.J. Ewell, A. Hector, P. Inchausti, … and D. A. Wardle. 2005. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecological Monograph* 75:3-35.

Isbell, F., D. Tilman, S. Polasky, S. Binder, and P. Hawthorne. 2013. Low biodiversity state persists two decades after cessation of nutrient enrichment. *Ecology letters*, 16(4):454-460.

Janssens, F., A. Peeters, J. R. B. Tallowin, J. P. Bakker, R. M. Bekker, F. Fillat, and M. J. M. Oomes. 1998. Relationship between soil chemical factors and grassland diversity. *Plant and Soil* 202:69-78

Klingman, D. L., Miles, S. R., Mott, G. O. 1943. The cage method for determining consumption and yield of pasture herbage. *Journal of the American Society of Agronomy*, 35:739-746.

Knapp, C. N., M. Fernandez-Gimenez, E. Kachergis, and A. Rudeen. 2011. Using participatory workshops to integrate state-and-transition models created with local knowledge and ecological data. *Rangeland Ecology and Management*, 64(2):158-170.

Laycock, W. A. 1991. Stable states and thresholds of range condition on North American rangelands: a viewpoint. *Journal of Range Management* 44(5):427-433.

Lambers, H., M. C. Brundrett, J. A. Raven, and S.D. Hopper. 2010. Plant mineral nutrition in ancient landscapes: high plant species diversity on infertile soils is linked to functional diversity for nutritional strategies. *Plant and Soil* 334:11-31.

Lemaire G. 2012. lntensification of animal production from grassland and ecosystem. In: (D. Hemming [eds.]. Animal Science Reviews 2012, CABI, Oxfordshire, UK, p. 45-52.

Llobet, M., O. R. Vignolio, R. Save, and C. Biel. 2012. Above-and below-ground interactions between Lotus tenuis and Cynodon dactylon under different fertilization levels. *Canadian Journal of Plant Science* 92(1):45-53.

Mallarino, A. P., and W. F. Wedin. 1990. Nitrogen fertilization effects on dinitrogen fixation as influenced by legume species and proportion in legume-grass mixtures in Uruguay. *Plant and Soil*, 124(1):127-135.

Mueller-Dombois, D., and H. Ellenberg. 1974. Aims and Methods of Vegetation Ecology. Wiley, New York. 547 p.

Muir, J. P., W. D. Pitman, and J. L. Foster. 2011. Sustainable, low input, warm season, grass–legume grassland mixtures: mission (nearly) impossible?. Grass and Forage Science, 66(3):301-315.

Murphy, J., and Riley, J. P. 1962. A modified single solution method for the determination of phosphate in natural waters. *Analytica chimica acta*, 27:31-36.

Overbeck, G.E., S. C. Muller, A. Fidelis, J. Pfadenhauer, V. D. Pillar, C. C. Blanco, I. I. Boldrini, R. Both, and E. D. Forneck. 2007. Brazil's neglected biome: the South Brazilian Campos. *Perspectives in Plant Ecology, Evolution and Systematics* 9:101-116.

Palacio, R. 1999. Respuesta del campo natural a la siembra de leguminosas y fertilización fosfatada en términos de forraje y producto animal. Trabajo de pasantía, Escuela Agraria "Piraraja", Lavalleja, Uruguay, 18 p.

Pallares, O. R., E. J. Berretta, and G. E. Maraschin. 2005. The south american campos ecosystem. *In:* Suttie, J., S. G. Reynolds, C. Batello [eds.]. Grasslands of the world. FAO: 171-219.

Paruelo, J. M., and M. Vallejos. 2013. Ecosystem Services Related to Carbon Dynamics: Its Evaluation Using Remote Sensing Techniques. *In:* Alcaraz-Segura, D., C. Di Bella, J. Straschnoy [eds.]. Earth Observation of Ecosystem Services, 17-32.

Pezzani, F., A. Del Pino, F. Lezama, A. Rodriguez, G. Parodi, S. García, M. Alchurrut, and M. Jaurena. 2012. Long-term impacts of phosphorous fertilization on natural grasslands in Uruguay: nutrient contents and arbuscular mycorrhizal status of dominant species. *In:* Proceedings 19th ISTRO Conference, p. 227.

Richardson, D. M., P. Pyšek, M. Rejmánek, M. G. Barbour, F. D. Panetta, and C. J. West. 2000. Naturalization and invasion of alien plants: concepts and definitions. *Diversity and Distributions* 6:93-107.

Risso, D., and E. J. Berretta.1997. Animal productivity and dynamics of native pastures improved with oversown legumes in Uruguay. *In:* Proceedings 18th International Grassland Congress p. 22-30.

SAS Institute Inc. 2010. JMP Software, Cary, NC: SAS Institute Inc. 145 p.

Schwinning, S., and A. J. Parsons. 1996. Analysis of the coexistence mechanisms for grasses and legumes in grazing systems. *Journal of Ecology*, 799-813.

Shannon, C., and W. Weaver. 1949. The mathematical theory of communication. University of Illinois Press, Urbana-USA.

Soca, P., W. Ayala, and R. Bermúdez. 2002. The effect of herbage allowance of Lotus pedunculatus cv. Grasslands Maku on winter and spring beef heifer performance. *In:* Proceedings of the conference-New Zealand Grassland Association p. 81-84.

Soriano, A. 1991. Río de la Plata grasslands. *In:* Coupland, R.T (ed.) Natural grasslands: Introduction and Western Hemisphere. Elsevier, Amsterdam, The Netherlands. p. 367-407.

Suding, K.N., S. L. Collins, L. Gough, C. Clark, and E.E. Cleland. 2005. Functional-and abundance-based mechanisms explain diversity loss due to N fertilization. *In:* Proceedings of the National Academy of Sciences of the United States of America 102:4387-4392.

Thompson, G. R. 1995. The direct determination of phosphorus in citric acid soil extracts by colorimetry and direct-current plasma emission spectroscopy.*South African Journal of Plant and Soil*, *12*(4):152-157.

Tiecher, T., L. B. Oliveira, D. S. Rheinheimer, F. L. F. Quadros, L. C. Gatiboni, G. Brunetto, J. Kaminski. 2014. Phosphorus application and liming effects on forage production, floristic composition and soil chemical properties in the Campos biome, southern Brazil. *Grass and Forage Science*, 69(4):567-579.

Tognetti, P. M. and E. J. Chaneton. 2012. Invasive exotic grasses and seed arrival limit native species establishment in an old-field grassland succession. *Biological Invasions*, 14(12):2531-2544.

Toms, J. D., and M. L. Lesperance. 2003. Piecewise regression: a tool for identifying ecological thresholds. *Ecology* 84.8:2034-2041.

Vallano, D. M., P. C. Selmants, and E. S. Zavaleta. 2012. Simulated nitrogen deposition enhances the performance of an exotic grass relative to native serpentine grassland competitors. *Plant Ecology* 213(6):1015-1026.

Van der Heijden, M. G., J. N. Klironomos, M. Ursic, P. Moutoglis, R. Streitwolf-Engel, T. Boller, A. Wiemken, and I. R. Sanders. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* 396(6706):69-72.

Westoby, M., B. H. Walker, and I. Noy-Meir. 1989. Opportunistic management for rangelands not at equilibrium. *Journal of Range Management* 42:266-274.

Willems, J. H., and M. G. L. Van Nieuwstadt, 1996. Long-term after effects of fertilization on above-ground phytomass and species diversity in calcareous grassland. *Journal of Vegetation Science*, 7(2):177-184.

CAPÍTULO IV

CONSIDERAÇÕES FINAIS

CONSIDERAÇÕES FINAIS

A investigação em experimentos de médio e longo prazo permitiu quantificar o efeito de práticas de melhoramento na diversidade, resiliência e estabilidade do campo nativo. Neste sentido, os estudos da presente tese constituem um dos primeiros esforços para compreender os impactos de práticas de melhoramento de pastagens de campo nativo em indicadores ecossistêmicos. Os resultados da tese têm variadas implicações práticas e sugestões para futuras pesquisas que se detalham na continuação.

O estudo apresentado no capítulo II representa um novo enfoque para o entendimento da sustentabilidade de práticas de manejo em campos nativos do bioma Pampa. Além das tradicionais avaliações produtivas, neste experimento foram avaliados os efeitos combinados da adição de N, P e água na diversidade e estabilidade da vegetação. Esta pesquisa possibilitou avançar no entendimento dos mecanismos que regulam a estabilidade temporal da produção de forragem dos campos nativos. Este trabalho é um dos poucos experimentos a nível mundial que avalia a estabilidade da produção a partir de uma comunidade natural altamente diversa tanto em número de espécies como de grupos funcionais. A influência da constância das espécies dominantes na estabilidade temporal ratifica a teoria proposta por Grime (1988) em que os processos são regulados em função da abundância das espécies e seus atributos. O contraste entre os resultados encontrados e os esperados em base a teoria dominante “diversidade-estabilidade” poderia ser explicado pelo fato de que os experimentos manipulativos de diversidade dificilmente imitam o que acontece em ecossistemas reais (Wardle, 2016).

Embora as constatações deste experimento representem avanços significativos para o conhecimento de ecossistemas reais, os resultados não deveriam ser extrapolados a outras condições como, por exemplo, pastagens exóticas. A partir do descobrimento da influência dos efeitos da constância das espécies dominantes e da asincronia das espécies na estabilidade temporal emergem importantes questões. Por um lado seria relevante continuar o experimento para avaliar os efeitos a prazos mais longos, assim como também para determinar outros importantes atributos como, por exemplo, a resistência e resiliência às secas, ou resolver perguntas específicas como os efeitos na abundância de fungos micorrízicos nas espécies dominantes ou detectar efeitos de condições climáticas extremas na dinâmica da composição destas espécies.

O estudo apresentado no capítulo III permitiu classificar os estados da vegetação de melhoramentos de campos nativos com leguminosas. A partir desta classificação se elaborou uma proposta de modelo de estados e transições da vegetação em função da proporção de leguminosas e a dose de fertilização fosfatada. O modelo representa em forma geral a dinâmica de estados da vegetação de acordo com a proposta de Westoby et al. (1989), mas poderia ser ainda melhorado definindo os níveis específicos dos limiares de proporção de leguminosas, abundância de espécies nativas dominantes e exóticas invasoras, assim como a concentração de P relacionadas com a mudança de estado do campo nativo melhorado a campo degradado dominado por espécies exóticas.

O desenvolvimento de novos experimentos com níveis baixos de fertilização fosfatada em interação com diferentes intensidades de pastejo emerge como uma prioridade de pesquisa. Em paralelo, também seria relevante quantificar por separado a contribuição dos efeitos diretos e indiretos da concentração de P no solo nas mudanças tanto em diversidade, estabilidade e resiliência como em persistência das leguminosas exóticas. Especificamente, isto permitirá identificar o efeito direto do P no solo, e os efeitos indiretos relacionados com o incremento da competição das leguminosas e o aumento do N nos solos devido à fixação biológica de nitrogênio.

Do ponto de vista de manejo e conservação do ambiente pastoril, existe uma necessidade de dispor de critérios objetivos que auxiliem na tomada de decisões sobre fertilização nitrogenada e fosfatados das pastagens do campo nativo. Neste sentido, o desenvolvimento de índices de nutrição seria relevante para definir de forma precisa as doses de fertilizante, considerando-se a eficiência de utilização dos nutrientes e levando-se em conta importantes aspectos ambientais como o risco de contaminação. Em síntese, esta tese aporta novos conhecimentos sobre o impacto integral de práticas de melhoramento em atributos de sustentabilidade (produtividade, estabilidade, e resiliência) nas pastagens de campo nativo do bioma Pampa. A continuação destes esforços permitirá avanços na definição dos limites da intensificação baseados em critérios ecológicos, e assim contribuir com o desafio de desenvolver estratégias de manejo sustentável para superar o dilema produção-conservação.

REFERÊNCIAS

AVOLIO, M. L. et al. Changes in plant community composition, not diversity, during a decade of nitrogen and phosphorus additions drive above-ground productivity in a tallgrass prairie. **Journal of Ecology**, London, v. 102, n. 6, p. 1649-1660, 2014.

AYALA, W.; CARÁMBULA, M. Mejoramientos extensivos en la Región Este: manejo y utilización. In: Risso, D.F., Berretta, E.J. and Morón, A. (eds) **Producción y Manejo de Pasturas**. Montevideo: INIA, 1996. p. 177-182. (INIA, Serie Técnica 80)

BAI, Y. F. et al. Tradeoffs and thresholds in the effects of nitrogen addition on biodiversity and ecosystem functioning: evidence from inner Mongolia Grasslands. **Global Change Biology**, Urbana, IL, v. 16, n. 1, p. 358-372, 2009.

BEGON, M. **Population ecology**: a unified study of animals and plants. Oxford: Blackwell Science, 1996. 247 p.

BEMHAJA, M.; BERRETTA, E.J.; BRITO, G. Respuesta a la fertilización nitrogenada de campo natural en basalto profundo. In: REUNIÓN DEL GRUPO TÉCNICO REGIONAL DEL CONO SUR EN MEJORAMIENTO Y UTILIZACIÓN DE LOS RECURSOS FORRAJEROS DEL AREA TROPICAL Y SUBTROPICAL: Grupo Campos, 14., 1998, Uruguay. **Anales...** Montevideo: INIA, 1998. p. 119-122. (INIA, Serie Técnica 94)

BERRETTA, E.J. et al. Mejoramiento de campo natural de basalto fertilizado con nitrógeno y fósforo. In: BERRETTA E.J. (Ed.) **Seminario de Actualización en Tecnologías para Basalto en Uruguay**. Montevideo: INIA, 1998. p. 63-73, (INIA, Serie Técnica 102)

BETTOLLI, M.L. et al. Pastura natural de Salto (Uruguay): relación con la variabilidad climática y análisis de contextos futuros de cambio climático. **Revista Brasileira de Meteorologia,** São José dos Campos, SP, v. 25, n. 2, p. 248-259, 2010.

BOGGIANO, P.; ZANONIANI, R.; MILLOT, J.C. Respuestas del campo natural a manejos recientes de intervención. In: GÓMEZ MILLER R., ALBICETTE M. M. (Ed.). **Seminario de Actualización Técnica en manejo de campo natural.** Montevideo: INIA, 2005. p. 105-114. (INIA, Serie Técnica 151)

BOLDRINI, I.I. A flora dos Campos do Rio Grande do Sul. In: PILLAR V.D. et al. (Ed.). **Campos Sulinos:** Conservação e Uso Sustentável da Biodiversidade. Brasília: Ministério do Meio Ambiente, 2009. p. 63-77.

BOMMARCO, R.; KLEIJN, D.; POTTS, S. Ecological intensification: harnessing ecosystem services for food security. **Trends in ecology and evolution**, Amsterdam, v. 28, n. 4, p. 230-238, 2013.

BRAMBILLA, D.M. **Efeito de adubação nitrogenada sobre campo nativo sobressemeado com azevém anual.** 2014. 137 f. Tese (Doutorado) - Programa de Pós-Graduação em Zootecnia, Universidade Federal do Rio Grande do Sul, Faculdade de Agronomia, Porto Alegre, 2014.

BRISKE, D.D. et al. Recommendations for development of resilience-based state-and-transition models. **Rangeland Ecology and Management,** Oregon, v. 61, n. 4, p. 359-367, 2008.

BURKART, S.E. et al. Plant species diversity in remnant grasslands on arable soils in the cropping Pampa. **Plant Ecology***,* Cham, v. 212, p.1009-1024, 2011.

CARVALHO, P.C.F.; BATELLO, C. Access to land, livestock production and ecosystem conservation in the Brazilian Campos biome: the natural grasslands dilemma. **Livestock Science**, Amsterdam, v. 120, n. 1, p. 158-162, 2009.

CRAWSHAW, D. et al. Caracterização dos campos sul-rio-grandenses: uma perspectiva da ecologia da paisagem. **Boletim Gaúcho de Geografia**, Porto Alegre, n. 33, p. 233-252, 2007.

CAUDLE, D. **Interagency ecological site handbook for rangelands**. US Department of the Interior, Bureau of Land Management, Lakewood, Co. 2013. 109 p.

CHAPIN, F.S. et al. Consequences of changing biodiversity. **Nature,** London, v. 405, p. 234-242, 2000.

CHAPIN, F.S.; MATSON, P.A.; MOONEY H.A. **Principles of Terrestrial Ecosystem Ecology.** New York: Springer-Verlag, 2002. 423 p.

CRAINE, J.M. et al. Global diversity of drought tolerance and grassland climate-change resilience. **Nature Climate Change**, London, v. 3, n. 1, p.63-67, 2013.

CRUZ, P. et al. Leaf traits as functional descriptors of the intensity of continuous grazing in native grasslands in the south of Brazil. **Rangeland Ecology & Management,** Oregon, v. 63, n. 3, p. 350-358. 2010.

DAVIS, M.A.; GRIME, J.P.; THOMPSON, K. Fluctuating resources in plant communities: a general theory of invasibility. **Journal of Ecology**, London v. 88, p. 528-534, 2000.

DE ÁVILA, M.R. **Efeito do nitrogênio sobre a dinâmica da composição florística de uma pastagem natural sobressemeada com azevém anual**. 2012. 169 f. Tese (Mestrado) - Programa de Pós-Graduação em Zootecnia, Universidade Federal do Rio Grande do Sul, Faculdade de Agronomia, Porto Alegre, 2012.

ESKELINEN, A.; HARRISON, S.P. Resource co limitation governs plant community responses to altered precipitation. **Proceedings of the National Academy of Sciences**, Palo Alto, v. 112, n. 42, p.13009-13014, 2015.

FAY, P.A. et al. Grassland productivity limited by multiple nutrients. **Nature plants**, London, v. 1, p. 7, 2015.

FERREIRA, E.T. et al. Fertilization and oversowing on natural grassland: effects on pasture characteristics and yearling steers performance. **Revista Brasileira de Zootecnia,** Viçosa, v. 40, n. 9, p. 2039-2047, 2011.

FOLEY, J.A. et al. Global consequences of land use. **Science**, New York, v. 309 n.5734, p. 570-574, 2005.

GASTAL, F.; LEMAIRE, G. N uptake and distribution in crops: an agronomical and ecophysiological perspective. **Journal of Experimental Botany**, Oxford, v.53, n. 370, p. 789-799, 2002.

GEERTSEMA, W. et al. Actionable knowledge for ecological intensification of agriculture. **Frontiers in Ecology and the Environment**, Hoboken, v. 14, n. 4, p.209-216, 2016.

GRMAN, E. et al. Mechanisms contributing to stability in ecosystem function depend on the environmental context. **Ecology Letters**, Hoboken, v. 13, p. 1400-1410, 2010.

GOMES, L.H. **Produtividade de um campo nativo melhorado submetido à adubação nitrogenada**. 2000. 124 f. Tese (Doutorado) - Programa de Pós-Graduação em Zootecnia, Universidade Federal do Rio Grande do Sul, Faculdade de Agronomia, Porto Alegre, 2000.

GONZALEZ, A.; LOREAU, M. The causes and consequences of compensatory dynamics in ecological communities. **Annual Review of Ecology, Evolution, and Systematics**, Palo Alto, n. 40, p.393-414, 2009.

GOUGH, L. et al. Fertilization effects on species density and primary productivity in herbaceous plant communities. **Oikos**, Hoboken, v. 89, p. 428–439, 2000.

GRIME, J.P. Benefits of plant diversity to ecosystems: immediate, filter and founder effects. **Journal of Ecology**, Hoboken, v. 86, n. 6, p. 902-910, 1998.

HARPOLE, W.S. et al. Nutrient co-limitation of primary producer communities. **Ecology Letters**, Hoboken, v. 14, n. 9, p. 852-862, 2011.

HARPOLE, W.S. et al. Addition of multiple limiting resources reduces grassland diversity. **Nature**, London, v. 537, n.7618, p.93-96, 2016.

HAUTIER, Y. et al. Eutrophication weakens stabilizing effects of diversity in natural grasslands. **Nature**, London, v. 508, n. 7497, p. 521-525, 2014.

HAUTIER, Y. et al. Anthropogenic environmental changes affect ecosystem stability via biodiversity. **Science**, New York, n. 348, p. 336-340, 2015.

HECTOR, A. et al. General stabilizing effects of plant diversity on grassland productivity through population asynchrony and overyielding. **Ecology**, Hoboken, v. 91, n.8, p. 2213-2220, 2010.

HEJCMAN, M. et al. The Rengen Grassland Experiment: plant species composition after 64 years of fertilizer application. **Agriculture, ecosystems & environment**, Palo Alto, v. 122, n. 2, p. 259-266, 2007.

HENLE, K. Identifying and managing the conflicts between agriculture and biodiversity conservation in Europe, A review. **Agriculture, Ecosystems & Environment**, Palo Alto, v. 124, n. 1, p.60-71, 2008.

HOLLING, C.S. The resilience of terrestrial ecosystems: local surprise and global change. In: CLARK, W.C.; MUNN, R.E. (Ed.) **Sustainable development of the biosphere**. Cambridge: Cambridge University Press, 1986. p. 292-317.

HOOVER, D.L.; KNAPP, A.K.; SMITH, M.D. Resistance and resilience of a grassland ecosystem to climate extremes. **Ecology**, Hoboken, v. 95, n. 9, p. 2646-2656, 2014.

HULME, P.E. Adapting to climate change: is there scope for ecological management in the face of a global threat? **Journal of Applied Ecology**, Hoboken, v. 42, n. 5, p. 784-794, 2005.

HUSTON, M.A. Hidden treatments in ecological experiments: re-evaluating the ecosystem function of biodiversity. **Oecologia**, New York, v. 110, p. 449-60, 1997.

ISBELL, F.I.; POLLEY, H.W; WILSEY, B.J. Biodiversity, productivity and the temporal stability of productivity: patterns and processes. **Ecology Letters**, Hoboken, v. 12, n. 5, p. 443-451, 2009.

ISBELL, F. et al. Nutrient enrichment, biodiversity loss, and consequent declines in ecosystem productivity. **Proceedings of the National Academy of Sciences**, Whashington, v. 110, n. 29, p. 11911-11916, 2013.

IVES, A.R.; CARPENTER, S.R. Stability and diversity of ecosystems. **Science,** New York, v. 317, n. 5834, p. 58-62, 2007.

JAURENA, M. et al. Campo natural: patrimonio del país y fundamento de la estabilidad productiva de la ganadería. **Revista INIA**, Montevideo, n. 32, p. 31-35, 2013.

JOHNSON, N.C. Tansley Review: resource stoichiometry elucidates the structure and function of arbuscular mycorrhizas across scales. **New Phytologist**, Hoboken, v. 185, p. 631-647, 2010.

KNAPP, A.K.; SMITH M.D. Variation among biomes in temporal dynamics of aboveground primary production. **Science**, New York, v. 291, p. 481-484, 2001.

LAMBERS, H. et al. Plant mineral nutrition in ancient landscapes: high plant species diversity on infertile soils is linked to functional diversity for nutritional strategies. **Plant and Soil**, Dordrecht, v. 334, p. 11-31, 2010.

LATERRA, P.; RIVAS, M. Bases y herramientas para la conservación in situ y el manejo integrado de los recursos naturales en los campos y pampas del Cono Sur. **Agrociencia**, Montevideo, v. 9, n. 1-2, p. 169-178, 2005.

LEMAIRE, G. Intensification of animal production from grassland and ecosystem. In: HEMMING, D. (Ed.) **Animal Science Reviews 2012**. Oxfordshire: CABI, 2012. p. 45-52.

LEZAMA, F. **Las comunidades herbáceas de un área de pastizales naturales de la región basáltica de Uruguay**. 2005. 62 f. Tesis (Maestría) - Programa PEDECIBA, UdelaR-Uruguay, 2005.

LOREAU, M.; DE MAZANCOURT, C. Biodiversity and ecosystem stability: a synthesis of underlying mechanisms. **Ecology Letters**, Hoboken, v. 16, p. 106-115, 2013.

MacDOUGALL, A.S. et al. Diversity loss with persistent human disturbance increases vulnerability to ecosystem collapse. **Nature**, London, v. 494, n.7435, p.86-89, 2013.

McNAUGHTON, S.J. et al. Ecosystem-level patterns of primary productivity and herbivory in terrestrial habitats. **Nature**, London, 341, p. 142-144, 1989.

MORÓN, A. **El ciclo del nitrógeno en el sistema suelo-planta-animal.** Seminario de actualización técnica. Montevideo: INIA, 1994. 12 p. (Serie Técnica 51)

MOUQUET, N.; MOORE, J.L.; LOREAU, M. Plant species richness and community productivity: why the mechanism that promotes coexistence matters. **Ecology Letters**, Hoboken, v. 5, n. 1, p. 56-65, 2002.

NABINGER, C.; CARVALHO, P.C.F. Ecofiosiología de los sistemas pastoriles: aplicaciones para su sustentabilidad. **Agrociencia**, Montevideo, v. 13, n. 3, p. 18-27, 2009.

OLIVER, T.H. et al. Biodiversity and resilience of ecosystem functions. **Trends in ecology & evolution**, London, v. 30, n. 11, p. 673-684, 2015.

OVERBECK, G.E. Brazilian's neglected biome: the South Brazilian Campos. **Perspectives in Plant Ecology, Evolution and Systematics**, Jena, v. 9, n. 2, p. 101-116, 2007.

OVERBECK, G.E. et al. Conservation in Brazil needs to include non forest ecosystems. **Diversity and Distributions**, Hoboken, v. 21 n. 12, p.1455-1460, 2015.

PALLARES, O.R.; BERRETTA, E.J.; MARASCHIN G.E. The south american campos ecosystem. In: SUTTIE, J.; REYNOLDS, S.G.; BATELLO, C. [Ed.]. **Grasslands of the world.** FAO, 2005. p. 171-219.

PARUELO, J.M.; VALLEJOS M. Ecosystem services related to carbon dynamics: its evaluation using remote sensing techniques. In*:* ALCARAZ-SEGURA, D.; DI BELLA, C.; STRASCHNOY, J. [Ed.]. **Earth Observation of Ecosystem Services**. Boca Raton: CRC Press, 2013. p. 17-32.

PICKETT, S.T.; WU, J.; CADENASSO, M.L. Patch dynamics and the ecology of disturbed ground: a framework for synthesis. In: WALKER, L.R. (Ed.). **Ecosystems of Disturbed Ground.** Amsterdam: Elsevier, 1999. p.707-722.

PIMM, S.L. The complexity and stability of ecosystems. **Nature,** London, n. 307 n.5949, p. 321-326, 1984.

RICHARDSON, D.M. et al. Naturalization and invasion of alien plants: concepts and definitions. **Diversity and Distributions,** Hoboken, v. 6, p. 93-107, 2000.

RISSO, D.; BERRETTA. E.J. Animal productivity and dynamics of native pastures improved with oversown legumes in Uruguay. In: INTERNATIONAL GRASSLAND CONGRESS, 18th, Winnipeg and Saskatoon, Canada 1997. **Proceedings...** Winnipeg: IGC, 1997. p. 22-30.

RODRÍGUEZ PALMA, R.; RODRÍGUEZ, T. Fertilización de campo natural: forraje crecido. **Agrociencia,** Montevideo, v. 14, n. 3, p. 133, 2010. (Memorias del III Congreso de la Asociación Uruguaya de Producción Animal)

RUBIO, G. et al. Acumulación de biomasa, nitrógeno y fósforo en un pastizal natural fertilizado de la Pampa Deprimida, Argentina. **Revista Ciencia del Suelo,** Buenos Aires, v. 15, p. 48-50, 1997.

SADRAS, V.O. A quantitative top-down view of interactions between stresses: theory and analysis of nitrogen–water co-limitation in Mediterranean agro-ecosystems. **Crop and Pasture Science**, Clayton, v. 56, n. 11, p. 1151-1157, 2005.

SALA, O.E. et al. Primary production of the central grassland region of the United States. **Ecology**, Hoboken, v. 69, p. 40-45, 1988.

SALA, O.E.; PARUELO, J.M. Ecosystem services in grasslands. In: DAILY, G.C. (Ed.) **Nature's Services:** Societal Dependence on Natural Ecosystems. Washington: Island Press, 1997. p. 237-252.

SALA, O.E. et al. Global biodiversity scenarios for the year 2100. **Science**, New York, v. 287, n.5459, p. 1770-1774, 2000.

SHI, Z. et al. Dual mechanisms regulate ecosystem stability under decade-long warming and hay harvest. **Nature Communications**, London, v. 7, n.11973, 2016.

SMITH, M.D.; KNAPP, A.K.; COLLINS, S.L. A framework for assessing ecosystem dynamics in response to chronic resource alterations induced by global change. **Ecology**, Hoboken, v. 90, n. 12, p. 3279-3289, 2009.

SNYMAN, H.A. Short-term response of rangeland botanical composition and productivity to fertilization (N and P) in a semi-arid climate of South Africa. **Journal of Arid Environment,** London, v. 50, p.167-183, 2002.

SOCA, P.; AYALA, W.; BERMÚDEZ R. The effect of herbage allowance of *Lotus pedunculatus* cv. Grasslands Maku on winter and spring beef heifer performance. **Proceedings of New Zealand Grassland Association**, West Coast, v.64, p.81-84, 2002.

SORIANO, A. Río de la Plata grasslands. In: COUPLAND, R.T (Ed.) **Natural grasslands:** Introduction and Western Hemisphere. Amsterdam: Elsevier, 1991. p. 367-407.

SUDING, K.N. et al. Functional-and abundance-based mechanisms explain diversity loss due to N fertilization. **Proceedings of the National Academy of Sciences,** Washington, v.102, p.4387-4392, 2005.

STEFFEN, W. et al. Planetary boundaries: Guiding human development on a changing planet. **Science**, New York, v. 347, n.6223, p.736-736, 2015.

SUDING, K.N.; HOBBS, R.J. Threshold models in restoration and conservation: a developing framework. **Trends in ecology & evolution**, London, v. 24, n. 5, p. 271-279, 2009.

TILMAN, D.; DOWNING, J.A. Biodiversity and stability in grasslands. **Nature,** London, v. 367, p. 363-365, 1994.

TILMAN, D.; REICH, P.B.; KNOPS, J.M.H. Biodiversity and ecosystem stability in a decade-long grassland experiment. **Nature,** London, v. 441, p. 629-632, 2006.

TOGNETTI, P.M.; CHANETON E.J. Invasive exotic grasses and seed arrival limit native species establishment in an old-field grassland succession. **Biological Invasions**, Dordrecht, v. 14, n. 12, p. 2531-2544, 2012.

VILÁ, M.; SARDANS, J. Plant competition in Mediterranean-type ecosystem. **Journal of Vegetation Science**, Hoboken, v. 10, p. 281-294, 1999.

VITOUSEK, P.M. et al. A. Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen–phosphorus interactions. **Ecological Applications,** Hoboken, v. 20, n. 1, p. 5-15, 2010.

YACHI, S.; LOREAU, M. Biodiversity and ecosystem productivity in a fluctuating environment: the insurance hypothesis. **Proceedings of the National Academy of Sciences,** Washington, v. 96, p. 1463-1468, 1999.

YANG, Z.L.; VAN RUIJVEN, J.; DU, G.Z. The effects of long-term fertilization on the temporal stability of alpine meadow communities. **Plant and Soil**, Dordrecht, v. 345, p. 315-324, 2011.

WILLEMS, J.H.; VAN NIEUWSTADT, M.G.L. Long-term after effects of fertilization on above-ground phytomass and species diversity in calcareous grassland. **Journal of Vegetation Science**, Hoboken, v. 7, n. 2, p. 177-184, 1996.

WAGNER, A.P.L. et al. Tendências temporais de índices de vegetação nos campos do Pampa do Brasil e do Uruguai. **Pesquisa Agropecuária Brasileira**, Brasília, v. 48, n. 9, p. 1192-1200, 2013.

WARDLE, D.A. Do experiments exploring plant diversity–ecosystem functioning relationships inform how biodiversity loss impacts natural ecosystems? **Journal of Vegetation Science**, Hoboken, v. 27, n. 3, p. 646-653, 2016.

WU, J.; LOUCKS, O.L. From balance of nature to hierarchical patch dynamics: a paradigm shift in ecology. **Quarterly review of biology**, Chicago, v. 70, n. 3, p. 439-466, 1995. .

ZANONIANI, R.A.; BOGGIANO, P.; CADENAZZI, M. Respuesta invernal de un campo natural a fertilización nitrogenada y ofertas de forraje. **Agrociencia**, Montevideo, v. 15, n. 1, p. 115-124, 2011.

APÊNDICES

Apêndice 1. Normas para preparação de trabalhos científicos para publicação no periódico *Ecology Letters (*Normas utilizadas para escrever o Capítulo II).

Link of authors guidelines of Ecology Letters
http://onlinelibrary.wiley.com/store/10.1111/(ISSN)1461-0248/asset/homepages/ELE_Author_guidelines_20-Nov-2015.pdf?v=1&s=e20d3e888d4802697c83ad2f6e5e80119f0f7a86&isAguDoi=false

Apêndice 2: Normas para preparação de trabalhos científicos para publicação no periódico *Rangeland Ecology & Management (*Normas utilizadas para escrever o Capítulo III).

Link of authors guidelines of Rangeland Ecology and Management
https://www.elsevier.com/wps/find/journaldescription.cws_home/734882?generatepdf=true

VITA

Martín Alejandro Jaurena Barrios nasceu em Montevideo – Uruguay, no primeiro dia do mês de setembro de 1971. Ingressou na Facultad de Agronomia - Universidad de la República de Uruguay (UdelaR) em março 1991, onde desenvolveu atividades de becario na área de forragicultura e fitotecnia de 1994 a 1998. Recebeu o título de Ingeniero Agrónomo em fevereiro de 1997. De 1998 a 2002 foi assistente técnico do programa de conservação ambiental PROBIDES localizado em Rocha – Uruguay. Entre 2003 a 2005 foi consultor de projetos na área de rizobiología do Ministerio de Ganadería Agricultura y Pesca e ajudante docente na área de fitotecnia na Facultad de Agronomia – UdelaR em Montevideo - Uruguay. Em 2005 ingressou no curso de Mestrado junto ao Programa de Pós Graduação da Facultad de Agronomia – UdelaR na área de concentração Biologia Vegetal, recebendo o grau de Magister em Ciências Agrárias em Dezembro de 2009. De 2006 à atualidade é pesquisador do programa Pasturas y Forrajes do Instituto Nacional de Investigación Agropecuaria, INIA – Uruguay na área de Ecologia e Manejo de Campo Nativo, radicado em Tacuarembó - Uruguay. Em dezembro de 2012 foi aprovado no processo seletivo de Doutorado em Zootecnia/UFRGS na área de Plantas Forrageiras com bolsa da Agencia Nacional de Investigación e Innovación (ANNI – Uruguay).