Universidade Federal do Rio Grande do Sul
Instituto de Biociências
Programa de Pós-Graduação em Botânica

Tese de Doutorado

Interação solo-vegetação campestre:
Estudos de caso em diferentes escalas ecológicas

Bianca Ott Andrade

Porto Alegre, Março de 2014

Universidade Federal do Rio Grande do Sul
Instituto de Biociências
Programa de Pós-Graduação em Botânica

Tese de Doutorado

Interação solo-vegetação campestre:
Estudos de caso em diferentes escalas ecológicas

Bianca Ott Andrade

Tese apresentada ao Programa de Pós-Graduação em Botânica, Universidade Federal do Rio Grande do Sul como requisito para obtenção do título de Doutora em Ciência – ênfase em Botânica.

Orientadora: Prof^{a}. Dra. Ilsi Iob Boldrini
Co-Orientador: Prof. Dr. Alberto Vasconcellos Inda

Banca Examinadora:
Prof. Dr. Jaime Antônio de Almeida
Prof^{a}. Dra. Lúcia Dillenburg
Prof^{a}. Dra. Sandra Müller

Defesa Pública: 11 de Abril de 2014

“Plant ecologist would benefit from learning more about soil science, geomorphology and geology. Although soil science terminology seems forbidding, it is no worse than that of plant anatomy and systematic”.

(Sollins, 1988).

SUMÁRIO

AGRADECIMENTOS

Como diria Raul, “Nunca se vence uma guerra lutando sozinho”. Foram muitas as pessoas das quais recebi apoio, confiança, incentivo, orientação e que tornaram os meus momentos mais leves e agradáveis.... enfim, a todos que de alguma forma colaboraram para que essa tese ganhasse forma, agradeço!

Agradeço à minha orientadora Ilsi Iob Boldrini e meu co-orientador, Alberto Inda Vasconcellos.

Ao Gerhard Overbeck que foi um co-orientador não-oficial.

Aos membros da banca: Jaime Almeida, Sandra Müller e Lúcia Dillenburg.

Aos meus colegas e amigos (pela parceria, ajuda, incentivo e/ou inspiração): Pedro Maria de Abreu Ferreira, Adriane Esquivel, Luciana Menezes, Juliana Gonçalves, Camila Bonilha, Mariana Souza, Cleusa Vogel, Rosângela Rolim, Pedro Joel S. Silva Filho, Patricia Rosa, Fernanda Schmidt, Rodrigo Ardissone, Cristiano Buzzato, Michele Nervo, Rodrigo León Cordero, Silviane Pesamosca, Marlon Facco, Diober Borges Lucas, Paula Medeiros, Jessé Fink, Michael Mazurana, Vanessa Bertolazi, Jessica Oliveira, Luis Fernando Silva, Gabriele Pilger, Guilherme Iecker, Christiane Koch, Rafael Machado, Vanderlei Debastiani, Bethânia Azambuja, Eduardo Vélez, Vinícius Bastazini, Anaclara Guido, Mariana Rockenbach, Marcelo Fett, Julio Azambuja, Eduardo Pasini, Jalise Tontini, Marcel Lacerda, David Silva, Alessandra Boos, Julia Maria Hermann, Timo Conradi, Emer Walker, Florencia Yannelli, Mayara Caddah.

Aos Professores e colaboradores: Valério de Patta Pillar, Johannes Kollmann, Heinrich Hasenack.

Aos proprietários das áreas de estudo do PELD, gerentes das Unidades de Conservação (Deonir Zimmermann, Daniel Slomp e Ketulyn Fuster) e demais funcionários da SEMA por todo o apoio e confiança. Ao Rogério Jaworski, Fernando Quadros e Pedro Trindade pelo auxílio na procura pelas áreas de estudo e todo apoio a nós conferido.

Aos meus pais Maurício e Elza, e minha irmã, Beatriz.... meus exemplos de força, perseverança, amor, união.... de tudo....

Ao Vitor pela parceria, paciência e por tornar mais fácil os momentos mais difíceis.

Às agências financiadoras: CAPES (PNADB) pela bolsa de doutorado; CNPq pela bolsa e auxilio para pesquisa durante o estágio no exterior. O projeto PELD é financiado pelo CNPq. O projeto de cooperação internacional Brasil-Alemanha foi financiado pelo CNPq e DFG.

A todos meu muito obrigada!!!

RESUMO

Enquanto em regiões temperadas o conhecimento sobre a relação solo-vegetação é consolidado, nos trópicos e subtrópicos é preliminar. É urgente a necessidade de se determinar os fatores abióticos que controlam padrões vegetacionais visando dar suporte a estudos de recuperação e conservação. O presente estudo analisa a relação entre fatores abióticos e vegetação campestre na forma de três artigos científicos (capítulos I, II e III) e um artigo de revisão (capítulo IV). Nos primeiros três artigos, analisou-se a variância da vegetação em diferentes escalas ecológicas; e no artigo de revisão, discutiu-se de forma aplicada a variância de fatores bióticos e abióticos em resposta à degradação. Dessa forma, a presente tese objetivou responder as seguintes questões: (I) Que diferenças podem ser observadas dentro de uma mesma espécie quanto à funcionalidade e suas estratégias de alocação sob diferentes graus de disponibilidade de recursos?; (II) Quão variáveis são as propriedades físicas e químicas do solo em diferentes escalas espaciais; e existem propriedades dos solos que podem explicar com maior precisão a distribuição das espécies em diferentes escalas espaciais? (III) Que porcentagem de variância da vegetação pode ser explicada por propriedades pedológicas e climáticas; e quais características de solo e clima melhor explicam esses padrões de vegetação? No capítulo IV é apresentado um modelo conceitual sobre degradação dos campos e sua aplicação aos campos do Rio Grande do Sul (RS). Para responder as questões acima usei dados ao nível de espécie de campos calcáreos da Alemanha (capítulo I); dados ao nível de comunidade em seis áreas campestres do Rio Grande do Sul, sul do Brasil (capítulos II e III); e através da revisão de literatura relacionada à degradação, quanto à capacidade de recuperação dos campos do RS (capítulo IV). Os resultados evidenciaram que: (I) dentro de espécies ficaram evidentes duas estratégias frente à limitação de recursos, enquanto a resposta dos atributos aos diferentes tratamentos se mostrou constante; (II) a variação dos parâmetros do solo relaciona-se à escala espacial aplicada e a variância da vegetação geralmente responde a diferentes parâmetros de solo em diferentes escalas; (III) 45% da variância da vegetação entre biomas nos campos do RS foi explicada por características pedológicas e climáticas, sendo em grande parte governada pela precipitação anual e a porcentagem de saturação por alumínio do solo; e (IV) o modelo conceitual apresenta variações ao longo de dois eixos (biótico e abiótico) e poderá servir de suporte a estudos de conservação e recuperação de campos tropicais e suptropicais, bem como facilitar a tomada de decisões quanto ao manejo e conservação. Como conclusão geral, verificou-se que a vegetação campestre responde a variações ambientais em diferentes escalas espaciais e pode adotar diferentes estratégias para sobrepor filtros ambientais e processos de degradação. O entendimento da relação entre a vegetação e o meio abiótico é de grande importância para tomada de decisões quanto ao emprego de formas alternativas de manejo e conservação.

Palavras-chave: variação intraespecífica; experimento multi-específico; plasticidade; atributos funcionais de raíz; ecossistemas campestres; relação solo-vegetação; heterogeneidade do solo; Bioma Pampa; campos de altitude; recuperação de campos; limiares; ecologia campestre.

SUMMARY

Whereas in temperate regions the abiotic-biotic relationship is well-known, in the tropics and subtropics our understanding is still preliminary. There is an urgent need to determine abiotic factors that control vegetation patterns in order to give support to restoration and conservation approaches. The present thesis analyses the relationship between abiotic factors and grassland vegetation in three original research papers (chapters I, II and III) and a review paper (chapter IV). In the first three papers, vegetation variance in response to abiotic factors was analyzed at different ecological scales; and in the fourth, the variance in biotic and abiotic factors in response to degradation process was discussed with a more applied view. Thus in this thesis the aim is to answer the following questions: (I) Which differences can be found in functional plant traits and allocation strategies within species at different levels of water and nutrient availability?; (II) How variable are physical and chemical parameters in different spatial scales; and are there soil parameters that can more accurately explain plant distribution in different spatial scales? (III) How much of RS grassland vegetation variance can be explained by soil and climatic properties; and which climatic and soil properties better explain these vegetation patterns? In chapter IV a conceptual model of grassland degradation is presented and applied to Rio Grande do Sul (RS) grasslands. To address these questions I used species-level data in a calcareous grassland in Germany (chapter I); community-level data in six sites in RS, South Brazilian grasslands (chapter II and III); and a review of literature studies concerning RS grassland degradation and restorability (chapter IV). The results showed that: (I) at a intraspecific level, the study species showed two allocation strategies in relation to resource stress, while the responses of individual traits to the soil treatments were consistent across species; (II) soil parameters variation are related to the measurement scale applied and the vegetation variance often responds to different soil parameters at different scales; (III) climatic and soil properties explained 45% of vegetation variance between biomes in RS grasslands and the main factors controlling its variance are annual precipitation and percent aluminum saturation; and (IV) the conceptual model is displayed as biotic and abiotic changes along the axes and can serve as a general framework to study degradation and restorability of tropical and subtropical grasslands, and further it may facilitate decisions on alternative management and conservation. As a general conclusion, the grassland vegetation responds to changes in the environment in different scales and may use different strategies to overcome environmental selective forces and degradation process. The understanding of this relationship is of high importance to facilitate decisions on alternative management and conservation.

Keywords: intraspecific trait variation; Multi-species experiment; plasticity; root traits; grassland ecosystems; plant-soil relationship; soil heterogeneity; Pampa biome; Highland grasslands; grassland restoration; thresholds; grassland ecology.

PREFÁCIO

A presente tese é composta por quatro capítulos. Cada capítulo corresponde a um artigo, os quais serão submetidos a publicações em diferentes revistas científicas. Cada capítulo (artigo) foi redigido em inglês e originalmente formatado de acordo com a revista a ser submetida. Para facilitar a leitura, na tese as tabelas e figuras encontram-se no corpo do texto e os capítulos foram formatados de uma mesma forma (tamanho de fonte, espaçamento entre linhas). Os ítens resumo, referências e as citações no texto foram mantidos no formato original, o que acarreta em mínimas diferenças entre os capítulos. Tabelas e figuras foram enumeradas de forma independente em cada capítulo.

INTRODUÇÃO GERAL

A compreensão dos fatores que regem os padrões de organização das espécies é um tema central na ecologia (Greig-Smith, 1979; Keddy, 1991) e baseia-se na dependência escalar de padrões ecológicos e processos. A vegetação varia em resposta a fatores ambientais como altitude, umidade (Lezama et al., 2006), fertilidade do solo (Santos et al., 2008; Brambilla et al., 2012), e fatores de uso da terra, como histórico, regime de uso e pressão de pastejo (Nabinger et al., 2000; Carvalho et al., 2006). Já a heterogeneidade do solo se expressa amplamente em diferentes escalas: em escala continental e regional a variabilidade do solo reflete a formação do solo (principalmente clima, relevo e material de origem) enquanto em escala de poucos hectares (catenas ou topossequências) o relevo e a sucessão vertical de horizontes são as características de destaque (Lavelle and Spain, 2001).

Gradientes ambientais, que se caracterizam pela clara zonação em virtude de variações no meio abiótico e na fisionomia da vegetação, são de grande valia na avaliação da heterogeneidade desses ambientes. Testes de hipóteses e verificação de correlações entre variáveis dependentes e independentes são indicados para avaliação (Keddy, 1991). A escolha de váriáveis independentes é de grande importância, visto que com frequência é difícil traduzir medidas de determinadas variáveis ambientais (que variam ao longo de gradientes), as quais influem na resposta fisiológica dos organismos (Keddy, 1991). Cuidados especiais também devem ser conferidos às variáveis dependentes, que devem estar em coerência com a escala espacial estudada. A escala adotada deve ser considerada como um segundo fator de grande importância, pois pode ter profundos efeitos nos padrões encontrados (Wiens, 1989). No presente trabalho, gradientes ambientais foram analisados sob diferentes escalas ecológicas e espaciais, objetivando-se avaliar a resposta da vegetação à variação do ambiente. As seguintes perguntas foram levantadas e respondidas no presente estudo:

Como se dá a variação intraespecífica ao nível local, frente a condições físicas e químicas do solo restritivas ao desenvolvimento das plantas?

Já, ao nível de comunidades, características químicas e físicas do solo podem explicar com maior acurácia a variação da vegetação em diferentes escalas espaciais?

Ainda em nível de comunidade, quais variáveis climáticas e químicas melhor explicam a variação da vegetação ao nível regional (entre biomas) e local (dentro de um mesmo bioma), e qual a porcentagem da variação da vegetação por elas explicada?

E ao nível de ecossistema, de que forma essa variação de componentes bióticos e abióticos do sistema podem representar diferentes estágios de conservação e degradação?

Referências

Brambilla, D.M., Nabinger, C., Kunrath, T.R., Carvalho, P.C.d.F., Carassai, I.J., Cadenazzi, M., 2012. Impact of nitrogen fertilization on the forage characteristics and beef calf performance on native pasture overseeded with ryegrass. Revista Brasileira de Zootecnia 41, 528-536.

Carvalho, P.C.d.F., Fisher, V., Santos, D.T.d., Ribeiro, A.M.L., Quadros, F.L.F.d., Castilhos, Z.M.S., Poli, C.H.E.C., Monteiro, A.L.G., Nabinger, C., Genro, T.C.M., Jacques, A.V.A., 2006. Produção Animal no Bioma Campos Sulinos. Brazilian Journal of Animal Science 35(n. Supl. Esp.), 156-202.

Greig-Smith, P., 1979. Pattern in vegetation. The Journal of Ecology, 755-779.

Keddy, P.A., 1991. Working with heterogeneity: an operator's guide to environmental gradients, Ecological heterogeneity. Springer, pp. 181-201.

Lavelle, P., Spain, A., 2001. Soil ecology. Springer, Dordrecht.

Lezama, F., Altesor, A., León, R.J., Paruelo, J.M., 2006. Heterogeneidad de la vegetación en pastizales naturales de la región basáltica de Uruguay. Ecología austral 16, 167-182.

Nabinger, C., Moraes, A., Marschin, G.E., 2000. Campos in Southern Brazil. In: G. Lemaire, J.G. Hodgson, A. Moraes, C. Nabinger, P.C.F. Carvalho (Eds.), Grassland Ecophysiology and Grazing Ecology. CABI, Wallingford, pp. 355-376.

Santos, D.T.d., F., C.P.C.d., C., N., Carassai, I.J., Gomes, L.K., 2008. Bioeconomic efficiency of native pasture fertilization in southern Brazilian. Ciência Rural 38(2), 437-444.

Wiens, J., 1989. Spatial scaling in ecology. Functional Ecology 3, 385-397.

Capítulo I

INTRASPECIFIC TRAIT VARIATION AND ALLOCATION STRATEGIES OF CALCAREOUS GRASSLAND SPECIES: RESULTS FROM A RESTORATION EXPERIMENT

Co-autores: Gerhard E. Overbeck, Gabriele E. Pilger, Julia-Maria Hermann, Timo Conradi, Ilsi I. Boldrini & Johannes Kollmann

* Artigo submetido para publicação em *Basic and Applied Ecology*

Abstract

Intra- and interspecific trait variation express the response of plants dealing with different environmental conditions. We measured root and leaf traits on 14 species of calcareous grasslands in a restoration experiment. We aimed at identifying intraspecific differences in biomass allocation and functional plant traits under contrasting soil conditions by comparing plants growing in ancient grassland and two restored grasslands on ex-arable land, one of them with topsoil removal. Relative importance of trait variation within and among species, and among sites was assessed by variance partitioning. Interspecific variation was more important than intraspecific variation, but the contribution of the latter to total variation was considerable, especially for specific leaf area. Changes in soil properties due to topsoil removal resulted in lower values of plant height, specific leaf area and specific root length compared to the control (ancient grassland). Soil fertility found in the treatment without top soil removal did not affect plant plasticity compared to the control. The study species showed two allocation strategies in relation to resource stress, while the responses of individual traits to the soil treatments were consistent across species. We conclude that caution must be taken when using mean trait values for plastic species or when working with environmental gradients.

Keywords: Biomass; Leaf traits; Multi-species experiment; Plasticity; Root traits; Topsoil removal

Zusammenfassung

Intra- und interspezifische Variation von Pflanzeneigenschaften (‚plant traits‘) ist Ausdruck der Reaktion von Pflanzenarten auf unterschiedliche Standortverhältnisse. Kontrastierende Standortbedingungen eines Renaturierungsexperimentes bildeten ein geeigneter Rahmen für eine Untersuchung der Wurzel- und Blatteigenschaften von 14 Kalkmagerrasenarten. Die Studie fokussierte auf Unterschiede in der Biomasse-Allokation und ausgewählten Pflanzenmerkmalen zwischen Individuen, die auf einem erhaltenen (historischen) Kalkmagerrasen wuchsen, und solchen auf zwei renaturierten Flächen (mit oder ohne Oberbodenabtrag) mit Acker als Vornutzung. Untersucht wurde die relative Variation der Pflanzeneigenschaften zwischen Individuen, Arten und Untersuchungsflächen. Die interspezifische Variation war stärker als die intraspezische, besonders bei der spezifischen Blattfläche. Oberbodenabtrag führte zu geringerer Pflanzenhöhe, und niedrigeren Werten für spezifische Blattfläche und spezifische Wurzellänge verglichen mit dem historischen Magerrasen. Die höheren Nährstoffgehalte der Ackerflächen ohne Bodenabtrag beeinflussten die Plastizität der Pflanzenarten nicht. Die Magerrasenarten zeigten zwei unterschiedliche Allokationsstrategien als Reaktion auf ungünstige Bodenverhältnisse, während die Veränderungen der einzelnen Pflanzeneigenschaften bei allen Arten ähnlich ausfielen. Die Ergebnisse legen nahe, dass die Verwendung von gemittelten Werten von Pflanzeneigenschaften bei plastischen Arten entlang von Umweltgradienten problematisch ist.

Introduction

Trait variability is an important element of plant strategies because it allows plants to grow and to reproduce under environmental conditions with different degrees of stress, disturbance and strength of biotic interactions (Weiner 2004; Craine 2009; Fort, Cruz & Jouany 2014). If the expression of a plant trait is an indicator of the ecological niche filled by a species (Mason, de Bello, Doležal, & Lepš 2011), examining the ecological niche breadth and the resulting consequences for population and community dynamics (Bolnick, Amarasekare, Araújo, Bürger, Novak et al. 2011) requires a quantification of the plasticity of species' traits across varying environmental conditions (Jung, Violle, Mondy, Hoffmann, & Muller 2010; Albert, Grassein, Schurr, Vieilledent, & Violle 2011).

Recent studies on the functional structure of plant communities suggest that competitive hierarchies exist, in which there is an optimal range of trait values that allow species to successfully compete for limiting resources (Kunstler, Lavergne, Courbaud, Thuiller, Vieilledent, Zimmermann et al. 2012; Fort et al. 2014; Herben & Goldberg 2014), but this trait optimum may shift along environmental gradients. If a species is sufficiently plastic to adjust the expression of traits relevant for resource acquisition and turnover towards the required optimum, it will be more likely to persist in a broad range of environments.

In ecological restoration, species are often transferred to sites that show marked differences in soil conditions compared to the source habitat. For example, in restoring calcareous grasslands in Central Europe, a well-established method is transferring hay to ex-arable land, sometimes preceded by topsoil removal (Kiehl & Pfadenhauer 2007). The objective of the latter is to reduce nutrient loads and seed banks from previous land use to establish species-rich calcareous grasslands (Kiehl, Thormann & Pfadenhauer 2006). This restoration practice results in three types of sites with strongly contrasting soil moisture and nutrient contents, i.e. nutrient-poor ancient grasslands, and restored grasslands either with or without nutrient-rich soil. Plant species occurring in these sites should show some trait plasticity relevant to the respective resource supply.

In this study, we make use of the contrasting site conditions of a nutrient-poor ancient grassland and two restoration sites on ex-arable land, one of which restored by topsoil removal. The aim was to study the effects of different levels of water and nutrient supply on intraspecific trait variability and allocation strategies. For this purpose, we investigated a set of above- and below-ground traits related to resource acquisition, growth rate and competitive vigour in 14 species. We hypothesized that (i) intraspecific trait variability is higher among than within sites; (ii) intraspecific trait variability is species- and trait-dependent, i.e. species respond differently to the environmental constraints; (iii) traits related to resource acquisition and growth rate have lower values in the harsher environment; and (iv) investment in below-ground structures is higher under harsher conditions.

Multi-species experiments are a powerful approach to identify general ecological patterns (van Kleunen, Dawson, Bossdorf & Fischer 2014). Our study is one of the first multi-species experiments to analyze the extent of functional trait variability of plant traits under varying levels of abiotic stress, especially in a restoration context. Importantly, we also provide information on the variability of root traits, which play a significant role for acquisition, storage and cycling of resources (Freschet, Cornelissen, van Logtestijn & Aerts 2010), especially at sites with rare disturbance, and low availability of water and nutrients.

Materials and methods

Study location

The study was conducted in the 'Garchinger Heide' nature reserve (48°18'N, 11°39'E, 469 m a.s.l., 27 ha), one of the few remnants of species-rich calcareous grasslands on calcaric leptosol (IUSS 2006) in the Munich Gravel Plain, Germany. The shallow soils are nutrient-poor and relatively dry, despite mean annual precipitation around 880 mm and mean annual temperature 8.1 °C (Kiehl & Pfadenhauer 2007). Areas surrounding the remnant grassland were converted into arable land in the early 20th century (Pfadenhauer 2001). These areas were restored in 1993, aiming to re-establish the characteristic community of reference grasslands. Three adjacent sites were chosen for our study (Fig. 1): two restoration sites on ex-arable land, i.e. one with (TR) and another without topsoil removal (NR), and a control site (AG, ancient grassland; Table 1). On TR, 30–40 cm topsoil was removed in 1993 down to the calcareous gravel. The remaining soil consisted of 11% fine particles (<2 mm) and 89% gravel, with little organic matter and low water holding capacity (Jeschke & Kiehl 2008). In 1993, soon after these restoration measures, the sites received transfer of fresh seed-containing hay from the ancient grassland. Species richness, composition and abundance of species became different among sites (Kiehl & Pfadenhauer 2007). The current management of the sites is annual mowing, done in mid-late August; the site with topsoil removal is not mown because of its low productivity.

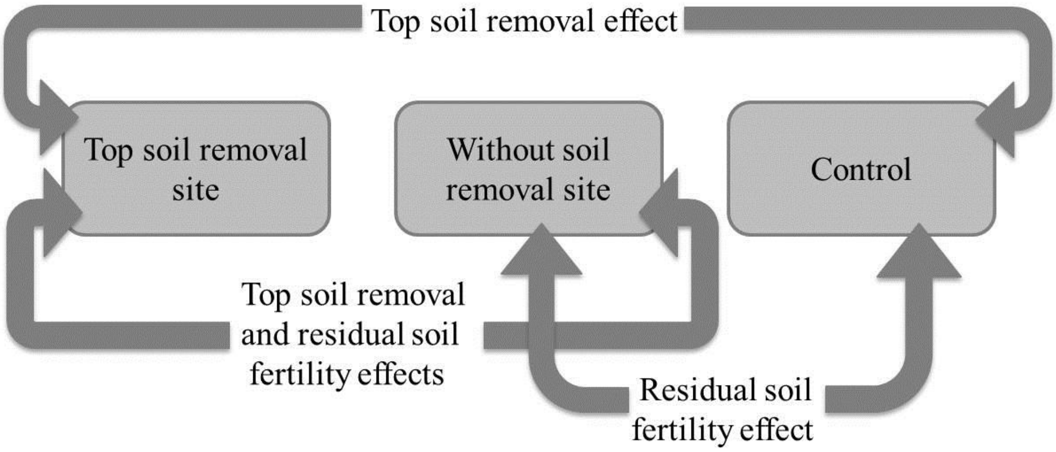

Fig. 1. Schematic overview of factors determining plant biomass allocation and trait plasticity between restoration (with and without topsoil removal) and control sites.

Soil sampling and analysis

In March 2013, one composite soil sample consisting of six cores (3–8 cm depth) was taken per site and mixed by sieving. Soils were analysed for pH_{CaCl_2} and (CAL-)extractable phosphorous and potassium (VDLUFA 1991, 2012); soil NO_3^- was determined using ion chromatography.

Table 1. Overview of soil conditions and grassland communities of the three study sites (TR, topsoil removal; NR, no topsoil removal; AG, ancient grassland; -, no data).

Sites	Soil depth (cm)	pH ($CaCl_2$)	NO_3^- (mg/100g)	P_2O_5 (mg/100g)	K_2O (mg/100g)	Bare soil (%)	Species density (m^{-2})
TR	<1	7.6	-	2	4	60	18
NR	20	7.3	0.13	36	38	30	29
AG	11*	6.1	0.12	1	4	29*	33*

* T. Conradi, unpubl. data

Selection and measurements of species and traits

Between July and September 2012, we sampled trait data of 14 species belonging to eleven families and different functional groups (graminoids, legume forbs, non-legume forbs, chamaephytes). All species were present in at least two sites and are target species of calcareous grassland restoration; eight of the 14 species were found on all study sites (Table 2). Cover of the species differed among the sites, but together they represented >70% of cover at each site.

Plant individuals were collected for trait and biomass sampling along transects, covering the whole area. We avoided sampling of the same individual by keeping a minimum of 5 m distance, which is sufficient also for clonal species (Tamm, Kull & Sammul 2002). All individuals of a given species were collected at the point of maximum development (i.e. flowering, except early-flowering *Carex humilis*), in most cases on the same day. Ten individuals of each species were randomly sampled per site for above- and below-ground biomass, plant height and specific leaf area (SLA). A minimum of five individuals were used for those species for which a sufficient number of plants could not be found due to their rarity. We selected non-senescent, intact leaves for trait measurements. Following Cornelissen et al*.* (2003), two leaves per individual were collected, except for plants with very small leaves (*Festuca rupicola*, n = 5; *Helianthemum nummularium*, n = 10; *Linum perenne*, n = 10). Because of legal restrictions on sampling, we could only measure above-ground traits (SLA, height) within the ancient grassland. Below-ground traits, and root and leaf nitrogen concentrations were taken from five individuals per species; plant excavation was done with a 20 cm-wide shovel, and the soil around of the root was carefully loosened, avoiding the loss of fine roots. Above- and below-ground samples were oven dried for 48 h at 60 ^{o}C before weighing.

For each individual, we measured above- and below-ground biomass as well as six functional traits that are strongly related to the acquisition and economics of resources and therefore are directly related to the contrasting levels of resource supply: (1) Maximum vegetative height, associated with competitive vigour (Cornelissen et al. 2003). (2) SLA is strongly correlated with relative growth rate (Cornelissen et al. 2003) and tends to increase with site productivity (Westoby et al. 2002). Laminar surface area was measured with ImageJ software from digital camera images. (3) Specific root length (SRL), the ratio of root length to mass, describes the absorptive tissue deployed per unit of mass invested and is also strongly correlated to relative growth rate (Cornelissen et al. 2003). (4) Fine root diameter (RD) was measured, since it is related to soil physical conditions and water content (Cornelissen et al. 2003). Finally, (5, 6) total N concentrations (%N) in leaves and roots (hereafter LNC and RNC) were measured from oven-dried and milled samples using an element analyser; both are related to photosynthetic rate and growth (Aerts & Chapin III 1999).

Data analyses

To decompose trait variation, we used linear models at different levels of sampling (among sites, among and within species) and for each species (among and within sites). In the first model, traits varied among and within species, and species were nested within sites, whereas in the second model, traits varied only among sites. Analysis of variance was used for summarizing the linear models. For these analyses, we only used the eight species common to all treatments. The amount of variation among and within sites, and among and within species was calculated as the sum of squares of the effect divided by the total sum of squares of the model unit equivalent to R^2.

To test for significant differences in trait values between sites, one-way ANOVA and t-test were carried out. We tested for homogeneity of variance and normality prior to the analysis. When necessary, data were transformed to reach normality, and when prerequisites for parametric statistic were not met, the Mann-Whitney U-test was used. To evaluate phenotypic variation in resource acquisition and growth traits as a response to environmental variation, we calculated the ratio of TR and NR treatment values for those traits that differed significantly between the TR and NR treatments. A Student t-test was used to evaluate whether relative above- and below-ground biomass, as well as total biomass, were significantly related to restoration treatments.

Due to the design of the restoration experiment, we could not generate true replicates (cf. Temperton, Märtin, Luecke, Röder, & Kiehl 2012), and there were no comparable fragments of this grassland type in the region. The absence of true replicates does not invalidate the study (Oksanen 2001), but requires special attention when interpreting results, as it allows the discussion of differences between sites, not between experimental treatments. All statistical analyses were carried out with R 2.15.1 (R Development Core Team 2010).

Results

Inter- and intraspecific trait variation

The first model partitioned the trait variation into within and among species, and among sites components (Fig. 2A): 10, 28, 24, 32, 18 and 11% of the total variance in plant height, SLA, SRL, RD, LNC and RNC, respectively, were explained by intraspecific trait variation. Interspecific variability in height, SLA, SRL, RD, LNC and RNC was 7, 2, 3, 2, 5 and 8-fold higher than intraspecific variation. SLA and height varied significantly among sites as opposed to the other traits. Species differed considerably regarding contribution of among- and within-site variation.

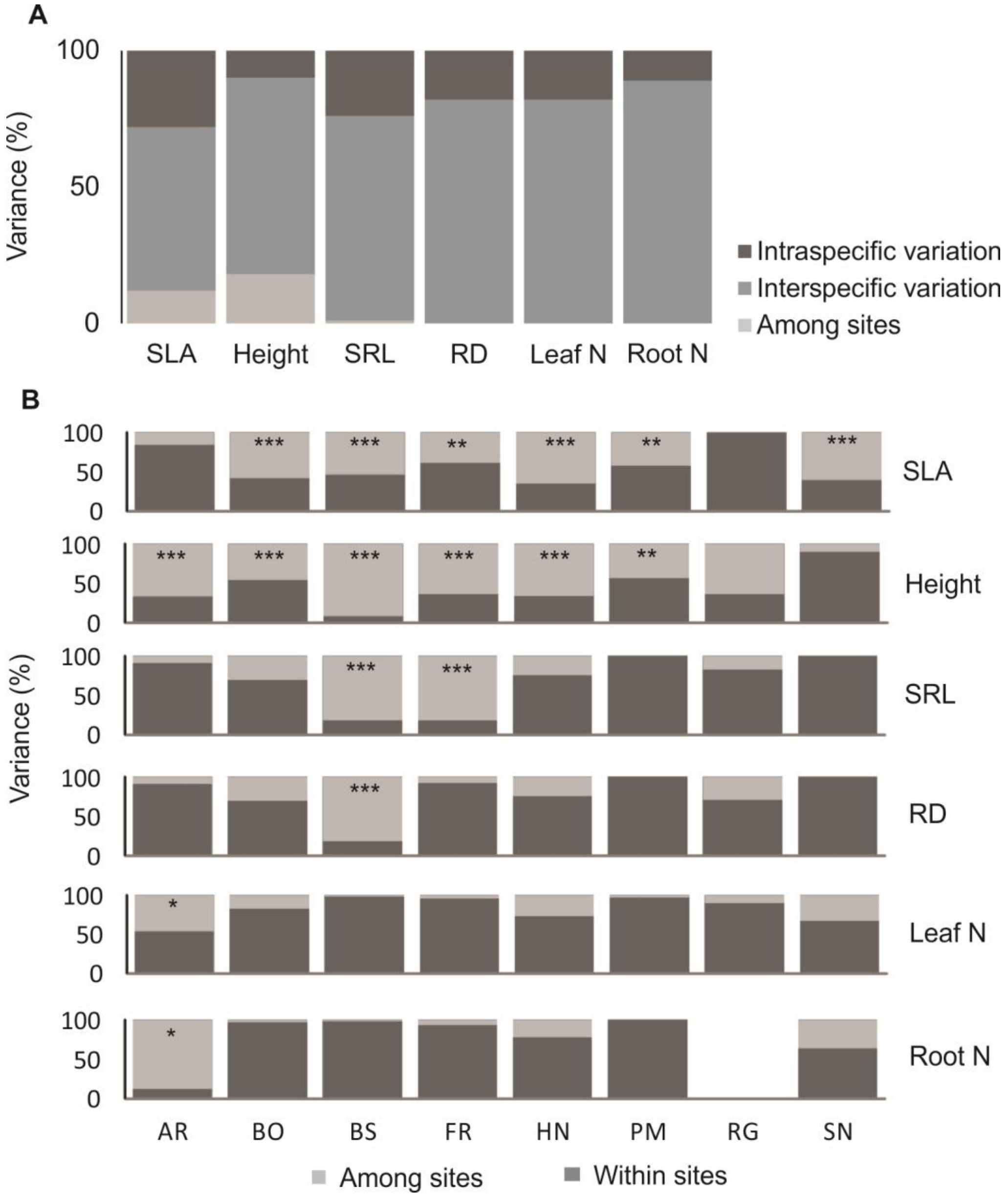

Fig. 2. Variance decomposition in inter- and intraspecific and among sites contributions for single-trait pattern (A), and among and within sites (B) in the eight common species found in the three treatments. For figure (A), the coefficient of variation were significantly different within traits in all levels ($P < 0.01$), except for SRL, RD, Leaf N and Root N at site level. Root N data is absent for RG because of a too low number of measurements. Grassland species: AR, *Anthericum ramosum*; AV, *Anthyllis vulneraria*; BO, *Betonica officinalis*; BS, *Buphthalmum salicifolium*; FR, *Festuca rupicola*; HN, *Helianthemum nummularium*; PM, *Plantago media*; RG, *Rhinanthus glacialis,* and SN, *Silene nutans*.

At the species levels, SLA and height were generally more variable among sites than the other traits, with exception of *Festuca rupicola* and *Plantago media* for SLA, and *Buphthalmum salicifolium* and *Plantago media* for height (Fig. 2B). RD, LNC and RNC showed no significant differences between the restoration sites, with few exceptions (*Buphthalmum salicifolium* and *Rhinanthus glacialis* for RD, *Festuca rupicola* for LNC and *Anthyllis vulneraria* for RNC).

Comparison of restoration sites with ancient grassland

Despite phosphorus and potassium content being 36- and 10-fold higher at the NR and TR site, respectively, compared to the AG site, almost no differences were found for plant height and SLA. *Buphthalmum salicifolium* and *Linum perenne* were taller at NR, and *Bromus erectus* at AG, while *Betonica officinalis, Plantago media* and *Buphthalmum salicifolium* had higher SLA values at NR. In general, individuals (within NR and AG) with higher SLA also were taller and had higher SRL values.

At the TR site, under water and nutrient limitation, SLA, SRL and height decreased or remained mostly the same in comparison with the site without topsoil removal (NR) and the control (AG). The comparison of SLA and SRL, i.e. functional traits relevant to resource uptake, between the two restoration sites showed a decrease in SLA and SRL in TR compared to NR. The exceptions were *Festuca rupicola* and *Rhinanthus glacialis*, which showed a higher SRL at the TR site (Fig. 3, Table 2). Most species showed lower mean values for traits related to resource acquisition and growth rate at harsher environmental conditions (TR in comparison to NR; Fig. 3), albeit with considerable variation and three exceptions.

Table 2. Study species of calcareous grasslands, including ecological indicators for soil nitrogen and moisture (Ellenberg, Weber, Düll, Wirth, Weber et al. 1992: scale 1–9; x, no information), functional groups (C, chamaephyte; F, forb; LF, legume forb; G, graminoids), plant abundance (r, <10%; a, ≥10% frequency), and six plant traits in the study sites (TR, topsoil removal; NR, no topsoil removal; AG, ancient grassland). Empty cells indicate that the species was not present in the respective site; different letters represent significant differences at $P < 0.05$ level within trait and species.

Species	Ellenberg N, moisture	Functional group	Plant distribution				Height			SLA			SRL		Root diameter		Leaf nitrogen		Root nitrogen	
			TR	NR	AG		TR	NR	AG	TR	NR	AG	TR	NR	TR	NR	TR	NR	TR	NR
Anthericum ramosum	3, 3	F	a	r	a	mean	17.8^{a}	30.3^{b}	28.6^{b}	14.5^{a}	17.2^{a}	15.3^{a}	9.2^{a}	18.1^{a}	0.53^{a}	0.74^{a}	1.58^{a}	1.63^{a}	0.21^{a}	0.26^{b}
						σ	3.3	4.9	4.0	3.2	2.0	2.8	6.5	11.2	0.2	0.5	0.2	0.2	0.03	0.03
Anthyllis vulneraria	2, 3	LF	r	r	-	mean	19.6^{a}	34.0^{b}		8.7^{a}	12.6^{b}		13.9^{a}	16.7^{b}	0.3^{a}	0.3^{a}	1.6^{a}	1.39^{b}	1.66^{a}	1.56^{a}
						σ	2.9	9.6		0.8	3.5		3.4	10.1	0.06	0.12	0.1	0.1	0.2	0.3
Betonica officinalis	3, x	F	r	r	a	mean	4.6^{a}	7.2^{b}	9.4^{b}	9.3^{a}	15.1^{b}	12.7^{c}	16.6^{a}	43.4^{b}	0.3^{a}	0.2^{a}	1.43^{a}	1.57^{a}	0.53^{a}	0.57^{a}
						σ	2.1	2.5	2.1	1.1	2.6	2.4	6.6	9.6	0.06	0.02	0.2	0.1	0.1	0.1
Bromus erectus	3, 3	G	-	a	a	mean		21.0^{a}	32.0^{b}		12.4^{a}	11.2^{a}		12.8		0.3				
						σ		5.9	7.5		3.7	2.0		3.8		0.1				
Buphthalmum salicifolium	3, 4	F	a	a	a	mean	6.4^{a}	41.3^{b}	28.7^{c}	17.1^{a}	18.9^{a}	13.0^{b}	7.6^{a}	46.2^{b}	0.6^{a}	0.2^{b}	0.71^{a}	0.76^{a}	1.57^{a}	1.52^{a}
						σ	2.2	6.9	3.2	1.6	3.6	1.3	1.6	20.2	0.1	0.03	0.1	0.2	0.2	0.1
Carex humilis	3, 2	G	a	-	a	mean	16.1^{a}		13.8^{a}	9.5^{a}		13.5^{b}	62.3		0.1					
						σ	4.3		3.9	1.7		4.9	25.3		0.02					
Festuca rupicola	2, 3	G	a	a	r	mean	9.3^{a}	19.8^{b}	21.4^{b}	6.6^{a}	10.1^{b}	11.4^{b}	160.1^{a}	102.7^{b}	0.1^{a}	0.1^{a}	0.84^{a}	0.8^{a}	0.95^{a}	0.89^{b}
						σ	1.9	3.4	6.1	1.5	2.7	3.5	74.3	24.2	0.02	0.01	0.1	0.1	0.2	0.05

Helianthemum nummularium	2, 3	C	a	r	r	mean	11.2^{a}	18.8^{b}	19.2^{b}	8.7^{a}	12.9^{b}	12.0^{b}	29.7^{a}	32.0^{a}	0.2^{a}	0.2^{a}	1.9^{a}	2.1^{a}	0.57^{a}	0.45^{a}
						σ	2.6	2.1	3.3	0.8	2.0	0.9	10.0	6.2	0.02	0.01	0.2	0.2	0.1	0.1
Hippocrepis comosa	2, 3	LF	a	-	r	mean	3.6^{a}		8.2^{b}	13.0^{a}		14.1^{a}	37.3		0.3					
						σ	1.2		2.3	4.7		2.9	18.2		0.03					
Linum perenne	2, 3	F	-	a	r	mean		38.3^{a}	26.8^{b}		12.5^{a}	15.3^{a}		42.7		0.2				
						σ		5.2	7.3		1.5	5.6		10.7		0.1				
Plantago media	3, 4	F	r	a	r	mean	1.4^{a}	6.8^{b}	7.6^{b}	11.0^{a}	16.4^{b}	12.9^{a}	32.6^{a}	34.9^{a}	0.3^{a}	0.3^{a}	1.2^{a}	1.13^{a}	0.72^{a}	0.72^{a}
						σ	0.6	3.2	3.5	2.1	3.6	1.8	15.4	9.9	0.05	0.06	0.3	0.2	0.2	0.1
Rhinanthus glacialis	2, 5	F	a	a	r	mean	15.0^{a}	24.6^{b}	32^{b}	13.4^{a}	13.4^{a}	12.9^{a}	15.5^{a}	10.4^{a}	0.2^{a}	0.3^{b}	2.33^{a}	2.04^{a}	0.76	-
						σ	6.1	2.9	6.8	1.5	2.8	2.4	12.6	5.0	0.1	0.06	0.6	0.4	0.3	
Salvia pratensis	4, 3	F	-	a	r	mean		9.8^{a}	6.5^{a}		19.0^{a}	13.5^{a}		156.1		0.2				
						σ		3.0	2.2		5.9	3.1		94.1		0.02				
Silene nutans	3, 3	F	r	r	r	mean	4.1^{a}	3.2^{a}	3.6^{a}	9.2^{a}	34.1^{b}	30.5^{b}	61.7^{a}	110.8^{a}	0.2^{a}	0.2^{a}	1.34^{a}	1.7^{a}	0.41^{a}	0.65^{a}
						σ	1.1	1.0	1.2	0.8	8.6	10.3	22.0	57.1	0.03	0.03	0.2	0.4	0.2	0.1

Allocation patterns in response to external drivers

Above-ground biomass was higher (though mostly not significantly) at the NR site for most species for which we had data from both restoration sites, with the exception of *Silene nutans* and *Festuca rupicola*, that responded the opposite way (Table 3). *Anthericum ramosum*, *Silene nutans* and *Betonica officinalis* showed a higher amount of below-ground instead of above-ground biomass under conditions of nutrient and water limitation. The root/shoot ratio was significantly higher at the TR site, except for three species that could maintain their relative biomass values under both environmental conditions (*Anthyllis vulneraria*, *Helianthemum nummularium*, *Plantago media*). *Rhinanthus glacialis*, a hemiparasitic plant, showed the highest relative allocation into above-ground tissue, both at TR and NR sites, with low root biomass values at both sites. *Silene nutans* had very contrasting values between the two sites, with absolute biomass both above- and below-ground significantly lower at the NR site.

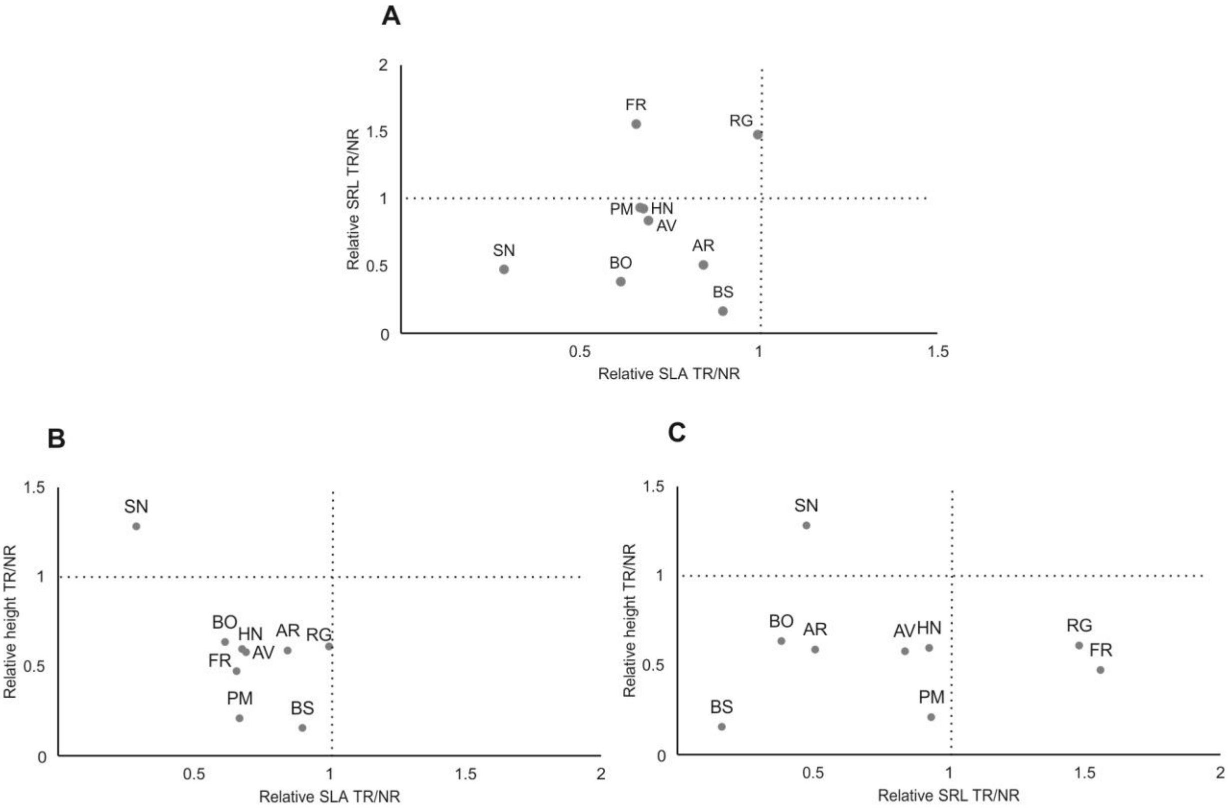

Fig. 3. Relative trait averages (A: SLA vs. SRL, B: SRL vs. height, C: SLA vs. height) displaying phenotypic variation between restoration treatments (TR/NR). If a species is situated above 1 on any of the axes, trait values are lower on the site with harsher conditions. Significant differences are indicated (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$); for full species names, see Fig. 2.

Table 3. Means of above- and below-ground biomass (g) in the two restoration sites (TR, topsoil removal; NR, no soil removal), and the below-ground / above-ground biomass ratio (B/A). Species were ordered according to the column 'B/A TR'.

	Above-ground			Below-ground			B/A		
Species	TR		NR	TR		NR	TR		NR
Anthericum ramosum	0.40	**	0.88	0.75		0.82	1.88	*	0.93
Silene nutans	0.82	***	0.06	1.41	***	0.01	1.72	***	0.17
Betonica officinalis	0.49		0.59	0.65		0.58	1.33	*	0.98
Buphthalmum salicifolium	0.24	**	1.87	0.23	*	0.86	0.96	**	0.46
Festuca rupicola	1.93		0.68	1.37	**	0.33	0.71	***	0.49
Helianthemum nummularium	4.58		1.99	2.28		1.04	0.50		0.52
Plantago media	0.91		1.18	0.34		0.32	0.37		0.27
Anthyllis vulneraria	1.23		2.11	0.21		0.34	0.17		0.16
Rhinanthus glacialis	0.32	*	0.98	0.03		0.06	0.09	*	0.06
Carex humilis	3.43		-	2.8		-	0.82		-
Hippocrepis comosa	2.72		-	1.02		-	0.38		-
Salvia pratensis	-		0.73	-		0.65	-		0.89
Bromus erectus	-		1.51	-		0.68	-		0.45
Linum perenne	-		0.97	-		0.33	-		0.34

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; -, no data

Discussion

The overall objective of this study was to quantify intraspecific trait variation and biomass allocation patterns under contrasting conditions as a way to assess how plants cope with varying levels of environmental stress. These environmental constraints were the result of a 20-year restoration experiment in calcareous grassland. Water stress conditions led to clear changes of plant traits and allocation patterns compared to nutrient stress conditions, i.e. ancient grassland compared to the site without topsoil removal.

It is often assumed that slow-growing plants from infertile habitats show lower morphological plasticity than fast-growing species from fertile habitats (Grime 2006). In our study system, considerable intraspecific trait variation in response to water and nutrient stress exists. SLA was the trait with highest intraspecific variability, and in general, leaves on the topsoil removal site had lower SLA. The range of intraspecific variation of SLA found in our study resembles that in studies evaluating gradients over several kilometres (Albert et al. 2010;

Hulshof & Swenson 2010; Jung, Violle, Mondy, Hoffmann, & Muller 2010). SLA often shows considerable plasticity in response to environmental factors (Wilson, Thompson, & Hodgson 1999), which may enhance plant performance under a range of conditions and be a desirable characteristic in restoration of degraded areas where species will have to be introduced.

The limiting conditions on TR (shallow stony soil, low water holding capacity) most likely led to higher investments in leaf physical defence and to a longer leaf lifespan, as indicated by lower SLA at the TR site. Relative allocation into above-ground biomass was lower on TR compared to NR for most species. Thicker leaves indicated a greater investment in structural rather than metabolic components, enhancing leaf strength and durability (Farrell, Szota, Williams, & Arndt 2013). Our results show that SRL decreased at the topsoil removal site (though not always significantly); *Rhinanthus glacialis* showed the opposite trends in SRL and root diameter, probably because it is a hemiparasite. These species often develop only small root systems due to the limited need to assimilate inorganic solutes (Press, Scholes, & Watling 1999).

Plants with high SRL are able to build longer roots for a given dry mass investment (Cornelissen et al. 2003). Enhanced nutrient availability reduces the need for explorative fine root length growth and thus accounts for a decrease in SRL (Ostonen, Püttsepp, Biel, Alberton, Bakker et al. 2007). Under drought the same pattern is found, as thinner roots are more severely affected (Davies & Bacon 2003).

Plant performance is determined by morphological, physiological and phenological traits that contribute to overall fitness (Violle, Enquist, McGill, Jiang, Albert et al. 2012). Considering all traits analysed, two biomass allocation strategies in response to water and nutrient stress can be distinguished for our study species: (i) maintenance of root/shoot biomass ratio, enhancing resource acquisition (*Anthyllis vulneraria*, *Helianthemum nummularium*, *Plantago media*), and (ii) higher below-ground biomass investment (*Anthericum ramosum*, *Betonica officinalis*, *Buphthalmum salicifolium*, *Festuca rupicola*, *Silene nutans*). The maintenance of biomass ratios in the first species group could be explained by the close relationship between SLA and SRL, which indicates stronger changes in resource acquisition efficiency, resulting in the maintenance of below- and above-ground biomass ratio, instead of higher investment in below-ground production.

Most of studied species could not enhance their trait values under water and nutrient constraints (Fig. 3). The majority followed a conservative pattern of resource-use with slower tissue turnover (Fig. 3A) and lower investment in above-ground parts (Fig. 3B and C), in consequence of the lower capacity of below-ground resource acquisition (Fig. 3B). Turnover of tissues is a major mechanism by which the efficiency of resource use is maximized in resource-poor environments (Craine 2009). The benefits of short root life span are reduced in soils of low fertility (Eissenstat, Wells, Yanai, & Whitbeck 2000).

Leaf nitrogen content (LNC) has been shown to be related to relative growth rate of plants (Diaz, Hodgson, Thompson, Cabido, Cornelissen et al. 2004). Studies on ^{15}N in leaves and soil have shown that N dynamics in ecosystems can change markedly even along short environmental gradients, in response to different drivers such as abiotic factors (e.g. climate or soil properties) or species interactions when competing for soil N (Huber et al. 2007, Kahmen et al. 2007). However, in our study no overall differences for LNC and RNC were found, in contrast to Temperton et al. (2012), who evaluated LNC in five different species in the same study site than ours, and found that the N values content differed significantly between TR and NR sites for three species. These contrasting results indicate that these responses may be species-specific.

Environmental heterogeneity on a small scale is common in many ecosystems (Rogers & O'Keeffe 2003; Stöhr 2007), and different disturbance regimes may also influence trait expression (Zheng, Lan, Li, Shao, Shan et al. 2011). This means that caution has to be taken when working with mean trait values in studies that analyze trait composition along environmental gradients. Depending on the research question, the use of mean traits neglecting intraspecific variation may have consequences for the predictive ability of community ecology and can lead to misinterpretations (Violle et al. 2012). To overcome this problem, new methods comprising intra- and interspecific variation were proposed (e.g. Siefert 2012; de Bello, Lavorel, Albert, Thuiller, Grigulis, et al. 2011). In a restoration context, future research should address whether or not the observed intraspecific trait variation between sites translates into fitness differences between populations inhabiting these sites, thus influencing their long-term persistence in restored habitats.

Acknowledgements

The study was supported by a CNPq ('Science without Borders Program') scholarship to BOA, and a German Research Foundation grant to JK (KO 1741/2-1,2). We acknowledge the nature conservancy authorities for permission to work in the study area. We thank Adriane E. Muelbert for valuable discussion and Vanderlei Debastiani for help with the statistical analyses. The reviewers made valuable suggestions on an earlier version of this manuscript.

References

Aerts, R., & Chapin III, F. S. (1999). The mineral nutrition of wild plants revisited: A re-evaluation of processes and patterns. *Advances in Ecological Research, 30*, 1–67.

Albert, C. H., Grassein, F., Schurr F. M., Vieilledent, G., & Violle, C. (2011). When and how should intraspecific variability be considered in trait-based plant ecology? *Perspectives in Plant Ecology, Evolution and Systematics, 13,* 217–225.

Albert, C. H., Thuiller, W., Yoccoz, N. G., Soudant, A., Boucher, F., Saccone, P., et al. (2010). Intraspecific functional variability: extent, structure and sources of variation. *Journal of Ecology, 98,* 604–613.

Bolnick, D., Amarasekare, P., Araújo M. S., Bürger, R., Novak, M., Rudolf, V. H. et al. (2011). Why intraspecific trait variation matters in community ecology. *Trends in Ecology and Evolution, 26,* 185–194.

Cornelissen, J. H. C., Lavorel, S., Garnier, E., Diaz, S., Buchmann, N., Gurvich, D. E., et al. (2003). A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Australian Journal of Botany, 51,* 335–380.

Craine, J. M. (2009). *Resource strategies of wild plants*. New Jersey: Princeton University Press.

Davies, W., & Bacon, M. (2003). Adaptation of roots to drought. *Root Ecology, 168,* 173.

de Bello, F., Lavorel, S., Albert, C. H., Thuiller, W., Grigulis, K., Dolezal, J., Janeček, Š., Lepš, J. (2011). Quantifying the relevance of intraspecific trait variability for functional diversity. *Methods in Ecology and Evolution, 2,* 163–174.

Diaz, S., Hodgson, J. G., Thompson, K., Cabido, M., Cornelissen, J. H. C., et al. (2004). The plant traits that drive ecosystems: Evidence from three continents. *Journal of Vegetation Science, 15,* 295–304.

Eissenstat, D. M., Wells, C. E., Yanai, R. D., & Whitbeck, J. L. (2000). Building roots in a changing environment: implications for root longevity. *New Phytologist, 147,* 33–42.

Ellenberg, H., Weber, H. E., Düll, R., Wirth, V., Weber, W., & Paulissen, D. (1992): Zeigerwerte von Pflanzen in Mitteleuropa. *Scripta Geobotanica, 18,* 1–248.

Farrell, C., Szota, C., Williams, N. S., & Arndt, S. K. (2013). High water users can be drought tolerant: using physiological traits for green roof plant selection. *Plant and Soil, 372*, 177–193.

Fort, F., Cruz, P., & Jouany, C. (2014). Hierarchy of root functional trait values and plasticity drive early-stage competition for water and phosphorus among grasses. *Functional Ecology*. DOI: 10.1111/1365-2435.12217.

Freschet, G. T., Cornelissen, J. H. C., van Logtestijn, R. S. P., & Aerts, R. (2010). Evidence of the ‘plant economics spectrum’ in a subarctic flora. *Journal of Ecology, 98,* 362–373*.*

Grime, J. P. (2006). *Plant strategies, vegetation processes, and ecosystem properties*. Chichester: Wiley.

Herben, T., & Goldberg, D. E. (2014). Community assembly by limiting similarity vs. competitive hierarchies: testing the consequences of dispersion of individual traits. *Journal of Ecology, 102,*156–166.

Huber, E., Wanek, W., Gottfried, M., Pauli, H., Schweiger, P., Arndt, S. K., Reiter, K., & Richter, A. (2007). Shift in soil–plant nitrogen dynamics of an alpine–nival ecotone. *Plant and Soil*, *301*, 65–76.

Hulshof, C. M., & Swenson, N. G. (2010). Variation in leaf functional trait values within and across individuals and species: an example from a Costa Rican dry forest. *Functional Ecology, 24,* 217–223*.*

IUSS (2006). *World Reference Base for Soil Resources*. (2nd ed.). World Soil Resources Reports 103. Rome: FAO.

Jeschke, M., & Kiehl, K. (2008). Effects of a dense moss layer on germination and establishment of vascular plants in newly created calcareous grasslands. *Flora, 203,* 557–566.

Jung, V., Violle, C., Mondy, C., Hoffmann, L., & Muller, S. (2010). Intraspecific variability and trait-based community assembly. *Journal of Ecology, 98,* 1134–1140.

Kahmen, A., Wanek, W., & Buchmann, N. (2008). Foliar delta(15)N values characterize soil N cycling and reflect nitrate or ammonium preference of plants along a temperate grassland gradient. *Oecologia*, *156*, 861–870.

Kiehl, K. & Pfadenhauer, J. (2007). Establishment and persistence of target species in newly created calcareous grasslands on former arable fields. *Plant Ecology, 189,* 31–48.

Kiehl, K., Thormann, A., & Pfadenhauer, J. (2006). Evaluation of initial restoration measures during the restoration of calcareous grasslands on former arable fields. *Restoration Ecology, 14,* 148–156.

Kunstler, G., Lavergne, S., Courbaud, B., Thuiller, W., Vieilledent, G., Zimmermann, N. E., Kattge, J., & Coomes, D. A. (2012). Competitive interactions between forest trees are driven by species' trait hierarchy, not phylogenetic or functional similarity: implications for forest community assembly. *Ecology Letters, 15*, 831–840.

Mason, N. W. H., de Bello, F., Doležal, J., & Lepš, J. (2011). Niche overlap reveals the effects of competition, disturbance and contrasting assembly processes in experimental grassland communities. *Journal of Ecology, 99,* 788–796*.*

Oksanen, L. (2001). Logic of experiments in ecology: is pseudoreplication a pseudoissue? *Oikos, 94,* 27–38.

Ostonen, I., Püttsepp, Ü., Biel, C., Alberton, O., Bakker, M. R., Lõhmus, K., et al. (2007). Specific root length as an indicator of environmental change. *Plant Biosystems, 141,* 426–442.

Pfadenhauer, J. (2001). Some remarks on the socio-cultural background of restoration ecology. *Restoration Ecology, 9,* 220–229*.*

Press, M., Scholes, J., & Watling, J. (1999). Parasitic plants: physiological and ecological interactions with their hosts. *Physiological Plant Ecology, 13,* 175–197.

R Development Core Team. (2010). *R: A language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing.

Rogers, K. H., & O'Keeffe, J. (2003). River heterogeneity: ecosystem structure, function and management. In: Biggs, H. C. (Ed.), *The Kruger experience: Ecology and management of savanna heterogeneity*. Washington: Island Press.

Siefert, A. (2012). Incorporating intraspecific variation in tests of trait-based community assembly. *Oecologia, 170,* 767–775*.*

Stöhr, D. (2007). Soils – heterogeneous at a microscale. In G. Wieser & M. Tausz (Eds.), *Trees at their upper limit: treelife limitation at the alpine timberline* (pp. 37–56)*.* Netherlands: Springer.

Tamm, A., Kull, K., & Sammul, M. (2002). Classifying clonal growth forms based on vegetative mobility and ramet longevity: a whole community analysis. *Evolutionary Ecology, 15,* 383–401.

Temperton, V. M., Märtin, L. L., Luecke, A., Röder, D., & Kiehl, K. (2012). Effects of four different restoration treatments on the natural abundance of 15N stable isotopes in plants. *Frontiers in Plant Science, 3,* 70.

van Kleunen, M., Dawson, W., Bossdorf, O., & Fischer, M. (2014). The more the merrier: Multi-species experiments in ecology. *Basic and Applied Ecology, 15*, 1–9.

VDLUFA (1991). *Methodenbuch Band I, Bodenuntersuchung, Methode A 5.1.1*. Bonn: VDLUFA Verlag.

VDLUFA (2012). *Methodenbuch Band I, Bodenuntersuchung, Methode A 6.2.1.1*. Bonn: VDLUFA Verlag.

Violle, C., Enquist, B. J., McGill, B. J., Jiang, L., Albert, C. H., Hulshof, C., et al. (2012). The return of the variance: intraspecific variability in community ecology. *Trends in Ecology & Evolution, 27,* 244–252.

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

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

Wilson, P. J., Thompson, K. & Hodgson, J. G. (1999). Specific leaf area and leaf dry matter content as alternative predictors of plant strategies. *New Phytologist, 143,* 155–162*.*

Zheng, S., Lan, Z., Li, W., Shao, R., Shan, Y., Wan, H., et al. (2011). Differential responses of plant functional trait to grazing between two contrasting dominant C3 and C4 species in a typical steppe of Inner Mongolia, China. *Plant and Soil, 340,* 141–155*.*

Capítulo II

VARIABILITY OF SOIL PROPERTIES IN RELATION TO SCALE – LESSONS FOR ECOLOGICAL STUDIES

Co-autores: Alberto Vasconcellos Inda, Ilsi Iob Boldrini

Summary

1. We aimed to determine how variable physical and chemical properties are in different scales, and which soil properties can more accurately explain plant distribution in different scales, for better use of current soil methods in plant ecology studies.

2. Soil and vegetation survey were performed in different sample scales: regional, local and plot-level. Variance partitioning allowed us to decompose soil properties variance in the different sample scales. Then, to better understand how much of these soil properties variation explains the variation in vegetation structure in regional and local scale was performed redundancy analysis (RDA) and partial RDA (pRDA). Principal component analysis (PCA) contributed with additional information, simplifying the interpretation of that complex data set.

3. Results indicate that soil properties variation is related to the measurement scale applied: chemical properties showed higher variability in a regional scale, while physical properties at local and plot-scale. The percentage of explanation of plant distribution by soil properties followed a similar pattern. At local and plot-level differences were verified in relation to the soil physical properties.

4. This study emphasizes the importance of choosing environmental descriptors depending on the spatial scale for soil-plant interaction studies.

Keywords: Grassland ecosystems; Spatial variability, Plant ecology, Plant-soil relationship, Soil heterogeneity.

Abbreviations

ACE	Aceguá municipality
ALE	Alegrete municipality
APA	Aparados da Serra National Park
ARA	Aratinga Ecological Station
LAV	Lavras do Sul municipality
TAI	Tainhas State Park
Al^{+3}	Aluminum
Al+H	ions Al^{+3} and H^{+}
BD	Bulk Density
Ca^{+2}	Calcium
CEC	Cation Exchange Capacity
M	macroporosity
K	Potassium
Mg^{+2}	Magnesium
P	Phosphorus
pH	Potential Hydrogen
SOC	Soil Organic Carbon
AWC	Available water content
%base	Percent Base Saturation
%Al	Percent Aluminum Saturation

Introduction

The soil-vegetation continuum is largely related to climate, topography, and underlying geology which affect the soil chemical and physical properties (Jeffrey 1987). The importance of soil features and processes for the environment has been directly and indirectly assessed, and an understanding of the landscape can clarify ecological relationships (Grigal et al. 1999). For instance, the soil-plant relationship determines the water and nutrient exchange between the plant community and the environment. From this interaction arises important ecosystem services, e.g. soil structure maintenance, water regulation, nutrient supply, biomass production (Sala & Paruelo 1997), with effects on global climate and food security (Lal 2004). This interaction can be achieved studying ecosystem process and patterns (Aerts & Chapin III 1999), an important link in conservation and restoration.

A vast amount of new information concerning soil-plant relationship has become available last decades. In a simple citation search in the Web of Science (WOS) database for articles published on plant-soil relationship, in the last three decades, we verified a marked increase in articles published. It increased almost five times in the nineties' in comparison to the eighties', and about two times in the last decade. This makes evident that a multidisciplinary approach has been desired in ecological studies. Aiming to give support to soil-plant relationship studies, Jeffrey (1987), Sollins (1998), Robertson et al. (1999), Perkins et al. (2013) and Wigley et al. (2013) can be useful as guides for methodological support. However, still lack information on preference scale for sampling chemical and physical soil properties that are important in an ecological context.

Chemical and physical soil properties are mutually complementary and related (Schoenholtz et al. 2000). While chemical properties determine the capacity to hold, supply and cycle nutrients and carbon, the soil physical properties determine the movement and availability of water and nutrients to the plants. Both soil properties vary at multiple scales (Garten Jr et al. 2007), however, soil physical properties are not frequently considered in ecological studies, mostly because determination of soil chemical properties measurements are easier and cheaper to sample by standard laboratory methods. Soil physical properties, despite their well-known importance for plant establishment and growth (Burke et al. 1998; Hillel 2003; Howard 2012), are not broadly used in ecological studies.

Often, ecological studies that include soil factors use standard analysis of chemical soil characteristics with the objective to relate vegetation features at a rather local scale to soil properties – and often fail to find clear relationships between the soil variables considered and vegetation patterns (e.g. Cruz Ruggiero et al. 2002; Overbeck et al. 2006; Dantas & Batalha

2011), even though it is well known that soil properties are one of the main factors influencing vegetation patterns (Janssens et al. 1998). According to Sollins (1998), failure to find a correlation with chemical properties may be due to lack of range in soil fertility across the sites studied, failure of test methods to measure availability of nutrients to plants or temporal and spatial variability in soil properties. Obviously, at a local scale, the lack of variation of soil chemical properties may be responsible for this. In this paper, we wish to explore the dependence of physical and chemical soil properties on the scale, based on data from a regional-scale study of grassland vegetation in subtropical southern Brazil. We hypothesized that soil chemical properties are not as variable as physical properties at a local and plot-scale, and that the opposite may occur in a regional scale; and soil chemical features can better describe vegetation distribution in a regional scale, while at a local scale soil physical properties may be more sensitive. We use our results for a discussion of which soil analyses are more promising for ecological studies at different spatial scales.

Material and Methods

Study area

Soil and vegetation survey was conducted at six sites across Rio Grande do Sul, the southernmost state of Brazil, in late 2010 and early 2011 (southern hemisphere spring and summer). The survey was conducted in species-rich grasslands on well-drained soils that have been grazed by domestic cattle. The sites were distributed among two vegetation biomes: Atlantic Forest in the northern portion of the state (Highland grasslands) and Pampa, in the southern (Table 1). Three sites were chosen in each one. Surveys were done in a nearly-flat surface in Pampa sites, and in a topographic relief pattern in Highland grasslands sites. The basic sampling design was an experimental plot per site. The experimental plot (70 x 210 m) was divided in three subplots of 70 x 70 m each. Each plot with three 1m^2 permanent sampling units in transect, with 17 m between them. Plant and soil survey was done in a total of 54 sample units. This experimental design allowed us to quantify the separate contributions of variance of the different soil properties in regional, local and plot-level. Differences between biomes were considered as regional level; differences within biomes and between sites as local level; and within sites as plot-level.

The Highland grasslands soils are developed on rhyodacite rock, with quartz and kaolinite predominance in fine earth and clay-size fractions. The soils in Pampa sites developed from different rocks, being predominately: orthogneiss metadiorite in Lavras do Sul municipality (LAV), andesite in Alegrete municipality (ALE), and siltitic-arenitic rocks in Aceguá municipality

(ACE). Soils are characterized by the presence of plagioclase and orthoclase, associated to quartz in air-dried fine earth. Highland grasslands soils have lower sum of bases and base saturation, and higher aluminum saturation in comparison to soils of the Pampa sites, which confers lower pH value and soil fertility to the former. In-depth soil analyses and soil-plant relationship is discussed in Andrade et al. (un pub.: **capítulo III)**.

The sites in Highland grasslands are located in higher elevations than Pampa grasslands, which lead to higher annual mean precipitation and lower annual mean temperature than in Pampa biome (Table 1). In Pampa biome, the landscape is dominated by grasslands, while in the Highland grasslands, grasslands are found in mosaics with forest patches (Overbeck et al. 2007). Average grassland species richness is higher at Pampa sites, while species dominance is higher in Highland grasslands sites (Ferreira et al. unpubl.). The dominant grass species in Highland grasslands is the tussock grass *Andropogon lateralis*, which comes associated with *Bulbostylis* sp, *Paspalum maculosum* and *Schizachyrium tenerum* in APA, ARA and TAI, respectively. At the Pampa biome sites, the dominant species differ between sites: *Piptochaetium montevidense* and *P. notatum* in ACE; *A. lateralis, P. montevidense* and *P. notatum* in ALE; *P. notatum* and *Baccharis crispa* in LAV.

Table 1 Study site location in Rio Grande do Sul state, Brazil, and its description

Site/ Municipality	**Coordinates**	**Altitude (m a.s.l.)**	**Temperature (°C)**	**Precipitation (mm)**	**Biome**	**Soil classification**
Aparados da Serra National Park (APA) São Francisco de Paula	29°08’10”S, 50°09’21”W	924	15.2	1898	Highland grasslands	Humic Dystrudept
Aratinga Ecological Station (ARA) Cambará do Sul	29°23’31”S, 50°14’30”W	900	15.1	2002	Highland grasslands	Humic Dystrudept
Tainhas State Park (TAI) Jaquirana	29°05’40”S, 50°22’03”W	843	15.7	1931	Highland grasslands	Lithic Udorthent
Aceguá municipality (ACE) Aceguá	31°38’55”S, 54°09’26”W	163	17.9	1152	Pampa	Vertic Hapludalf
Alegrete municipality (ALE) Alegrete	30°04’08”S, 55°59’27”W	189	18.6	1507	Pampa	Typic Udorthent
Lavras do Sul municipality (LAV) Lavras do Sul	30°41’55”S, 53°58’11”W	334	17.9	1449	Pampa	Cromic Hapludalf

Plant sampling

All plant species occurring within each $1m^2$ sampling unit had its percentage cover estimated using a decimal scale, similar to that of Londo (1976). For the smaller cover categories we used up to 1% and 1 to 5% estimated cover, and the remaining categories followed a decimal scale up to 90-100% estimated cover. Plants found in only one area with 1% coverage were not considered in the following analysis, thus a total of 198 taxa were considered.

Soil sampling

One soil sample (depth: 20 cm) was taken close to each permanent sample unit. Chemical and textural soil properties were determined after air drying and sieving at 2 mm according to Embrapa (1997). The pH value was determined in water solution (1:1). Exchangeable cations, Mg^{+2}, Ca^{+2} and Al^{+3} were extracted with KCl $1molL^{-1}$. P and K were determined using the Mehlich I extraction method. Cation exchange capacity (CEC) was determined at pH 7. Soil organic carbon (SOC) was measured using the wet combustion method. We also calculated percent base saturation (%base) and aluminum saturation (%Al). Soils were classified according to Soil Survey Staff (2010).

Soil physical properties were divided in two classes: texture and hydraulic properties. By texture we mean sand, silt and clay content, and by hydraulic properties: bulk density, available water content and macroporosity. This division allowed us to address several concerns about methods adopted for environmental description in ecological studies. Texture is more frequently employed in ecological studies because are easily and quickly sampled, and also use current standard laboratory equipment.

Soil texture was measured with densimeter method (Embrapa 1997). To assess hydraulic soil properties (bulk density, macroporosity and available water content) three undisturbed soil cores (7 cm in length and 10 cm in diameter) were collected per plot from 3−10cm depth, close to the permanent sample units, which follow the topographic contour lines. Soil water retention at suctions of 0, 6, 33 kPa was determined on standard pressure plate apparatus, and at suctions of 100, 500 and 1500 kPa a pressure membrane was used (Reynolds & Topp 2008). The difference in moisture content at 0 kPa and −6 kPa was calculated to estimate macroporosity. Available water content is the amount of water held between field capacity (−33 kPa) and wilting point (−1500 kPa). Soil bulk density was determined by calculating mass per unit volume.

Statistical analysis

Correlation analysis was performed for all soil variables to determine the relationship between these variables and keep out variables with high levels of autocorrelation in the further analyses. Pearson's correlation coefficient was calculated for each pair of variables of the data set of the 54 sampling considering both physical and chemical properties (Appendix A). P, K, Al, Ca, SOC, clay, silt, sand, AWC and M were the variables selected (coefficient = 0.8) for the partitioning variance analysis, which aims to evaluate how variable are soil physical (texture and hydraulic) and chemical properties in a regional, local and plot-scale. Soils chemical properties were standardized by marginal total, because contain variables with different metric units. The randomization test comparing sampling units (Pillar & Orloci 1996) was performed based on Euclidean resemblance matrix and with 10.000 iterations in bootstrap resampling.

Multivariate redundancy analysis (RDA) was used to test the second hypothesis and determine the likelihood of soil predictors in explaining patterns in community structure. Before that, Hellinger transformation was performed in vegetation matrix because it produces much more accurate estimations and valid comparisons between sets of factors in explaining community structure (Peres-Neto et al. 2006). Different explanatory and response tables were used according to the observed scales. Aiming to analyse data at regional and local scales we used raw matrices (sampling units described by species mean percentage cover values), with cover values averaged to the subplot level. Therefore, at regional scale we analysed 18 sample units (three subplots per site), and at local level we considered half of it, in reference to each biome. At local scale, for comparisons between sites, all sample units were considered (9 plots per site). We used summarized soil chemical and physical data to estimate the influence of abiotic factors on grassland vegetation coverage. For composing the explanatory table we used the ordination scores of sampling units from the first two ordination axis obtained in a Principal Coordinate Analysis of sampling units described by the abiotic matrix containing the variables described above. These variables were then used to explain variation in community structure with RDA. Using the R-language function "varpart", available in vegan library (Oksanen et al. 2008), was conducted the variation partitioning of the response tables with respect to the tables of explanatory variables, based upon RDA-adjusted R^2 values. The later analysis was performed twice: at first, two tables of explanatory variables were used (physical and chemical soil properties). To unravel the main trends of plant and soil properties variation among sample sites, we performed a principal component analysis (PCA). PCA produces uncorrelated multivariated axes that can be interpreted to represent the variance along the multiple scales evaluated. The first axis encompassed 65, 70, 71 and 48% of variation in texture, hydraulic and chemical properties, and vegetation patterns, respectively. The second axis explained 35, 27, 13 and 9%, respectively.

Statistical analyses were carried out with Multiv software (Pillar 2011) and R 3.0.2 (R Development Core Team 2010).

Results

Spatial variability of soil properties

Variance partitioning found that soil properties are significantly related to the measurement scale applied (*P*<0.001). Chemical properties showed higher variability (67.5 %) at a regional scale and smaller variance at the local and plot-level, with 15.5% and 17% variance, respectively. Textural properties presented higher variance at local scale (59.5%), while 17.5% and 23% of variance at regional and plot-level scale, respectively. Hydraulic features varied from lower to higher accuracy scale as follows: 34, 27 and 39%, respectively (Fig.1).

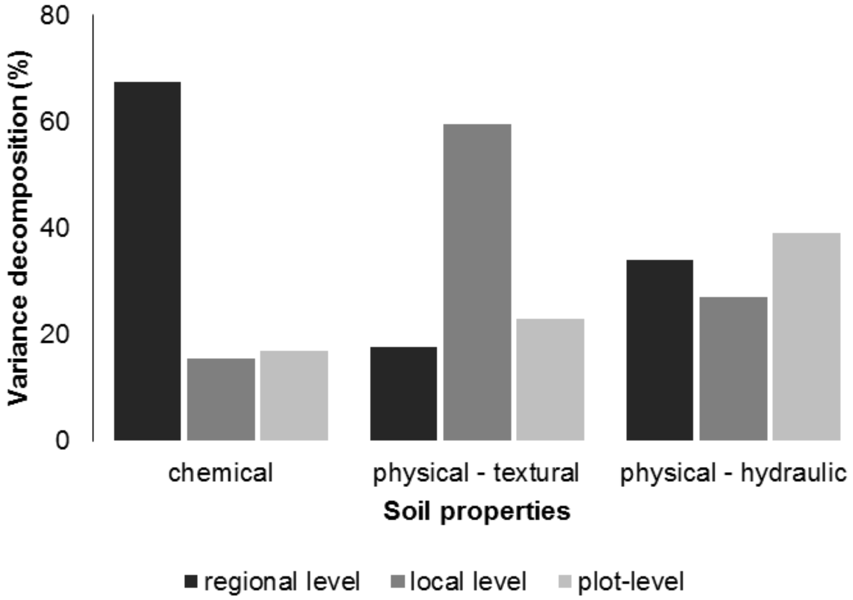

Fig 1. Variance particioning of soil properties between different measurement scales.

Soil-plant relationship at regional and local scales

The RDA analysis revealed a non-random concordance between vegetation and soil properties. Spatial heterogeneity of grassland vegetation was driven by regional and local variation of soil conditions. The best predictors of vegetation variance changed between the measurement scales (Table 3). The total variation of vegetation explained by the significant variables [Chemical+Physical], was about 20% in a regional and local scale, and about 10% in the plot-level (Table 3). In a regional scale, soil chemical and physical variables explained alone 9% and 8%, respectively, while the shared variation between them was 2% (Table 3).

At the local scale, the percentage of explanation varied between biomes (Table 3). The Highland grasslands had <0.01% of explanation only by chemical properties, 5% by physical properties and further 6% represent the shared variation between them. In Pampa biome, 3% of the vegetation variation is explained by soil chemical properties, 25% is explained by physical ones, while the shared variation between them was 4%.

Table 2 Partial redundancy analysis (pRDA) to determine how much of variation in vegetation community composition is explained by statistically significant variables. Different sample scales were evaluated.

Sample scales	**Sampled area**	**Chemical (Ch)**	**Physical (Ph)**	**Joint Ch and Ph**	**Residual**
Regional Level	Highland grasslands + Pampa	0.09***	0.08***	0.02	0.81
Local level	Highland grasslands	<0.01	0.05	0.06	0.89
	Pampa	0.03	0.25*	0.04	0.68
Plot level	TAI	0.04	0.08	<0.01	0.88
	ARA	0.03	0.04	0.03	0.90
	APA	0.04	0.07	0.04	0.85
	ACE	<0.01	0.08	0.05	0.87
	ALE	<0.01	0.05	0.06	0.89
	LAV	<0.01	0.02	<0.01	0.98

*P<0.05; **P<0.01; ***P<0.001

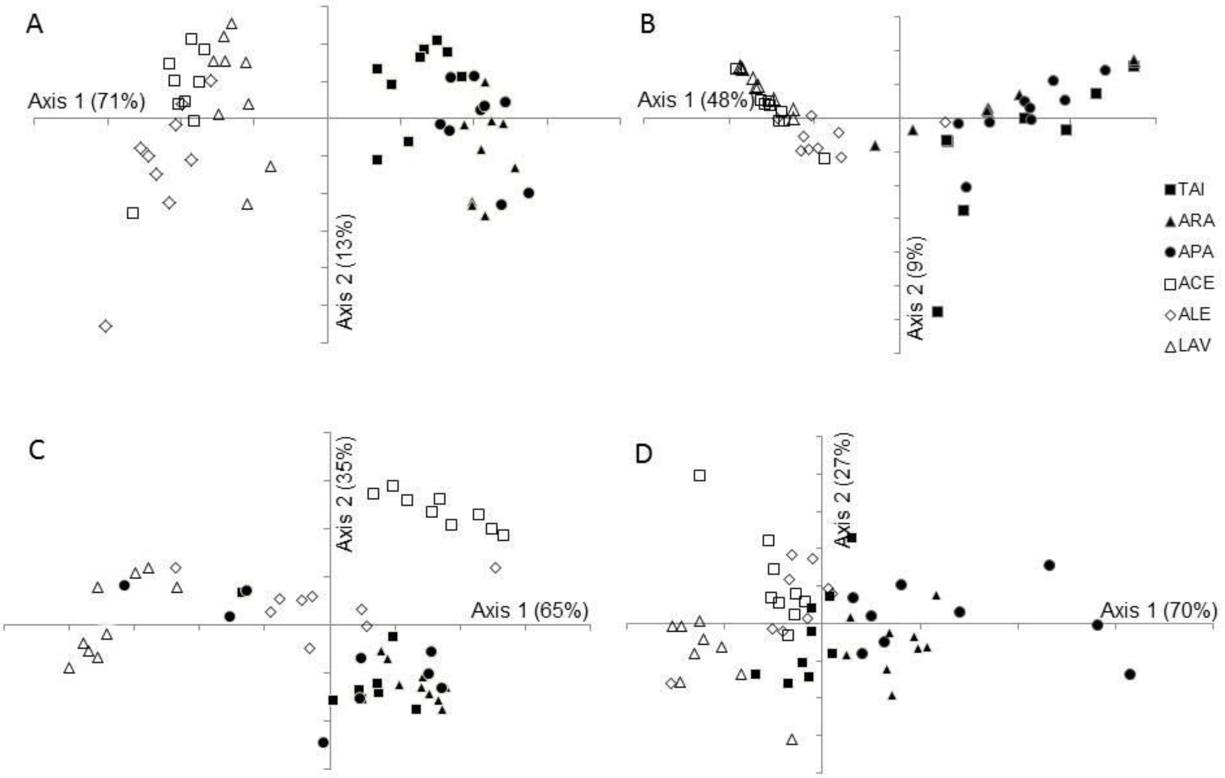

Fig 2. PCA ordination of the sample plots (n=54) along six areas in Rio Grande do Sul of selected soil variables and vegetation data: (A) soil chemical properties, (B) vegetation patterns, (C) soil physical properties (texture), and (D) soil physical properties (hydraulic).

Ordination analysis revealed two sharp groups for soil chemical properties (Fig. 2A) and vegetation data (Fig. 2B), both related to the biome distribution.The Highland grasslands sites have more homogenous bedrock in comparison to Pampa’s. Pampa’s sites remained in only one group, even being originated from different parent materials. Textural (Fig. 2C) and hydraulic properties (Fig. 2D), however, showed a continuous distribution between the two biomes.

Discussion

Heterogeneity of soil properties

We quantified patterns and scales of soils properties variation and considerable heterogeneity (i.e. variability with spatial structure) was verified. Soil chemical properties showed higher variability at a regional scale, in comparison to a local or plot-scale, and the opposite behavior was verified for soil physical properties, supporting the first hypothesis postulated. Spatial heterogeneity in soils occurs in different scales and reflects the effect of soil forming factors on their distribution (Lavelle & Spain 2001). A particular combination of the following five factors: parent material, climate, topography, organisms and time, will give rise to a certain soil (Van Breemen & Buuerman 2002). At the regional scale we have a large effect of climate and parent material, while in a local and plot-scale, the topography importance must be highlighted. Most mineral nutrient elements are derived ultimately from soil parent materials through weathering processes (Whitehead 2000), what confers strong spatial dependency of soil chemical properties on geological and climatic features. The importance of topography gets higher in a local and plot-scale and play an important role in determining soil moisture spatial pattern, water-holding capacity and soil microclimate (Hook & Burke 2000; Western et al. 2004; Poulsen et al. 2006; Brocca et al. 2007).

Soil-plant relationship at regional,local and plot-scales

Soil chemical and physical properties variation did not differ greatly at regional scales, explaining similar proportion of vegetation variance at the regional level. At the local and plot-level surveys soil physical properties explained a higher proportion of vegetation variance, supporting the second hypothesis. The spatial scale must be considered when defining the sample design for soil-plant interaction studies, since large ranges in soil chemical properties represent large effects on plant community characteristics (Sollins 1998). Topography position and soil texture explain much of soil nutrients and vegetation structure (Hook & Burke 2000) at local scale. Soils at a particular site can acquire materials and water from sites upslope and supply them, often in a modified form, to those downslope (Lavelle & Spain 2001). The effect of topography on vegetation patterns thus can be evaluated in local scale studies (geologically and climatically similar areas), following toposequences or catenas, where hydrologic and geomorphic process generate more or less consistent patterns of soil development and biogeochemistry along hillslopes, affecting soil water availability and vegetation structure (Hook & Burke 2000).

There are evidences that this general pattern found for vegetation variability, which is scale-dependent of different soil properties can be verified not only by grasslands ecosystems, but also for tropical rain forests (see discussion of Sollins 1998 about compiled data).

Recommendations for ecological studies

> *"Plant ecologist would benefit from learning more about soil science, geomorphology and geology."*
> (Sollins 1998)

Spatial hierarchies of environmental factors, intrinsic population process and disturbance regimes operate to influence the patchiness of grassland plant species and the ecosystem process that they carry out. Any attempt to study plant and environmental (soil) interactions must take into account the spatial scale. At a wide scale, chemical and textural properties may be enough for describing the higher percentage vegetation variability, however, at a local scale, where the variation in relief increases the complexity of the system, the importance of soil physical properties may get higher.

For studies developed at only one site or at several sites over homogenous parent material and climatic condition, the soil chemical properties should not be used on their own. Soil physical properties associated with chemical properties would explain the vegetation variability more accurately in a fine-scale, because of the greater importance of relief in local level and the variation in soil moisture, what was also pointed out by Dantas & Batalha (2011). We suggest the use of hydraulic properties, and not only textural ones. At a local scale the soil chemical features would be used to characterize the study site and confirm soil classification. One exception for finding remarkable chemical properties differences within a site would be due to a degradation process that can change representative characteristics of a class of soil (e.g. fertilization, heavy metal and sulfur contamination, Chapin III et al. 1986; Salemaa et al. 2001).

Conclusions

Summary statistic showed marked differences in soil properties variation among different scales, with direct influence on vegetation patterns. In this study we further refined the application of soil physical and chemical properties in ecological studies, particularly with regard to plant ecology. The wrong choice of soil properties depending on study scale may lead us to underestimate soil influence on plant distribution patterns or not correctly answer the research question pursued. This problem can be easily solved by defining the sample design with detail appropriate for the scale.

Acknowledgments

This research was supported by CNPq grant#2009−1/558282 (PELD Campos Sulinos). The authors wish to thank Gerhard Overbeck, which make valuable comments on the earlier version of this manuscript. BOA thanks CAPES for scholarship. IB and AVI receive support from CNPq.

References

Aerts, R., and F. S. Chapin III. 1999. The Mineral Nutrition of Wild Plants Revisited: A Re-evaluation of Processes and Patterns. Pages 1-67 in A. H. Fitter, and D. G. Raffaelli, editors. Advances in Ecological Research. Academic Press.

Brocca, L., R. Morbidelli, F. Melone, and T. Moramarco. 2007. Soil moisture spatial variability in experimental areas of central Italy. Journal of Hydrology **333**:356-373.

Burke, I., W. Lauenroth, M. Vinton, P. Hook, R. Kelly, H. Epstein, M. Aguiar, M. Robles, M. Aguilera, K. Murphy, and R. Gill. 1998. Plant-Soil interactions in temperate grasslands. Pages 121-143 in N. Breemen, editor. Plant-induced soil changes: Processes and feedbacks. Springer Netherlands.

Chapin III, F. S., P. M. Vitousek, and K. Van Cleve. 1986. The nature of nutrient limitation in plant communities. American Naturalist:48-58.

Cruz Ruggiero, P., M. Batalha, V. Pivello, and S. Meirelles. 2002. Soil-vegetation relationships in cerrado (Brazilian savanna) and semideciduous forest, Southeastern Brazil. Plant Ecology **160**:1-16.

Dantas, V. d. L., and M. A. Batalha. 2011. Vegetation structure: Fine scale relationships with soil in a cerrado site. Flora - Morphology, Distribution, Functional Ecology of Plants **206**:341-346.

Embrapa, editor. 1997. Manual de métodos de análise de solos. EMBRAPA-CNPS, Rio de Janeiro.

Garten Jr, C. T., S. Kang, D. J. Brice, C. W. Schadt, and J. Zhou. 2007. Variability in soil properties at different spatial scales (1 m–1 km) in a deciduous forest ecosystem. Soil Biology and Biochemistry **39**:2621-2627.

Grigal, D., J. Bell, R. Ahrens, R. Boone, E. Keley, H. Monger, P. Sollins, G. Robertson, D. Coleman, and C. Bledsoe. 1999. Site and landscape characterization for ecological studies. Standard soil methods for long-term ecological research.:29-52.

Hillel, D. 2003. Introduction to environmental soil physics. Academic press.

Hook, P. B., and I. C. Burke. 2000. Biogeochemistry in a shortgrass landscape: Control by topography, soil texture, and microclimate. Ecology **81**:2686-2703.

Howard, E. 2012. Soil mediation in grasslands. Nature Climate Change **2**:711-712.

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.

Jeffrey, D. W. 1987. Soil-plant relationships: an ecological approach. Springer, Portland.

Lal, R. 2004. Soil Carbon Sequestration Impacts on Global Climate Change and Food Security. Science **304**:1623-1627.

Lavelle, P., and A. Spain 2001. Soil ecology. Springer, Dordrecht.

Londo, G. 1976. The decimal scale for releves of permanent quadrats. Vegetatio **33**:61-64.

Oksanen, J., R. Kindt, P. Legendre, B. O'Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, and H. Wagner. 2008. The vegan package. Page 190. Community ecology package.

Overbeck, G. E., S. C. Müller, A. Fidelis, J. Pfadenhauer, V. D. P. 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.

Overbeck, G. E., S. C. Müller, V. D. P. Pillar, and J. Pfadenhauer. 2006. Floristic composition, environmental variation and species distribution patterns in burned grassland in southern Brazil. Brazilian Journal of Biology **66**:1073-1090.

Peres-Neto, P. R., P. Legendre, S. Dray, and D. Borcard. 2006. Variation partitioning of species data matrices: Estimation and comparison of fractions. Ecology **87**:2614-2625.

Perkins, L. B., R. R. Blank, S. D. Ferguson, D. W. Johnson, W. C. Lindemann, and B. M. Rau. 2013. Quick start guide to soil methods for ecologists. Perspectives in Plant Ecology, Evolution and Systematics **15**:237-244.

Pillar, V. D. 2011. MULTIV sofware para análise multivariada, testes de aleatorização e autoreamostragem "bootstrap". Departamento de Ecologia, UFRGS, Porto Alegre.

Pillar, V. D., and L. Orloci. 1996. On randomization testing in vegetation science: Multifactor comparisons of releve groups. Journal of Vegetation Science **7**:585-592.

Poulsen, A. D., H. Tuomisto, and H. Balslev. 2006. Edaphic and Floristic Variation within a 1-ha Plot of Lowland Amazonian Rain Forest1. Biotropica **38**:468-478.

R Development Core Team. 2010. R: A language and environment for statistical computing. R Foundation for statistical computing., Vienna, Austria.

Reynolds, W., and G. Topp. 2008. Soil water desorption and imbibition: tension and pressure techniques. Soil sampling and methods of analysis. 2nd ed. CRC Press, Boca Raton, FL:981-998.

Robertson, G. P., D. C. Coleman, C. S. Bledsoe, and P. Sollins 1999. Standard soil methods for long-term ecological research. Oxford University Press.

Sala, O. E., and J. M. Paruelo. 1997. Ecosystem services in grasslands in G. C. Daily, editor. Nature`s service: Societal dependence on natural ecosystems. Island Press, Washington.

Salemaa, M., I. Vanha-Majamaa, and J. Derome. 2001. Understorey vegetation along a heavy-metal pollution gradient in SW Finland. Environmental Pollution **112**:339-350.

Schoenholtz, S. H., H. V. Miegroet, and J. A. Burger. 2000. A review of chemical and physical properties as indicators of forest soil quality: challenges and opportunities. Forest Ecology and Management **138**:335-356.

Soil Survey Staff. 2010. Keys to Soil Taxonomy. USDA-Natural Resources Conservation Service, Washington, DC.

Sollins, P. 1998. Factors influencing species composition in tropical lowland rain forest: Does soil matter? Ecology **79**:23-30.

Van Breemen, N., and P. Buuerman 2002. Soil formation. Springer.

Western, A. W., S.-L. Zhou, R. B. Grayson, T. A. McMahon, G. Blöschl, and D. J. Wilson. 2004. Spatial correlation of soil moisture in small catchments and its relationship to dominant spatial hydrological processes. Journal of Hydrology **286**:113-134.

Whitehead, D. C. 2000. Nutrient elements in grassland: soil-plant-animal relationships. CABI, Wallingford.

Wigley, B. J., C. Coetsee, A. S. Hartshorn, and W. J. Bond. 2013. What do ecologists miss by not digging deep enough? Insights and methodological guidelines for assessing soil fertility status in ecological studies. Acta Oecologica **51**:17-27.

APPENDIX A

A table presenting Pearson's correlation coefficient and *P*-values of soil chemical and physical properties.

	pH	P	K	SOC	Al	Ca	Mg	AlH	CEC	%bas	%Al	clay	silt	sand	dens	AWC	M
pH	0	0.177	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	0.034	0.195	**0.001**	**0.001**	0.275
P	-0.185	0	0.110	0.020	0.306	0.975	0.570	0.157	0.086	0.326	0.288	0.866	0.052	0.112	**0.003**	0.258	0.994
K	0.456	0.234	0	0.044	**0.006**	**0.007**	**0.001**	**0.003**	0.031	**0.001**	**0.002**	0.027	0.020	0.621	0.166	0.422	0.315
SOC	**-0.848**	0.318	-0.281	0	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	0.360	**0.013**	**0.001**	**0.001**	**0.011**
Al	**-0.894**	0.149	-0.373	**0.827**	0	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	0.112	0.046	**0.001**	**0.001**	0.066
Ca	**0.831**	0.005	0.346	-0.578	-0.739	0	**0.001**	**0.001**	**0.024**	**0.001**	**0.001**	**0.010**	0.111	0.597	**0.001**	**0.006**	0.335
Mg	**0.905**	-0.082	0.457	-0.682	**-0.803**	**0.958**	0	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	0.019	0.833	**0.001**	**0.001**	0.542
AlH	**-0.910**	0.194	-0.416	**0.925**	**0.866**	-0.677	-0.770	0	**0.001**	**0.001**	**0.001**	**0.001**	0.211	0.053	**0.001**	**0.001**	**0.003**
CEC	-0.689	0.244	-0.303	**0.864**	0.689	-0.306	-0.433	**0.905**	0	**0.001**	**0.001**	**0.001**	0.624	**0.009**	**0.001**	**0.001**	**0.001**
%bas	**0.964**	-0.139	0.482	**-0.841**	**-0.922**	**0.847**	**0.920**	**-0.915**	-0.685	0	**0.001**	**0.001**	0.033	0.212	**0.001**	**0.001**	0.124
%Al	**-0.938**	0.146	-0.422	**0.884**	**0.970**	-0.778	**-0.852**	**0.924**	0.742	**-0.965**	0	**0.001**	0.127	0.032	**0.001**	**0.001**	0.044
clay	-0.633	0.021	-0.290	0.717	0.714	-0.391	-0.462	0.673	0.641	-0.665	0.733	0	0.684	**0.001**	**0.001**	0.033	0.139
silt	0.282	-0.276	0.310	-0.131	-0.215	0.214	0.320	-0.178	-0.068	0.300	-0.216	-0.057	0	**0.001**	0.307	0.849	0.027
sand	0.169	0.211	-0.070	-0.344	-0.274	0.071	0.030	-0.279	-0.348	0.175	-0.285	-0.583	-0.778	0	0.215	0.157	**0.006**
dens	0.747	-0.362	0.190	**-0.838**	-0.692	0.473	0.579	**-0.806**	-0.775	0.700	-0.733	-0.456	0.141	0.172	0	**0.001**	0.112
AWC	-0.592	0.163	-0.115	0.700	0.588	-0.404	-0.461	0.718	0.714	-0.580	0.626	0.284	0.024	-0.198	**-0.844**	0	**0.001**
M	0.155	0.001	-0.134	-0.367	-0.252	0.133	0.093	-0.387	-0.453	0.206	-0.265	-0.209	-0.315	0.388	0.221	-0.529	0

Capítulo III

SOIL AND CLIMATIC PARAMETERS AS PREDICTORS OF GRASSLAND PLANT COMMUNITY IN SOUTH BRAZILIAN GRASSLANDS

Co-autores: Pedro Maria de Abreu Ferreira, Ilsi Iob Boldrini, Alberto Vasconcellos Inda

Abstract

Grassland structure and composition varies between biomes in Rio Grande do Sul, the southern-most state of Brazil, coincident with variation in geology and soil properties. This has resulted in the hypothesis that soil parameters may play an important role in explaining vegetation among and within biomes. There is an urgent need to determine the environmental filters that control grassland species composition to give support to restoration and conservation approaches. Thus, we aimed to determine the relative contribution of climate and soil on grassland community composition. Multivariate statistical methods were used to explore spatial patterns and driving factors of plant grassland community in Rio Grande do Sul, across two biomes: Atlantic Forest (Highland grasslands) and Pampa. Redundancy analysis (RDA) showed non-random concordance between vegetation and soil parameters. We found marked differences in the main factors that explained vegetation variance between biomes. Variation partitioning, based on partial RDA, revealed that climatic and soil factors played important roles in structuring the vegetation community by explaining together 45% of total vegetation variation. Soil and climate factors together explained the majority of the variance in plant species composition between and within biomes, in comparison to the pure factors contribution. The soil mineralogical data gave support to the discussion of the triple relationship among soil, climate and vegetation. Finally, we pointed out some abiotic characteristics which combined enhance the grassland ecosystem fragility in the face to the conversion to another land use, deserving more research attention.

Keywords**:** Plant community; Soil ecology; Plant-soil-climate interaction; Pampa grasslands; Highland grasslands.

Introduction

Grassland community composition and structure are greatly influenced by abiotic characteristics. The comprehension of factors that control vegetation patterns plays a central role in ecology (Greig-Smith, 1979). The species present in a community are the result of different mechanisms sorting them through biotic species-species interactions and environmental condition, functioning as a number of biotic and abiotic filters (Jung et al., 2010). Only species that display characteristics that allow them to persist under the specific environmental conditions at the site will occur in the plant community and only if they managed to reach the site. Likewise, physical, chemical and biogeochemical soil properties may vary in response to spatial vegetation variability (Ehrenfeld et al., 2005). The maintenance of the feedback relationship between soil-plant enhances ecosystem resilience (Chapin III et al., 2000), and can keep providing important ecosystem services such as nutrient cycling, primary production, maintenance of soil fertility and prevention of soil loss (Gibson, 2009). However, most grassland ecosystems are threatened or have already been replaced by crops or plantations (Bond and Parr, 2010) resulting in the loss of hundreds of species in addition to several ecosystem services. In the current century we are experiencing a series of adverse phenomena (Foley et al., 2005) which demand the appreciation of soil-plant relationships.

Most of the grassland plant-soil relationship studies were carried out in temperate ecosystems (e.g. Burke et al., 1998; Whitehead, 2000), but there are few studies which address the issue for the subtropics (e.g. Pillar et al., 1992; Lezama et al., 2006). In South America there are wide swaths of species-rich grasslands distributed in two biomes. Rio Grande do Sul, Brazil’s southernmost state, is divided between the Atlantic Forest (Highland grasslands) and Pampa biomes. The former is mostly a forest biome, in opposition to the Pampa biome, which features one of the largest continuous grasslands in South America, extending from Rio Grande do Sul into Argentina and Uruguay (Soriano et al., 1992; Bilenca and Miñarro, 2004). Geological (Holz and De Ros, 2000), climate and soil (Streck et al., 2008) patterns have been previously studied for Rio Grande do Sul, but the main factors that explain vegetation variability have not been determined so far. We hypothesize that the climate and soil factors are the main parameters explaining vegetation variance between biomes, while at within biome level, the higher geological variability in the Pampa biome may result in a higher contribution of soil properties in comparison to climatic ones. We also expect that different filters act among and within biomes. The lack of knowledge on the complexity of interactions of biotic and abiotic components makes it difficult (or impossible) to offer alternative management strategies as well as restoration techniques to supply the current necessity.

In this paper, we investigate how environment relates to the compositional variance in subtropical grasslands at within- and between-biome scales. To better understand the controls on grassland vegetation patterns, our objectives were: (i) determine the relative importance of climate and soil factors on plant species composition; (ii) identify the main climate and soil factors that explain grassland community variation among and within biomes; and (iii) to discuss the relationship between plant, soil, and climate, as well as the implications for grassland conservation and management.

Material and Methods

Sample design and sites description

This study is part of a long-term ecological research project (PELD Campos Sulinos; CNPq 558282/2009−1). Data here evaluated correspond to the first year survey of the project (2010/ 2011). The field work was carried out in six areas in Rio Grande do Sul (RS): São Francisco de Paula municipality (Aparados da Serra National Park, APA), Cambará do Sul municipality (Aratinga Ecological Station, ARA), Jaquirana municipality (Tainhas State Park, TAI), Aceguá municipality (ACE), Alegrete municipality (ALE), Lavras do Sul municipality (LAV) (Fig. 1, Table 1). The former three sites are conservation units, and the latter are private properties. Grasslands at all sites are under cattle grazing for at least 50 years, but probably much more. These sites are distributed in two biomes: Atlantic Forest in the northern portion of the state (Highland grasslands) and Pampa, in the southern. In the Pampa biome, the vegetation matrix is dominated by grasslands, while the Highland grasslands are found among a forest matrix. The basic sampling design was an experimental plot per site. The experimental plot (70 x 210 m) was divided into three subplots of 70 x 70 m each. Nine 1m^2 permanent sampling units were systematically arranged in the subplot in a 3x3 grid, with 17 m between them).

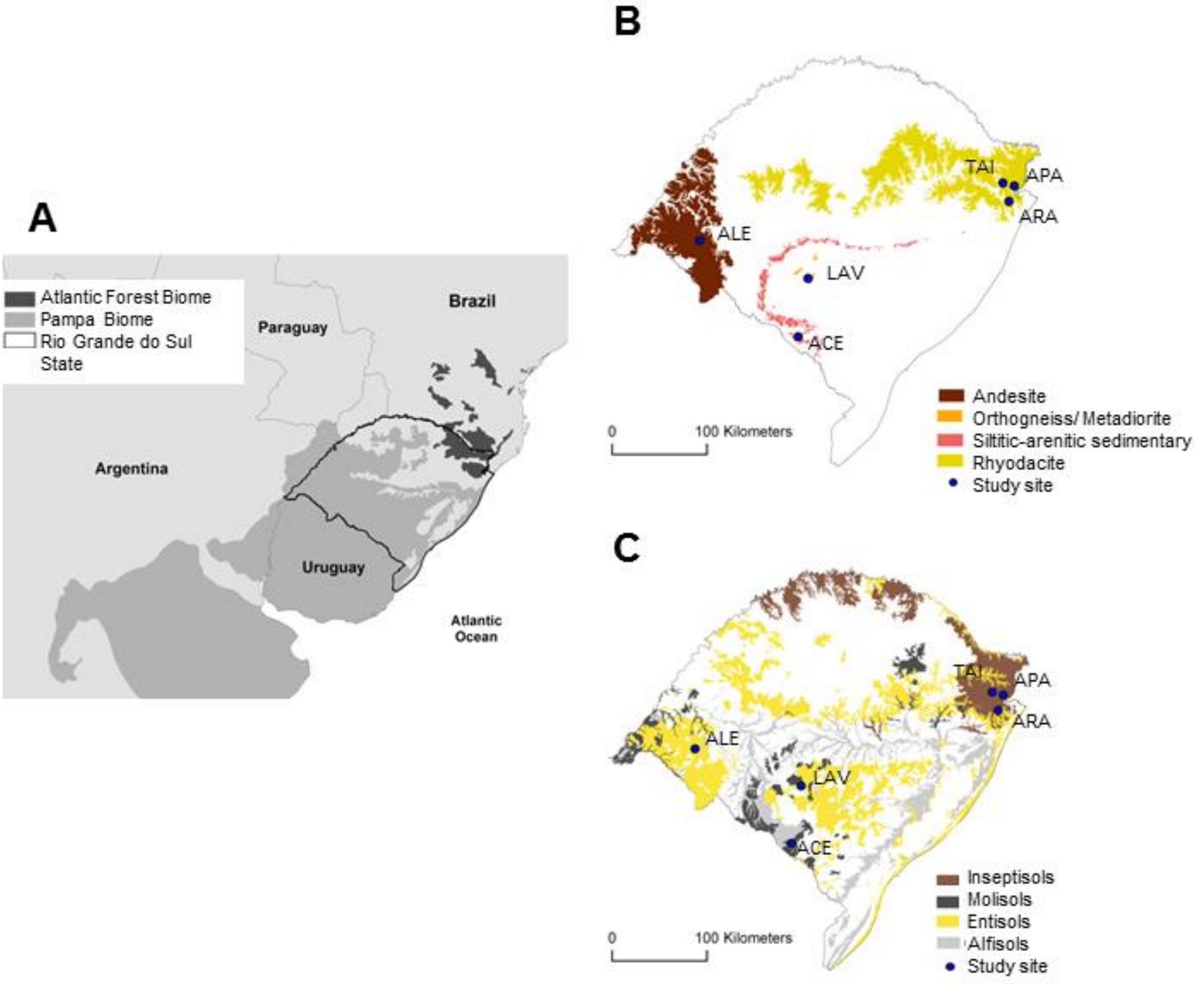

Fig. 1. Spatial distribution of sampling sites in South Brazilian grasslands according to: (A) the classification of vegetation into biomes; (B) geology (CPRM/SGB, 2008) and (C) soil occurrence (IBGE, 2002).

The sites in Highland grasslands, located at higher elevations than in Pampa biome (Table 1), have a higher annual mean precipitation (TAI: 1.931 mm, ARA: 2.222 mm and APA: 1.898 mm) and lower annual mean temperature (TAI: 15.7 ^{o}C, ARA: 15.1 ^{o}C and APA: 15.2 ^{o}C), showing a smaller annual temperature range than in Pampa biome. In Pampa biome the mean annual precipitation is 1.152, 1.507 and 1.449 mm, respectively in ACE, ALE, LAV, while the mean temperature is 17.9, 18.6 and 17.9 ^{o}C in ACE, ALE and LAV, respectively.

Table 1. Study sites and geographic location in Rio Grande do Sul state, Brazil. Management and brief floristic information are also shown.

Site	Coordinates	Altitude (m a.s.l.)	Parent material	Soil classification	Dominant species
			Highland grasslands		
APA	29°08’10”S, 50°09’21”W	924	rhyodacite	Humic Dystrudept	*Andropogon lateralis, Bulbostylis* sp. and *Paspalum maculosum*
ARA	29°23’31”S, 50°14’30”W	900	rhyodacite	Humic Dystrudept	*A. lateralis, P. maculosum* and *Axonopus affinis*
TAI	29°05’40”S, 50°22’03”W	843	rhyodacite	Lithic Udorthent	*A. lateralis* and *Schizachyrium tenerum*
			Pampa biome		
ACE	31°38’55”S, 54°09’26”W	163	siltitic-arenitic sedimentary	Vertic Hapludalf	*Piptochaetium montevidense, Paspalum notatum, Rhynchospora megapotamica* and *Paspalum plicatulum*
ALE	30°04’08”S, 55°59’27”W	189	andesite	Typic Udorthent	*A. lateralis, P. montevidense* and *P. notatum*
LAV	30°41’55”S, 53°58’11”W	334	orthogneiss/ metadiorite	Chromic Hapludalf	*P. notatum, Baccharis crispa* and *A. affinis*

Vegetation and environmental data

All plant species occurring within each 1m^2 sampling unit had its percentage cover estimated using a decimal scale (similar to that of Londo, 1976). We collapsed Londo’s smaller cover intervals into two categories (up to 1% and 1 to 5% estimated cover), and the remaining categories followed a decimal scale up to 90−100% estimated cover. Vegetation height was measured in five points per sampling unit. We also estimated cover of bare soil, rock outcrops and overall vegetation cover per sampling unit. In-depth discussion concerning these grassland communities can be found in Ferreira et al. (un pub).

Soil samples were collected in each sampling unit up to 20 cm depth. Chemical and textural soil properties were determined after air drying and sieving (Embrapa, 1997). The pH value was determined in water solution (1:1). Exchangeable cations, Mg^{+2}, Ca^{+2} and Al^{+3} were extracted with KCl 1molL^{-1}. P and K were determined using the Mehlich I extraction method. Cation exchange capacity (CEC) was determined at pH 7. Soil organic carbon (SOC) was measured using the wet combustion method. We also calculated cation percent base saturation (%base) and aluminum saturation (%Al).

X-ray diffraction (XRD) patterns of fine earth fraction (Ø < 2 mm) and clay (Ø < 0.002 mm) were obtained on Bruker D2-Phaser equipped with Cu Kα radiation (30 kV and 10 mA) using 800 mg samples. The diffracted intensities were measured over a 2θ range from 2 to

70°. The identification and semi-quantitative determination of minerals were based on the intensity of reflection patterns (Brown and Brindley, 1980).

Climate information was obtained for each sampled site and compiled from 1 km^2 resolution 'WorldClim' database during 1950−2000 period (Hijmans et al., 2005). Dataset of annual mean temperature (AMT), annual temperature range (ATR), annual precipitation (AP), precipitation seasonality (PS), precipitation of the wettest quarter (PWQ) and precipitation of the driest quarter (PDQ) were used in this study.

Data Analysis

Three matrices were prepared: one containing the response variables (plant community data), and two others with explanatory variables (soil and climate features).

Redundancy analysis (RDA) was performed to explore the relationships between plant grassland community and soil and climate predictors. We used the Hellinger-transformation as recommended by Peres-Neto et al. (2006) because it produces much more accurate estimations and valid comparisons between sets of factors in explaining community structure. Following this, we ran a forward selection procedure to select the minimum set of soil and climate variables that could explain the significant amount (α=0.05) of variation in plant grassland community. Following the exclusion of the non-significant explanatory variables, we then ran two separate RDAs for all the significant soil and climate variables remaining. It was done using the R-language function "varpart", available in vegan library (Oksanen et al., 2008), which conducted the variance partitioning of the response matrix with respect to the explanatory variables matrix, based upon RDA-adjusted R^2 values.

To evaluate whether soil variables means differ between biomes, we tested for significant differences by ANOVA with randomization testing (Euclidean distance as resemblance measure).

Statistical analyses were carried out with Multiv software (Pillar, 2011) and R 3.0.2 (R Development Core Team, 2010).

Results

Summary of vegetation community

A total of 337 plant species belonging to 40 families were recorded in the 162 plots. Poaceae, Asteraceae, Cyperaceae, Rubiaceae and Fabaceae are the main families with higher species number and coverage. Species richness differed significantly among biomes, as well as among families. Among all species surveyed, 152 can be found exclusively within the sites in Highland grasslands and 116 in the Pampa grasslands sites, while 69 species (20%) are present in both biomes. PCA ordination showed a clear separation of grassland community composition between biomes. All sites were almost completely covered by vegetation. The Highland grasslands sites showed about 1 % of bare soil per square meter. In Pampa, ACE showed low bare soil coverage similar to Highland grasslands sites, while ALE and LAV showed about 6 and 4 %, respectively. The rock outcrops coverage was in average 2, 6 and 4 % per square meter in TAI, ALE and LAV, respectively; while in the other sites it was absent or lower than 1 %.

Soil characteristics

The fine earth fraction had quartz (SiO_2) as the most abundant mineral in the grassland soils evaluated, with the highest value found in LAV, with soils derived from orthogneiss metadiorite rocks. Plagioclase ($(Na,Ca)(Al,Si)_4O_8$) and orthoclase ($KAlSi_3O_8$) were also found in this fraction only in Pampa sites, in the following decreasing order of importance: LAV, ACE, ALE. The clay fraction in ARA, APA and TAI consisted of fine-grained quartz, kaolinite ($Al_2Si_2O_5(OH)_4$) and lower proportions of gibbsite ($Al(OH)_3$). The clay fraction in ACE, ALE and LAV consisted of fine-grained quartz, plagioclase, orthoclase and small proportions of kaolinite and illite/mica. An indication of low-angle X-ray reflection of mica clay mineral or another 2:1 clay mineral was found in the former sites (Figure 2).

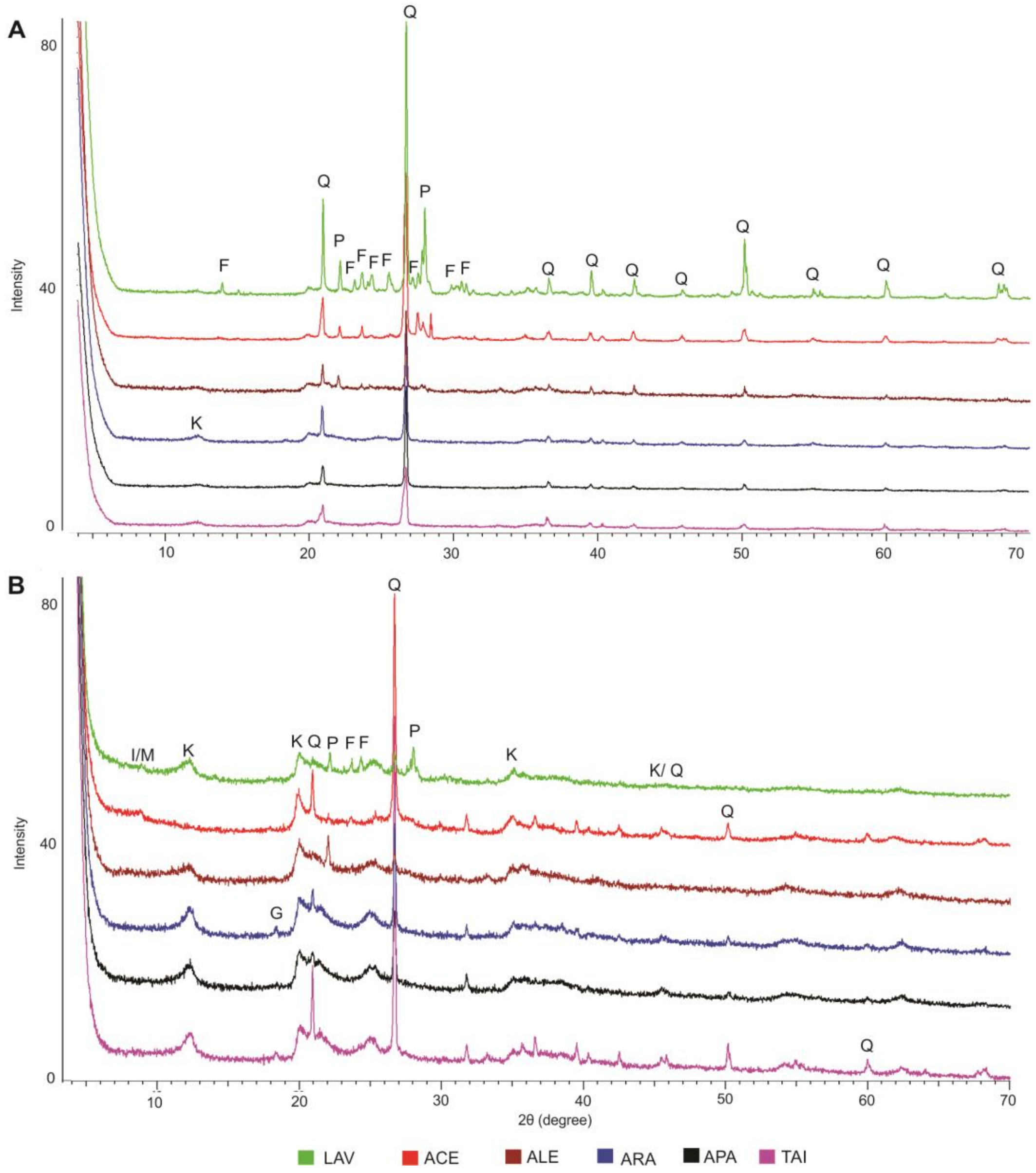

Fig. 2. X-ray diffraction pattern of fine earth (A) and clay fraction (B) of soils from six sites. Minerals detected: Q = quartz, I/M = illite/mica, G = gibbsite, K = kaolinite, F = K-feldspar (orthoclase), P= plagioclase.

Soil texture in the Highland grasslands did not vary significantly among sites, while within Pampa biome in ACE and ALE were found fine-textured soils, while in LAV, coarse particles predominate. All soil samples were found to be strongly to moderately acidic with pH values ranging from 4.1 to 4.8, and from 4.6 to 5.6 respectively in the Highland grasslands and Pampa biome. Percent aluminum saturation varied from 39.2 to 84.6 % in the Highland grasslands, while in Pampa, it varied from 0.5 to 16.4 %, with the least aluminum saturation percentage in ACE and ALE. Higher CEC and organic matter content were found in the Highland grasslands in comparison to Pampa grasslands, but lower base saturation (Table 2). A strong relationship was found between Al and SOC (R^2= 0.6286, P=0.001).

Table 2 Mean value (±SD) of soil properties in the six sites. Means with the same letter are not significantly (α=0.05) different between biomes.

	Highland grasslands			**Pampa biome**		
	TAI	**ARA**	**APA**	**ACE**	**ALE**	**LAV**
clay (%)	33.8±5^{a}	36.6±2.5^{a}	30.6±8^{a}	20.2±3.1^{b}	24.9±3.9^{b}	18.7±2.9^{b}
silt (%)	40.3±2.6^{a}	43.6±0.8^{a}	40.1±5.2^{a}	61.7±1.7^{b}	44.7±6.1^{b}	29.3±5.6^{b}
sand (%)	26±5.1^{a}	19.8±2.4^{a}	29.4±10.9^{a}	18.1±4.6^{b}	30.4±8.7^{b}	52±3.4^{b}
pH (H_2O)	4.5±0.1^{a}	4.2±0.1^{a}	4.3±0.1^{a}	5.1±0.1^{b}	5.2±0.1^{b}	4.9±0.1^{b}
P (mg/dm^3)	4.3±0.7^{a}	5±1^{a}	4.9±1.2^{a}	4.0±0.7^{a}	5.0±1.1^{a}	4.7±1^{a}
K (mg/dm^3)	113.4±54.9^{a}	119.9±23.3^{a}	115.2±39.3^{a}	187.3±58.5^{b}	181.5±73^{b}	121.6±41.4^{b}
SOC (%)	7.1±1.4^{a}	10±<0.1^{a}	9.6±0.6^{a}	4.3±0.6^{b}	5.4±1^{b}	4.1±0.7^{b}
Al troc. (cmol$_c$/dm^3)	4.9±1.1^{a}	4.4±0.3^{a}	3.8±0.7^{a}	0.3±0.1^{b}	0.2±0.1^{b}	0.5±0.2^{b}
Ca troc. (cmol$_c$/dm^3)	1.4±0.6^{a}	1±0.2^{a}	1±0.3^{a}	5.9±1.6^{b}	10.7±2.7^{b}	4.2±1^{b}
Mg troc. (cmol$_c$/dm^3)	0.8±0.3^{a}	0.7±0.1^{a}	0.6±0.2^{a}	2.7±0.5^{b}	3.7±0.6^{b}	1.7±0.4^{b}
Al+H (cmol$_c$/dm^3)	20.4±4.0^{a}	31.5±3.2^{a}	30.7±4.4^{a}	6±1.8^{b}	8.6±1.7^{b}	7.5±1.6^{b}
CEC (cmol$_c$/dm^3)	22.9±3.6^{a}	33.4±3.1^{a}	32.6±4.6^{a}	15.3±2.9^{b}	23.6±3.1^{b}	13.8±2^{b}
%bases	11.4±5.5^{a}	5.9±1.3^{a}	5.5±1.3^{a}	60.6±6.5^{b}	62.7±7.1^{b}	45.8±6.7^{b}
%Al	66.6±10.6^{a}	69.8±5.3^{a}	67.9±5.1^{a}	2.8±1^{b}	1.7±1^{b}	7.8±2.9^{b}

Climate and soil factors driving grassland community

Considering both biomes evaluated, the soil properties that most likely influenced species composition in grasslands are, in decreasing order of importance: percent aluminum saturation, silt, Mg^{+2}, [Al^{+3}+H^{+}], clay, pH, Al^{+3}, percent base saturation, CEC and soil organic carbon (SOC). While the climate characteristics are: annual precipitation (AP), precipitation seasonality (PS), annual mean temperature (AMT), annual temperature range (ATR), and precipitation of driest quarter (PDQ) (Table 3). In the Highland grasslands sites, the main soil properties that govern vegetation patterns are CEC, sand content, Al^{+3}, pH, clay content, SOC, percent base saturation, and the climatic conditions are AMT and PDQ. In Pampa grasslands, the main soil chemical and textural features are silt, CEC, pH and [Al^{+3}+H^{+}] and the climate conditions are ATR and PDQ.

Variation partitioning

The RDA analysis revealed a non-random concordance between vegetation and climate as well as between vegetation and soil parameters. The partial RDA revealed that the soil and climatic factors combined explained 45 % of the total variation in the grassland community considering the two biomes, and 20 and 29 % for the Highland grasslands and Pampa grasslands, respectively. The amount of variation explained by the pure climate factors (6 % for the two biomes, 2 % for Pampa biome, 3 % for Highland grasslands) was higher than the pure soil factors (2 % for the two biomes, 5 % for Pampa biome, 5 % for Highland grasslands). As expected, most of the vegetation variance (37 %) was explained by the correlation between soil and climate parameters for the two biomes, and the same pattern was found when analyzing each biome separately: 27 % for Pampa biome and 16% for the Highland grasslands.

Table 3. Importance of explanatory variables in RDA models for plant species composition between and within biomes. AMT= annual mean temperature, ATR= annual temperature range, AP= annual precipitation, PS= precipitation seasonality, PDQ= precipitation of driest quarter.

	Soil model			**Climate model**		
	Variable	**P**	**R^2**	**Variable**	**P**	**R^2**
Between biomes	%Al	0.001	0.250	AP	0.001	0.246
	silt	0.001	0.050	PS	0.001	0.073
	Mg^{+2}	0.001	0.043	AMT	0.001	0.066
	Al+H	0.001	0.024	ATR	0.001	0.034
	clay	0.001	0.013	PDQ	0.001	0.021
	pH	0.001	0.012			
	Al^{+3}	0.002	0.008			
	%base	0.004	0.007			
	CEC	0.009	0.006			
	SOC	0.022	0.006			
Within biomes						
Highland grasslands	SOC	0.001	0.077	AMT	0.001	0.112
	sand	0.001	0.039	PDQ	0.001	0.072
	pH	0.001	0.026			
	Al^{+3}	0.001	0.036			
	clay	0.010	0.019			
	K	0.021	0.017			
	P	0.039	0.016			
Pampa	silt	0.001	0.126	ATR	0.001	0.159
	CEC	0.001	0.107	PDQ	0.001	0.132
	pH	0.001	0.029			
	sand	0.040	0.014			
	Al	0.039	0.014			

Table 4. Partial redundancy analysis (pRDA) to determine how much of variation in vegetation community composition is explained by statistically significant variables. Different sample scales were evaluated.

Environmental properties	Between biomes	Within biomes: Highland grasslands	Within biomes: Pampa biome
Soil (S)	0.39***	0.14***	0.24***
[S+(C∩S)]	Silt, Clay, pH, SOC, Al, Mg, Al+H, CEC, %base, %Al	Clay, Sand, pH, C, Al, CEC, % base	Silt, pH, Al+H, CEC
Soil joint Climate (C∩S)	0.37	0.11	0.22
Climate (C)	0.43***	0.16***	0.27***
[C+(C∩S)]	AMT, ATR, AP, PS, PDQ	AMT, PDQ	ATR, PDQ
Total (C∪S)	0.45***	0.20***	0.29***

*P<0.05; **P<0.01; ***P<0.001

Discussion

We found a close relationship between biotic and abiotic parameters and marked differences in the main factors that explain vegetation variance among biomes. Variance partitioning showed that different factors act driving the vegetation patterns according to the scale adopted, and the majority of vegetation variance was explained by both climate and soil factors regardless of the observation scale. Soil mineralogical analysis confirmed the triple relationship between climate, soil properties and vegetation patterns.

Variation partitioning

In this study, we used variation partitioning to unravel the relative contribution of the environmental drivers of vegetation diversity. The combined effect of climate and soil factors clearly explained a large percentage of vegetation community variation (Table 3), since climate affects virtually all soil properties at scales ranging from local to global (Chapin III et al., 2011). Whereas the geological substrate influence on soil and vegetation is frequently clear, it is less easy to separate the effects of climate on soil properties from the direct effect of climate on vegetation dynamics or other aspects of ecosystem function (Jeffrey, 1987).

The proportion of the total variation in species composition accounted for by the explanatory variables, as well as the importance of explanatory variables, varied among biomes. Within biomes, even with more similar climatic regime among sites, the combination of climatic and soil factors still was a major factor governing grassland community composition. With decreasing scale, it becomes more difficult to identify correlated environment differences to vegetation patterns (Greig-Smith, 1979), which justifies the lower percentage of vegetation variance explained by environmental factors within biomes. Biotic interaction and management at small scale can probably explain an important fraction of vegetation heterogeneity (Adler et al., 2001).

Soil-climate-plant relationship

The soil mineralogical data gave support to the discussion of the triple relationship among soil, climate and vegetation. Clay mineralogy, the types and amounts of clay in the soil are dependent on its parent material and weathering history (Lavelle and Spain, 2001), and it is the prime determinant of a soil's chemical and physical properties (Sollins, 1998). Soil mineralogical data revealed that, in the Highland grasslands sites, rhyodacite parent material give rise to soils with secondary minerals, mostly kaolinite and a small amount of gibbsite, while in Pampa sites, soils that originated from siltitic-arenitic sedimentary, andesite and orthogneiss/ metadiorite parent materials show silicate minerals from the feldspar mineral group (orthoclase and plagioclase) and a small content of kaolinite and 2:1 clay minerals. The differences found resulted from the intense chemical weathering powered by the high annual precipitation in the Highland grasslands sites, while in Pampa are still found primary minerals, which highlight that the weathering process was not as intense as in the former. High quartz content was found in all soils sampled, and that is in accordance with the parent materials and their resistance to weathering (Jackson and Sherman, 1953).

Annual precipitation, which was found to be the main climatic variable acting as a filter of vegetation composition (Table 3), is also one of the main agents of weathering (Schaetzl and Anderson, 2005). In the Highland grasslands sites, the high annual precipitation and low evaporation is related to its higher altitude in comparison with Pampa sites. The soil thickness in the Highland grasslands sites, especially in TAI, is determined by the undulating to rolling relief which results in a higher soil erosion rate in opposition to the parent material weathering. The secondary minerals found in the Highland grasslands sites result from weathering of the primary minerals like orthoclases present in the rock.

In the Highland grasslands sites the higher proportion of positively charged ions are acidic cations H^+ and Al^{+3}, while in Pampa biome basic cations (Ca^{+2}, Mg^{+2}, K^+, Na^+) predominate. The P content showed no significant differences among sites. The lack of nutrients in the Highland grasslands former soils is attributed to low status of parent material and leaching losses from soil. Soil acidification can develop naturally when basic cations are leached from soils and the Al^{+3}, due to its low mobility, is retained in soil particles and SOC

(Berggren and Mulder, 1995), contributing to the lower pH in Highland grasslands sites, in opposition to Pampa sites.

The high air moisture and low temperature favor soil organic carbon (SOC) accumulation in the Highland grasslands sites (Streck et al., 2008). The SOM has also an important role in controlling aluminum solubility in strongly acidic soils (Berggren and Mulder, 1995; Schwesig et al., 2003; Silva et al., 2008). The percent aluminum saturation was found as the main soil factor acting as a filter in vegetation composition (Table 3). Elevated soil aluminum levels require highly specialized physiological adaptations of plants, and many evolve the capacity of cation hyperaccumulation (Ehrenfeld et al., 2005). Such soils were shown to impair root elongation and to interfere with the uptake, distribution and use of nutrients (Lambers et al., 2008). However, as highlighted by Haridasan (2008) which studied the interaction of Al with Brazilian *cerrado* plant species, high Al content is currently considered a limiting factor for crop production while not visibly affecting the structure of natural vegetation. Using the vegetation coverage associated to the mean vegetation height per plot as a proxy for productivity, we may confirm this prediction. However, it is noteworthy that the high percentage of Al saturation, in association to the relative influence of different floristic contingencies (Cabrera and Willink, 1980; Boldrini and Longhi-Wagner, 2011), are probably the main factors that explain the low percentage of common species between biomes. On the other hand, this soil property may be an important filter to prevent alien species establishment, since about 20 alien species was found in the Highland grasslands grasslands by Koch (un pub.) versus 153 alien species in the Pampa grasslands (Fonseca et al., 2013). It is currently known that environments with low soil nutrient availability and species community with attributes similar to potential invaders are less susceptible to invasion (Cleland et al., 2013).

In the Pampa biome, differences in soil texture were higher in comparison to soil chemical properties (Table 2). Soils such as rhyodacite (found in TAI, ARA, APA) are more acidic than those derived from andesite (found in ALE). Higher values of K^+ in ACE and ALE are related to common primary material like feldspars. The presence of 2:1 clay minerals in Pampa sites does not lead to a greater CEC in Pampa soil sites, in comparison to the Highland grasslands sites. The 2:1 clay minerals can show a higher amount of charged sites in comparison to 1:1 clay minerals (kaolinite) and aluminum hydroxides as gibbsite (Sposito, 2008), however, in the Highland grasslands sites, the high amount of SOM found in Highland grasslands exacerbates the properties shown by 2:1 clay minerals. Even with similar CEC values between biomes, Pampa sites have a higher ability to absorb and retain nutrients, as indicated by the higher percentage of base saturation and result in a non-aluminum related cation exchange capacity.

Implications for conservation and grassland management

The subtropical species-rich grasslands have been converted to other land use at an alarming rate in the past decades. Before this change occurs, soil-plant relationship must be considered; not only the soil conditions that benefit crop or tree plantation, but also the system’s degree of fragility and the losses and gains of ecosystem services would result. This could, in turn, avoid a cycle of grassland conversion and abandonment, since grassland restoration requires complex techniques that are currently not mastered for subtropical species-rich grasslands (Andrade et al. un pub.: **capítulo IV**).

The maintenance of livestock production is undoubtedly the key point for the maintenance of these species-rich grasslands (Nabinger et al., 2000; Carvalho et al., 2006) as well as to provide important ecosystem services. The maintenance of vegetation coverage is important in preventing losses in SOC, soil compaction and accelerated erosion. It is of high importance especially for soils with higher natural fragility, e.g. LAV site. The vegetation cover dampens the extremes of soil temperature variation: soils beneath a layer of vegetation are both cooler in summer and warmer in winter. It is especially important in the Pampa biome, since temperature extremes restrain plant growth and establishment.

The knowledge acquired in this study can also give support to ongoing public policies in the Highland grasslands (e.g. silvicultural zoning plan in Rio Grande do Sul) and new ones in the Pampa, which aim to avoid the conversion of high ecological vulnerability sites to pine or crop plantations, the main threats to South Brazilian grasslands. In cases where degradation has already happened, restoration strategies must also be well planned, controlling the traffic of genetic material between biomes, since apparently some genetic features may confer species adaptations to high aluminum saturation in the Highland grasslands.

Conclusion

The present research found strong evidence of soil and climate properties being the main factors causing variation in grassland vegetation between sites of the two biomes in South Brazilian grasslands. Aluminum saturation percentage was one of the most important factors influencing vegetation patterns, and our findings suggest that it may explain the low percentage of common species and low establishment of alien species, with effects on the management and restoration. Different soil-climate-plant relationships in a regional scale justify different management strategies, public policies, and restoration strategies between biomes.

Acknowledgments

This research was supported by CNPq grant#2009−1/558282 (PELD Campos Sulinos). BOA and PMAF thank CAPES for scholarships. IB and AVI receive support from CNPq.

References

Adler, P., Raff, D., Lauenroth, W., 2001. The effect of grazing on the spatial heterogeneity of vegetation. Oecologia 128(4), 465-479.

Berggren, D., Mulder, J., 1995. The role of organic matter in controlling aluminum solubility in acidic mineral soil horizons. Geochimica et Cosmochimica Acta 59(20), 4167-4180.

Bilenca, D., Miñarro, F., 2004. Identificación de areas valiosas de pastizal em las pampas y campos de Argentina, Uruguay y sur de Brasil. Fundación vida silvestre argentina, Buenos Aires.

Boldrini, I.I., Longhi-Wagner, H.M., 2011. Poaceae no Rio Grande do Sul: diversidade, importância na fisionomia e conservação. Ciência e Ambiente 42, 71-92.

Bond, W.J., Parr, C.L., 2010. Beyond the forest edge: Ecology, diversity and conservation of the grassy biomes. Biological Conservation 143(10), 2395-2404.

Brown, G., Brindley, G., 1980. X-ray diffraction procedures for clay mineral identification. Crystal structures of clay minerals and their X-ray identification 5, 305-359.

Burke, I., Lauenroth, W., Vinton, M., Hook, P., Kelly, R., Epstein, H., Aguiar, M., Robles, M., Aguilera, M., Murphy, K., Gill, R., 1998. Plant-Soil interactions in temperate grasslands. In: N. Breemen (Ed.), Plant-induced soil changes: Processes and feedbacks. Developments in Biogeochemistry. Springer Netherlands, pp. 121-143.

Cabrera, A.L., Willink, A., 1980. Biogeografia de America Latina. OEA, Washington.

Carvalho, P.C.d.F., Fisher, V., Santos, D.T.d., Ribeiro, A.M.L., Quadros, F.L.F.d., Castilhos, Z.M.S., Poli, C.H.E.C., Monteiro, A.L.G., Nabinger, C., Genro, T.C.M., Jacques, A.V.A., 2006. Produção Animal no Bioma Campos Sulinos. Brazilian Journal of Animal Science 35(n. Supl. Esp.), 156-202.

Chapin III, F.S., Chapin, M.C., Matson, P.A., Vitousek, P., 2011. Principles of terrestrial ecosystem ecology. Springer.

Chapin III, F.S., Zavaleta, E.S., Eviner, V.T., Naylor, R.L., Vitousek, P.M., Reynolds, H.L., Hooper, D.U., Lavorel, S., Sala, O.E., Hobbie, S.E., Mack, M.C., Diaz, S., 2000. Consequences of changing biodiversity. Nature 405(6783), 234-242.

Cleland, E.E., Larios, L., Suding, K.N., 2013. Strengthening Invasion Filters to Reassemble Native Plant Communities: Soil Resources and Phenological Overlap. Restoration Ecology 21(3), 390-398.

CPRM/SGB, C.D.P.D.R.M.S.G.D.B., 2008. Mapa geológico do Estado do Rio Grande do Sul. CPRM-SGB, Porto Alegre, pp. 1:750.000.

Ehrenfeld, J.G., Ravit, B., Elgersma, K., 2005. Feedback in the plant-soil system, 30. Annual Reviews, Palo Alto, CA, ETATS-UNIS.

Embrapa (Ed.), 1997. Manual de métodos de análise de solos. Documentos, 1. EMBRAPA-CNPS, Rio de Janeiro, 212 pp.

Foley, J.A., DeFries, R., Asner, G.P., Barford, C., Bonan, G., Carpenter, S.R., Chapin, F.S., Coe, M.T., Daily, G.C., Gibbs, H.K., Helkowski, J.H., Holloway, T., Howard, E.A., Kucharik, C.J., Monfreda, C., Patz, J.A., Prentice, I.C., Ramankutty, N., Snyder, P.K., 2005. Global Consequences of Land Use. Science 309(5734), 570-574.

Fonseca, C., Guadagnin, D., Emer, C., Masciadri, S., Germain, P., Zalba, S., 2013. Invasive alien plants in the Pampas grasslands: a tri-national cooperation challenge. Biological Invasions 15(8), 1751-1763.

Gibson, D.J., 2009. Grasses and grassland ecology. Oxford, New York.

Greig-Smith, P., 1979. Pattern in vegetation. The Journal of Ecology, 755-779.

Haridasan, M., 2008. Nutritional adaptations of native plants of the cerrado biome in acid soils. Brazilian Journal of Plant Physiology 20, 183-195.

Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G., Jarvis, A., 2005. Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology 25(15), 1965-1978.

Holz, M., De Ros, L.F., 2000. Geologia do Rio Grande do Sul. CIGO/ UFRGS, Porto Alegre.

IBGE, 2002. Mapa exploratório de solos do Rio Grande do Sul. IBGE/DGC, Rio de Janeiro, pp. 1:1.000.000.

Jackson, M., Sherman, G.D., 1953. Chemical weathering of minerals in soils. Adv. Agron 5(22), 317.

Jeffrey, D.W., 1987. Soil-plant relationships: an ecological approach. Springer, Portland.

Jung, V., Violle, C., Mondy, C., Hoffmann, L., Muller, S., 2010. Intraspecific variability and trait-based community assembly. Journal of Ecology 98(5), 1134-1140.

Lambers, H., Chapin III, F.S., Pons, T.L., 2008. Plant Physiological Ecology Springer, New York.

Lavelle, P., Spain, A., 2001. Soil ecology. Springer, Dordrecht.

Lezama, F., Altesor, A., León, R.J., Paruelo, J.M., 2006. Heterogeneidad de la vegetación en pastizales naturales de la región basáltica de Uruguay. Ecología austral 16, 167-182.

Londo, G., 1976. The decimal scale for releves of permanent quadrats. Vegetatio 33, 61-64.

Nabinger, C., Moraes, A., Marschin, G.E., 2000. Campos in Southern Brazil. In: G. Lemaire, J.G. Hodgson, A. Moraes, C. Nabinger, P.C.F. Carvalho (Eds.), Grassland Ecophysiology and Grazing Ecology. CABI, Wallingford, pp. 355-376.

Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Wagner, H., 2008. The vegan package, Community ecology package, pp. 190.

Peres-Neto, P.R., Legendre, P., Dray, S., Borcard, D., 2006. Variation partitioning of species data matrices: Estimation and comparison of fractions. Ecology 87(10), 2614-2625.

Pillar, V.D., 2011. MULTIV sofware para análise multivariada, testes de aleatorização e autoreamostragem “bootstrap". Departamento de Ecologia, UFRGS, Porto Alegre.

Pillar, V.D., Jacques, A.V.A., Boldrini, II, 1992. Environmental-factors related to the vegetational variation of a natural grassland. Pesquisa Agropecuaria Brasileira 27(8), 1089-1101.

R Development Core Team, 2010. R: A language and environment for statistical computing. R Foundation for statistical computing., Vienna, Austria.

Schaetzl, R.J., Anderson, S., 2005. Soils: Genesis and geomorphology. Cambridge University Press, New York.

Schwesig, D., Kalbitz, K., Matzner, E., 2003. Effects of aluminium on the mineralization of dissolved organic carbon derived from forest floors. European Journal of Soil Science 54(2), 311-322.

Silva, L.B., Dick, D.P., Inda, A.V., 2008. Solos subtropicais de altitude: Características químicas, teor de matéria orgânica e sua resistência à oxidação química. Ciência Rural 38, 1167-1171.

Sollins, P., 1998. Factors influencing species composition in tropical lowland rain forest: Does soil matter? Ecology 79(1), 23-30.

Soriano, A., León, R.J.C., Sala, O.E., Lavado, R.S., Deregibus, V.A., Cahuepé, O., Scaglia, A., Velazquez, C.A., Lemcoff, J.H., 1992. Río de la Plata grasslands. In: R.T. Coupland (Ed.), Natural Grasslands. Introduction and Western Hemisphere. Ecosystems of the World. Elsevier, Amsterdam, pp. 367-407.

Sposito, G., 2008. The chemistry of soils. Oxford university press.

Streck, E.V., Kämpf, N., Dalmolin, R.S.D., Klamt, E., Nascimento, P.C.d., Schneider, P., Giasson, E., Pinto, L.F.S., 2008. Solos do Rio Grande do Sul. 2nd ed. EMATER/RS-ASCAR, Porto Alegre.

Whitehead, D.C., 2000. Nutrient elements in grassland: soil-plant-animal relationships. CABI, Wallingford.

Capítulo IV

GRASSLAND DEGRADATION AND RESTORABILITY:
A CONCEPTUAL MODEL APPLIED TO SOUTH BRAZILIAN GRASSLANDS

Co-autores: Christiane Koch, Ilsi I. Boldrini, Eduardo Vélez-Martin, Heinrich Hasenack, Julia-Maria Hermann, Johannes Kollmann, Valério de P. Pillar, Gerhard E. Overbeck

* Artigo submetido para publicação em *Land Degradation and Development*

Abstract

Land degradation is a complex concept that integrates different aspects, including changes in soil conditions, biodiversity, productivity and socio-economic implications, compared to a reference state. We propose a new conceptual model to analyze degradation stages and thresholds in species-rich natural grasslands. The model is displayed as biotic and abiotic changes along two axes, which can be also interpreted in terms of ecosystem functions and services. The model integrates different degradation stages with their respective thresholds and describes key processes of land use change that lead to certain stages or the crossing of a threshold. We applied the model to South Brazilian grasslands, which are rich in biodiversity and suffer from a series of degradation processes. We discuss two scenarios of grassland degradation: unsuitable grassland management and complete change of land use. The conceptual model serves as a general framework to study degradation and restorability of tropical and subtropical grasslands after changes in management or transition to other land use; it will facilitate decisions on alternative management and conservation.

Keywords: Afforestation; Conservation; Grazing; Land use change; Restoration; Thresholds.

Introduction

Land degradation is a broad and complex concept that integrates different aspects, including changes in soil conditions, biodiversity, productivity and socio-economic implications compared to a reference stage (Stocking & Murnaghan 2001; Reed *et al.* 2011). Land degradation can be analyzed at different spatial scales, from a local focus on specific degradation processes to regional and biome scales, using various methods, including remote sensing, plot-based measurements, experiments, expert knowledge and assessment of stakeholder experience (Reed *et al.* 2011). The combination of abiotic factors with biotic measures allows a qualitative and quantitative assessment of the degree of degradation in comparison to non-degraded areas.

To provide a better understanding of the dynamics of degraded ecosystems and to facilitate mitigation of degradation processes and restoration, the concept of thresholds between alternative stages, i.e. original ('un-degraded') and altered ('degraded') stages, has been proposed (Suding *et al.* 2004). While the framework developed by Briske *et al*. (2006) focused on definition and description of thresholds, Hobbs *et al.* (2009) deal with stages between them ('historical', 'hybrid', 'novel'). A synthesis of these approaches is missing so far.

Models of degradation have been applied to rangeland systems (e.g. Bestelmeyer 2006). Grasslands are among the ecosystems with highest species richness in the world (Wilson *et al.* 2012) and provide a wide range of ecosystem services. Grasslands play an important role for the global carbon cycle, as 90% of their biomass is belowground, accumulation is high and decomposition of organic material slow (Gibson 2009). As main forage resource for livestock, grasslands are important for human wellbeing. They contribute to infiltration of water into the soil and thus to the maintenance of hydrological cycles. Finally, grasslands contribute to scenic beauty of many landscapes around the world. At the same time, large areas of grasslands are endangered due to land use changes or have already been lost (Sala *et al*. 2000), especially in tropical and subtropical regions (Bond & Parr 2010). A better understanding of land degradation is necessary to revert these trends and to allow for restoration of degraded areas.

In this paper, we present a conceptual model of grassland degradation that for the first time integrates different degradation stages with the respective thresholds, and includes the most important processes related to land use history that lead to stage transitions. As most grasslands are maintained by management or disturbances, such as grazing, fire or mowing, or combinations of these, we specifically integrate the management regime in the definition of the reference state that is the target for restoration, an aspect not considered in previous models. The model allows an assessment of the conservation status, supports informed decision making for improved management, and contributes to the evaluation of restoration potential for degraded grasslands. We apply the model to grasslands in Rio Grande do Sul (RS) state, southern Brazil, where biodiversity is well studied, but where conservation of grassland has been neglected and degradation processes and effects are poorly studied (Overbeck *et al*. 2007; Overbeck *et al*. 2013). We first introduce the conceptual model, illustrate it with data from the study region, and finally discuss its potential and limitations.

A new framework for grassland degradation and restoration thresholds

The novelty of our framework is to use traditionally managed grasslands as reference systems. As in Hobbs *et al*. (2009), changes of ecosystem properties can be displayed as biotic and abiotic changes at the local scale along two axes (Figure 1). We consider deviation in species composition and biomass production, resulting from altered management, species introduction or conversion of land use as biotic changes. As abiotic changes we understand alteration in soil chemical and physical properties, as caused by fertilization or soil cultivation. The reference stage is comprised by grasslands composed of native species, with a specific biodiversity and high conservation value, usually due to grazing and other management practices (e.g. fire, mowing). Changes in grassland management will cause properties of the system to change, resulting in a decrease in resilience, but with resumption of the historical management the original properties might be reached again, i.e. a self-recovery threshold is not crossed (Figure 1a, Scenario 1). If the grassland is converted to other land use, this will lead to an almost entire change of the original properties, e.g. complete loss of the aboveground plant community (Figure 1b, Scenario 2). Converted areas will have reduced resilience and active restoration will be necessary to re-establish a system similar to the original one, e.g. after abandonment of the new land use (Figure 1c). In case of very strong changes of the abiotic characteristics, restoration may not be possible, e.g. after mining. In the following, we apply the model to grasslands of RS, based on a synthesis of knowledge of effects of different types of management and degradation.

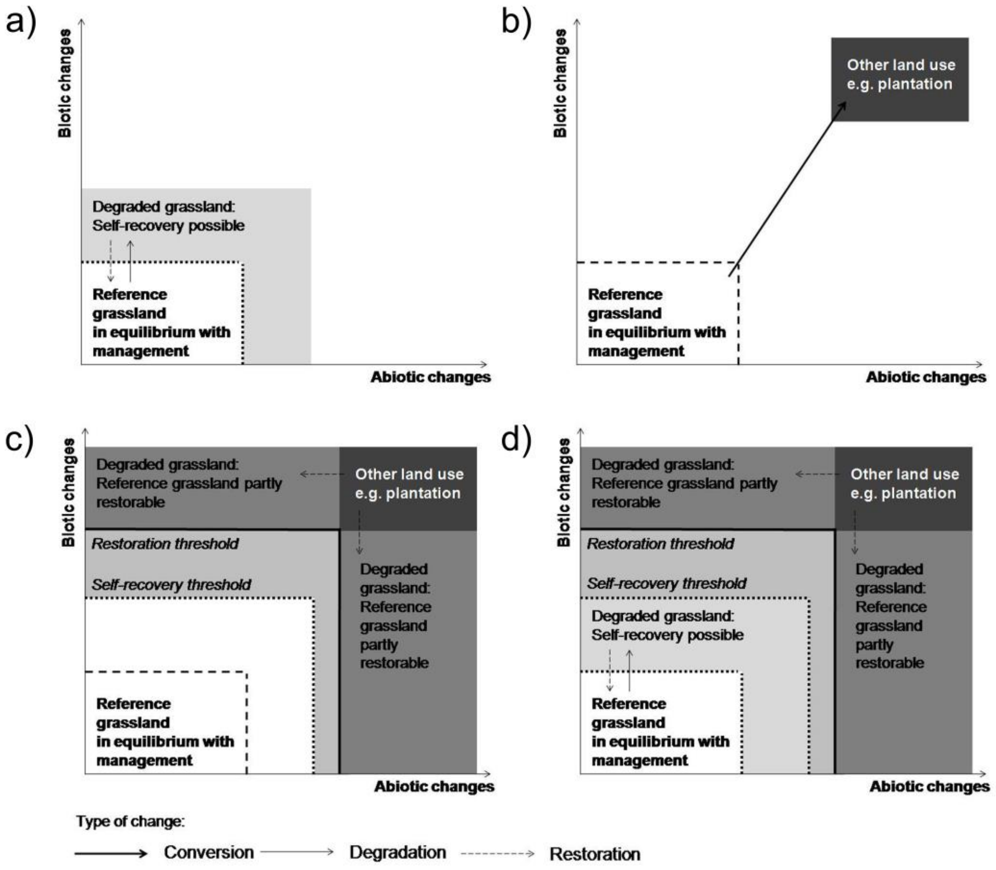

Figure 1. Conceptual model on degradation and restoration of species-rich natural grasslands: (a) Properties of the reference grassland are moderately altered if grassland management is changed, but modification is reversible. (b) After conversion to other land uses, the properties of the reference grassland are radically changed and ecosystem resilience is lost; (c) after abandonment some properties might recover ('self-recovery threshold') or be restorable ('restoration threshold'); and (d) integration of the various scenarios.

South Brazilian native grasslands: origin and development

In RS, grasslands occur in the highlands in the north, where they form mosaics with Araucaria forest, and in the south, in the Pampa biome, where they dominate the landscape (Overbeck *et al.* 2007), continuing in Uruguay and Argentina (Figure 2). South Brazilian grasslands are particularly rich in plant species, with about 2.200 grassland plant taxa known only for RS (Boldrini 2009).

In the first half of the 20th century, natural grasslands still covered 60% of RS (Cordeiro & Hasenack 2009). The grasslands are relicts from cooler and drier periods, and were affected by forest expansion since approximately 5.000 years BP, with increasing rates since 1.500 years BP (Behling 2002), favored by warmer and more humid climate. During the Holocene, after the extinction of large herbivores (Lima-Ribeiro & Felizola Diniz-Filho 2013), grasslands were maintained by anthropogenic fires and by grazing of small mammals (Cione *et al.* 2003; Behling & Pillar 2007), and since the 17th century by introduced livestock. Today, beef production is an important economic activity in the region, with native plant species constituting most grassland vegetation. Available data indicate that plant diversity and forage production reach maximum levels under intermediate levels of grazing (or fire) frequency (Overbeck *et al.* 2005; Nabinger *et al.* 2009).

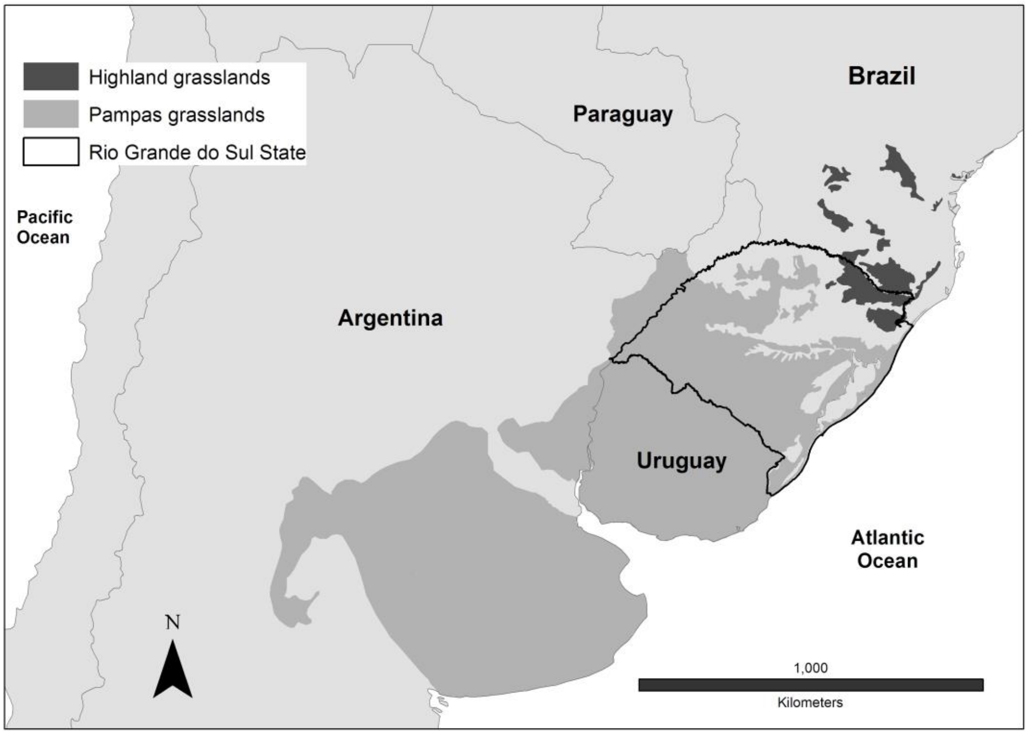

Figure 2. Location of the region used as an example for the proposed degradation model, grasslands in Rio Grande do Sul, southern Brazil. Shown is the original distribution of natural grasslands in southeastern South America.

Scenario 1: Degradation of grasslands after changes in management

Grazing

Grazing is one of the most important factors in the ecological history of grasslands around the world (Milchunas *et al.* 1988; Gibson 2009). Most studies evaluating effects of different grazing intensities in RS focus on effects on forage or beef production (e.g. Maraschin & Corrêa 1994; Moojen & Maraschin 2002; Pinto *et al*. 2008), and only few evaluate effects on species composition (Boldrini 1993; Boldrini & Eggers 1997) or soil properties (Bertol *et al*. 1998). Usually, grazed grasslands are formed by mosaics of intensively grazed patches dominated by prostrate grasses (e.g. *Axonopus affinis* Chase*, Paspalum notatum* Flüggé), and less grazed patches dominated by tussock grasses, small shrubs or other species less attractive for grazing animals (Boldrini & Eggers 1997; Diaz *et al.* 2007). This heterogeneity of the vegetation leads to structural complexity and diversity.

If grazing is excluded, grassland structure quickly changes: tall tussock grasses, e.g. *Andropogon* and *Sorghastrum* spp. (Boldrini & Eggers 1997) become dominant, drastically reducing plant species richness (Overbeck *et al.* 2005), litter accumulates and microclimate at soil surface changes (Pallarés *et al.* 2005). In northern RS, the encroachment of shrubs of the genus *Baccharis* (principally, *B. uncinella* DC.) and a slow invasion of forest pioneer species have been observed after abandonment (Oliveira & Pillar 2004), however, this likely only occurs in early stages of abandonment, as the accumulation of grass biomass hinders further recruitment of woody pioneers.

Overgrazing, on the other hand, can result in the replacement of productive forage species by species of lower forage quality, resulting in increasing cover of ruderal species and bare soil, while the contribution of highly nutritional C_3 grasses decreases (Pallarés *et al.* 2005). Ecosystem functions like water infiltration can be affected negatively as soil bulk density increased (Bertol *et al.* 1998). Either situation, when grazing is excluded or overgrazed, may be considered degraded due to changes in biotic and abiotic characteristics and the reduction of ecosystem resilience.

Introduction of alien species and fertilization

Overseeding of natural grasslands with introduced species, often combined with fertilization and liming, aims to increase forage quality and quantity especially in winter (Nabinger *et al.* 2000). Although many native species have high productivity and nutritive potential, they are not available on the seed market, and introduced species are used instead (Nabinger *et al*. 2000). Common species are *Lolium multiflorum* Lam., along with some European Fabaceae, e.g. *Trifolium repens* L. (Nabinger *et al*., 2000). Experience in other regions of the world shows that large-scale introduction of forage species may lead to losses of grassland biodiversity and changes in ecosystem functions (e.g. for North America: Christian & Wilson 1999; Marshall *et al*. 2012). For RS, it has been shown that forage yield increases linearly with nitrogen addition (Santos *et al*. 2008; Brambilla *et al*. 2012) and promotes the increase of animal live weight gain per area, but also leads to marked changes in the floristic composition, such as an increase of C_3 winter grasses if legumes are added (Pallarés *et al*. 2005; Brambilla *et al*. 2012). Phosphorus fertilization was also shown to increase pasture productivity (Gatiboni *et al*. 2000).

Fire

Fire is part of grassland ecosystems in many regions of the world (Belsky 1992; Anderson 2006), and has also shaped South Brazilian grasslands during the past millennia (Behling 2002). In general, grassland species in the region can be considered adapted to fire (Overbeck *et al*. 2005). The use of burns as management tools for livestock production, traditionally applied in the highland grasslands, is controversial due to concerns regarding possible negative impacts of fires, and fire had been prohibited by state legislation until recently. Effects of fire on soil biological, chemical and physical features and the feedbacks to aboveground processes are complex and highly variable (Neary *et al.* 1999). Regular fires select for different species groups compared to grazing (tussock vs. prostrated grasses, respectively), and selectively affect some species groups (e.g. C_3 grasses when burns occur in winter), but do not seem to cause reductions in grassland diversity (Overbeck *et al.* 2005). Fidelis *et al.* (2012) showed higher species richness in frequently burned grassland plots in comparison to sites where burning and grazing had been excluded for some years. Exclusion of fire in ungrazed areas, which is common practice in conservation units, leads to the accumulation of dead biomass and the risk of high-intensity fires increases. Current research suggests that fire could be a conservation tool where livestock production is not a management goal (Overbeck *et al.* 2005). Comparative studies on effects of fire and grazing on different grassland properties are mostly missing.

Invasion by alien species

Around the world, invasive alien species are considered a serious threat for biodiversity and many of these species have negative effects on grassland productivity, even though this may also be the case for some native (unpalatable) species as well. In southern Brazil, *Eragrostis plana* Nees, *Cynodon dactylon* (L.) Pers., *Ulex europaeus* L. and *Pinus* spp. are among the most problematic alien plant species in grasslands. Spread of these species is often linked directly or indirectly to management practices, i.e. they can serve as indicators of certain management practices or of past land use changes, but not as degradation cause itself, and spread of aliens can impact restoration possibilities.

Scenario 2: Degradation and restorability after complete conversion of grassland

Complete substitution of grassland by different land use will lead to losses or reduction of several ecosystem services (principally those related to biodiversity), while some may be maintained or increased. At any rate, biotic composition changes largely, and cultivation usually leads to changes in soil conditions, e.g. by fertilization or changes of soil physical properties (Figure 1b). The conversion of grasslands in RS has reached about 59% (104,553 km^2) in 2002 (Figure 3), mostly due to conversion to arable fields or exotic tree plantations. Remote sensing data can quantify the percentage of converted areas to some extent, as some signs of former agricultural activities or overgrazing can be detected (Figure 3), however, characteristics of vegetation itself (i.e. presence of alien species or not) cannot be observed at this scale.

Losses of native grassland have not been uniform in space, but reflect soil properties and topographic constraints. Especially in the Central Western Plateau region, native grasslands were nearly completely transformed into cropland, mostly for soybean. The coastal region has also seen high rates of transformation, principally due to rice and pine plantations. In the Northeastern Plateau, where soils are shallower, tree plantations and vegetable production are the main causes of grassland losses. Here, land use change has increased considerably within the past decade. In the Central Depression and in the Southwestern Grasslands of RS, a considerable proportion of the remaining grasslands have been overseeded with alien forage species.

For southern Brazil, some studies on ecosystem properties and ecological processes under different types of land use in former grassland areas are available. Table 1 synthesizes the available data, considering variables of importance for regeneration or restoration after the end of intensive land use (e.g. seed bank) or that may persist in a changed condition over long periods of time.

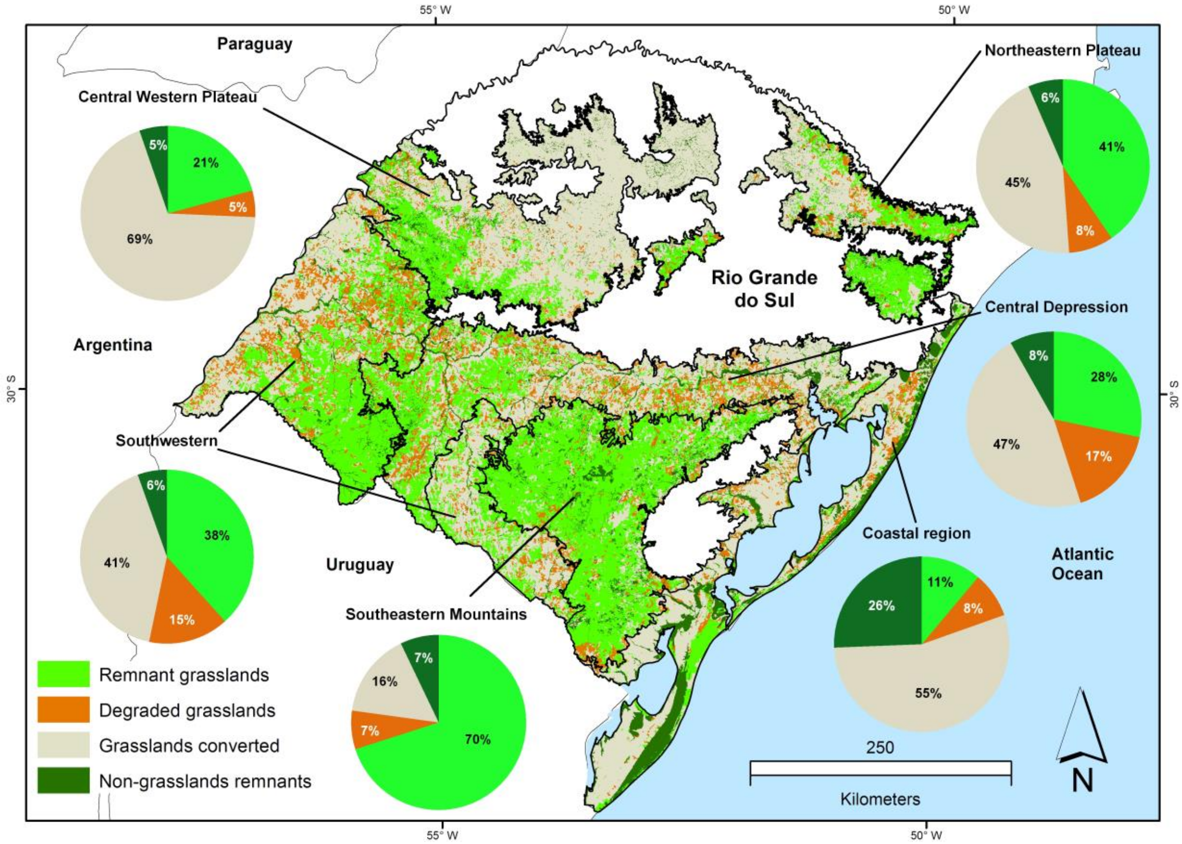

Figure 3. Distribution of grassland remnants and degraded grassland in RS state. The map is based on Landsat ETM+ images (spatial resolution: 30m). Only grassland areas with clearly visible signs of former land use change (e.g. use as agricultural field) are considered as degraded.

Arable land use and its effects

Studies on the seed bank of arable fields on former grasslands in Southern Brazil show that the number of grassland species decreases with management intensity and time, giving place to native or alien ruderal species (Favreto *et al*. 2007), thus reducing recovery potential of grassland. These results are in line with studies from other grassland ecosystems around the world that show higher abundance of weed species in the seed bank after agricultural use (Hutchings & Booth 1996; Kiehl & Pfadenhauer 2007). As vegetative recovery is the principal regeneration strategy of South Brazilian grasslands after disturbance (Fidelis *et al.* 2009), seed input from external sources as well as abiotic conditions are limiting for recovery of the former grassland community: the bud bank likely does not persist through periods of intensive agricultural use.

Grassland conversion also results in changes of soil properties. Arable land use increases nutrient levels of the soil (Rheinheimer *et al.* 1998; Perin *et al.* 2003), leading to different trajectories of vegetation recovery. It is well known that large quantities of carbon stored in grasslands may be rapidly transferred to the atmosphere and lost when the grassland is plowed and converted to agricultural land (Sala & Paruelo 1997). In a worldwide meta-analyses of carbon changes due to land-use changes, Guo & Gifford (2002) showed that a conversion of grasslands to crop rotation leads to a loss of 60% of belowground carbon. For Southern Brazil, a decrease in C-stock in soils under conventional-tilling has been shown, with magnitude depending on management intensity; no-tilling systems result in much lower losses of C in soils (Bertol *et al.* 2004; Diekow *et al.* 2005).

Tree plantations

By 2009, 6.000 km^2 (10%) of grasslands in RS were converted to plantations of pine, eucalyptus or acacia (Gautreau & Velez 2011). Observational data indicates that the type of vegetation that develops after logging and abandonment differs considerable from that of reference grassland and that species introduction likely is important if the objective is to restore grasslands. Zaloumis & Bond (2011) showed that species composition of grasslands established after logging of *Pinus elliottii* Engelm. plantations in South Africa was markedly changed in comparison to reference grasslands. Gonçalves *et al.* (2008) found relatively low species richness and dominance of a few ruderals and some alien species like the grass *Melinis minutiflora* P. Beauv. in the soil seed bank under tree plantations in the Central Brazilian *Cerrado*. Likewise, and in analogy to former agricultural fields, we can thus expect a low contribution of the seed bank in vegetation recovery.

Studies on effects of tree plantations on grassland soils give variable results (Table 1). Guo & Gifford (2002) stated that the conversion to plantations leads to a significant reduction of soil C stocks when coniferous species were used, while the effect with broadleaf species like eucalyptus was not significant. For RS, Wiesmeier *et al.* (2009) found lower C stocks under pine plantations while Mafra *et al.* (2008) could not show any changes. A growing number of literature examining potential for carbon sequestration in plantations is available, with changes based on the shift from belowground biomass dominance (grassland) to aboveground biomass with litter accumulation (plantation) (Guo *et al.* 2008). It has been shown that this potential strongly depends on soil types (Zinn *et al.* 2002), and might not be true for regions with high precipitation like RS, where a decrease in soil carbon was observed (Guo & Gifford 2002; Berthrong *et al.* 2012).

The few data available on vegetation regeneration after abandonment of other land uses indicate that ecosystem characteristics and services differ substantially from reference systems after land use transformation, and do not return to original conditions without assistance. Thus, it is important to distinguish two types of thresholds: a self-recovery threshold that describes until which point a recovery without additional management measures is possible (e.g. re-establishment of vegetation from the seed bank or from seed dispersal; Stage 1), and a restoration threshold that describes until which point an area can be restored with additional measures (e.g. modification of soil features, species introduction; Stage 2).

Table I. Review of studies on the effects of land use change on ecosystem processes in grasslands of southern Brazil (RS, Santa Catarina, Paraná). All trends in comparison to reference grasslands (-, no studies available)

	Conversion to arable land	**Conversion to forest plantation**
Aboveground vegetation	No-tillage systems: ↑ non-native species (1) After abandonment: ↓ floristic diversity , dominance of ruderal species (2) or alien species (Overbeck, unpubl. data)	usually no understory (3)
Seed bank	↓Density and diversity of native grassland species; ↑density of ruderals and non-natives (4,5)	-
Litter thickness and quality	-	Up to 7 cm needles; C/N ratio higher (6) or lower than reference grassland
C-stock and cycling	C stock better preserved under no-tillage (8, 14, 16) C stock ↓ under conventional tilling by 22% (17)	C stocks lower than in pasture (8), intermediate between grassland and shrub-grassland (6), or unchanged (10) Rio de la Plata grasslands (further to the South): tree plantation under high precipitation (level of RS) have reduced carbon stocks in soil when compared to grassland (13)
Soil pH and nutrient status	Increased nutrient load in topsoil (12); pH raised (15) or lowered (12)	Pine: pH lower (6,8,9) or unchanged (10) ; N_{tot} falling (8); P lower or higher; K lower; Al higher or unchanged (10) Eucalyptus: pH lower, K^+, Ca^{2+}, Mg^{2+} lower, increase in Na^+ and Al^{3+} (11)
Soil physical properties	Aggregate stability better preserved under no-tillage (7)	*Pinus* spp.: Soil density unchanged

1) Favreto *et al.* (2007); 2) Carmo *et al*. (2009); 3) Souza *et al.* (2013); 4) Favreto and Medeiros (2006) ; 5) Maia *et al*.(2008); 6) Dümig *et al*. (2008); 7) Bertol *et al*. (2004); 8) Wiesmeier *et al*. (2009); 9) Schumacher *et al*. (2008); 10) Mafra *et al*. (2008);11) Céspedes-Payret *et al*. (2012); 12) Rheinheimer *et al*. (1998); 13) Berthrong *et al*. (2012); 14) Pillar *et al*. (2012); 15) Almeida *et al*. (2005); 16) Diekow *et al*. (2005a), 17) Diekow *et al*. (2005b).

Discussion and conclusions

Land degradation studied on a regional scale often only considers conversion or complete losses of natural ecosystems, with limits to detect e.g. compositional changes, while at the local scale, finer effects of land management can be discussed. Both perspectives are necessary for an improved assessment of degradation and restoration potential, and differences between the two types of degradation likely imply in different perceptions regarding degradation and conservation or restoration. However, the assessment of both types of degradation processes requires availability of suitable data that allow a comparison of degraded grasslands with the reference systems.

Our conceptual model can serve as a framework to study degradation and restorability of tropical and subtropical grasslands after changes in management or substitution by other types of land use on different scales. For our model system, conclusive evidence is available that management is necessary for maintenance of diverse and productive grasslands in this region (e.g. Overbeck *et al.* 2007), and biodiversity conservation and livestock production can be considered as complementary management goals, allowing for sustainable use (e.g. Nabinger *et al.* 2009). Fire and grazing are selective forces that cause changes in grassland composition and structure, but their effects depend on frequency and intensity – both can contribute to conservation of biodiversity and productivity, but they can also be detrimental when frequency or intensity are too high or too low. A more systematic and large-scale quantification of effects of different management types (especially intensification, Scenario 1) on different properties of the grasslands in the region is still missing, making it difficult to define degradation more precisely at the moment.

Even though a considerable proportion of natural grasslands has been converted to other land use (Scenario 2), concern on restoration possibilities and necessities of these areas has been raised only recently (Overbeck *et al.* 2013) and is now entering into the agenda of conservation politics. Empirical data is necessary for the determination of degradation thresholds or limits to restoration. Once filled with more detailed data, the conceptual model can serve as a framework for decision-making and priority setting in nature conservation by identifying whether costly restoration measures will be needed or adaptation in management would be sufficient for self-recovery. In this, it is important to recognize that not only biotic and abiotic characteristics are covered by the model, but that these can also be interpreted in terms of ecosystem functions and services (e.g. carbon sequestration, forage production).

Bestelmeyer (2006) points out problems and risks associated with threshold models: for instance, no single predictive thresholds – which would greatly facilitate management decisions – should be expected to exist, and parameters may reflect measurability, and not long-term degradation processes. Threshold models may become ‘insidious’ (Bestelmeyer 2006) if they lead to the belief that certain areas are not restorable anymore, because some original features of the system cannot be recovered. This, however, is not a consequence of the model per se, but of a failure of recognizing the full range of features and processes of any type of ecosystem. The current debate on Novel Ecosystems (Hobbs *et al*. 2013) is centering exactly on the question of how to deal with this kind of system. Looking at a broad range of parameters that characterize processes and functions of degraded systems – including the potential to change to another stage – thus is of fundamental importance when working with thresholds. As in other regions of the world, different perspectives, e.g. that of livestock production (active management) and that of biodiversity conservation, are still not being considered in an integrative manner in the conservation debate in Brazil, which can be considered as a serious impediment for conservation and restoration policy (Overbeck *et al*. 2013).

Briske *et al.* (2006) defined thresholds as a switch from the dominance of negative feedbacks that contribute to ecosystem stability to the dominance of positive feedbacks that degrade resilience and promote the conversion to alternative post-threshold stages. In Brazil, ecological research is mostly based on positive feedbacks and few studies focus on understanding the process that confer resilience around desired stages. In contrast, some studies around the world encourage incorporating negative plant-soil feedback for better understanding density of plant invaders, and invader spread (Levine *et al*. 2006; Suding *et al*. 2013). Threshold categories can be used to identify the extend of threshold progression and to assess the potential for threshold reversibility (Briske *et al*. 2006). A conceptual model of degradation and restoration based on a variety of biotic and abiotic variables has the potential to include different functions and services and can contribute to a broader understanding of landscapes as multifunctional systems. We expect that our model can serve as a framework to study degradation and restoration processes, in southern Brazil and elsewhere.

Acknowledgements

This contribution results from a CNPq-DFG-cooperation project (490069/2011-8 and KO1741/2-1, respectively). CK receives support by the Evangelisches Studienwerk Villigst. EV and BA by CAPES. IB and VP receive support from CNPq.

References

Almeida JA, Bertol I, Leite D, Amaral AJ, Zoldan Júnior WA. 2005. Propriedades químicas de um Cambissolo Húmico sob preparo convencional e semeadura direta após seis anos de cultivo. *Revista Brasileira de Ciência do Solo* **29**: 437–445.

Anderson RC. 2006. Evolution and origin of the Central Grassland of North America: climate, fire, and mammalian grazers. *Journal of the Torrey Botanical Society* **133**: 626–647.

Behling H. 2002. South and southeast Brazilian grasslands during Late Quaternary times: a synthesis. *Palaeogeography Palaeoclimatology Palaeoecology* **177**: 19–27.

Behling H, Pillar VD. 2007. Late Quaternary vegetation, biodiversity and fire dynamics on the southern Brazilian highland and their implication for conservation and management of modern Araucaria forest and grassland ecosystems. *Philosophical Transactions of the Royal Society B-Biological Sciences* **362**: 243–251.

Belsky JA. 1992. Effects of grazing, competition, disturbance and fire on species composition and diversity in grassland communities. *Journal of Vegetation Science* **3**: 187–200.

Berthrong ST, Piñeiro G, Jobbágy EG, Jackson RB. 2012. Soil C and N changes with afforestation of grasslands across gradients of precipitation and plantation age. *Ecological Applications* **22**: 76–86.

Bertol I, Albuquerque JA, Leite D, Amaral AJ, Zoldan Junior WA. 2004. Propriedades físicas do solo sob preparo convencional e semeadura direta em rotação e sucessão de culturas, comparadas às do campo nativo. *Revista Brasileira de Ciência do Solo* **28**: 155–163.

Bertol I, Gomes KE, Denardin RBN, Machado LAZM, Maraschin GE.1998. Propriedades físicas do solo relacionadas a diferentes níveis de oferta de forragem numa pastagem natural. *Pesquisa Agropecuaria Brasileira* **33**: 779–786.

Bestelmeyer BT. 2006. Threshold Concepts and Their Use in Rangeland Management and Restoration: The good, the bad, and the insidious. *Restoration Ecology* **14**: 325–329.

Boldrini II. 1993. Dinâmica de vegetação de uma pastagem natural sob diferentes níveis de oferta de forragem e tipos de solos, Depressão Central, RS. PhD Thesis, 262 p.

Boldrini II. 2009. A flora dos Campos do Rio Grande do Sul. In *Campos Sulinos,* Pillar VDP, Müller SC, Castilhos ZMS, Jacques AVA (eds). MMA: Brasília; 63–77.

Boldrini II, Eggers L. 1997. Directionality of sucession after grazing exclusion in grassland in the south of Brazil. *Coenoses* **12**: 63–66.

Bond WJ, Parr CL. 2010. Beyond the forest edge: Ecology, diversity and conservation of the grassy biomes. *Biological Conservation* **143**: 2395–2404.

Brambilla DM, Nabinger C, Kunrath TR, Carvalho PCdF, Carassai IJ, Cadenazzi M. 2012. Impact of nitrogen fertilization on the forage characteristics and beef calf performance on native pasture overseeded with ryegrass. *Revista Brasileira de Zootecnia* **41**: 528–536.

Briske DD, Fuhlendorf SD, Smeins FE. 2006. A unified framework for assessment and application of ecological thresholds. *Rangeland Ecology & Management* **59**: 225–236.

Carmo MRB, Freitas DVB, Mizamoto SNA. 2009. Estrutura de um campo limpo em regeneração após duas décadas de uso agrícola no Sul do Brasil. In *Congresso Iberoamericano e do caribe sobre restauração ecológica*, Curitiba, 2008.

Céspedes-Payret C, Piñeiro G, Gutiérrez O, Panario D. 2012. Land use change in a temperate grassland soil: Afforestation effects on chemical properties and their ecological and mineralogical implications. *Science of the Total Environment* **438**: 549–557.

Christian JM, Wilson SD. 1999. Long-term ecosystem impacts of an introduced grass in the northern great plains. *Ecology* **80**: 2397–2407.

Cione AL, Tonni EP, Soibelzon L. 2003. The broken zig-zag: Late Cenozoic large mammal and tortoise extinction in South America. *Revista del Museo Argentino de Ciencias Naturales* **5**: 1–19.

Cordeiro JLP, Hasenack H. 2009. Cobertura vegetal atual do Rio Grande do Sul. In *Campos Sulinos*, Pillar VD, Müller S, Castilhos ZMS, Jaques AVA (eds). MMA: Brasília; 285–299.

Diaz S, Lavorel S, McIntyre S, Falczuk V, Casanoves F, *et al.* 2007. Plant trait responses to grazing - a global synthesis. *Global Change Biology* **13**: 313–341.

Diekow J, Mielniczuk J, Knicker H, Bayer C, Dick DP, Kögel-Knabner I. 2005. Soil C and N stocks as affected by cropping systems and nitrogen fertilisation in a southern Brazil Acrisol managed under no-tillage for 17 years. *Soil and Tillage Research* **81**: 87–95.

Favreto R, Medeiros R, Pillar VDP, Levien R. 2007. Vegetação espontânea em lavoura sob diferentes manejos estabelecida sobre campo natural. *Iheringia* **62**: 5–17.

Favreto R, Medeiros RB. 2006. Banco de sementes do solo em área agricola sob diferentes sistemas de manejo estabelecida sobre campo natural. *Revista Brasileira de Sementes* **28**: 34–44.

Fidelis A, Appezzato-da-Gloria B, Pfadenhauer J. 2009. A importância da biomassa e das estruturas subterrâneas nos Campos Sulinos. In *Campos Sulinos*, Pillar VDP, Müller SC, Castilhos ZMS, Jacques AVA (eds). MMA: Brasília; 88–100.

Fidelis A, Blanco CC, Müller SC, Pillar VDP, Pfadenhauer J. 2012. Short-term changes caused by fire and mowing in Brazilian Campos grasslands with different long-term fire histories. *Journal of Vegetation Science* **23**: 552–562.

Gatiboni LC, Kaminski J, Pellegrini JBR, Brunetto G, Saggin A, Flores JPC. 2000 Influência da adubação fosfatada e da introdução de espécies forrageiras de inverno na oferta de forragem de pastagem natural. *Pesquisa Agropecuaria Brasileira* **35**: 1663–1668.

Gautreau P, Velez E. 2011. Strategies of environmental knowledge production facing land use changes: Insights from the Silvicultural Zoning Plan conflict in the Brazilian state of RS. *Cybergeo* **577**: 1.

Gibson DJ. 2009. Grasses and grassland ecology. Oxford, New York.

Gonçalves AR, Martins RdCC, Martins IS, Felfili JM. 2008. Bancos de Sementes do Sub-bosque de *Pinus* spp. e *Eucalyptus* spp. na Flona de Brasília. *Cerne* **14**: 23–32.

Guo L, Gifford R. 2002. Soil carbon stocks and land use change: a meta analysis. *Global Change Biology* **8**: 345–360.

Guo LB, Cowie AL, Montagu KD, Gifford RM. 2008. Carbon and nitrogen stocks in a native pasture and an adjacent 16-year-old Pinus radiata D. Don. plantation in Australia. *Agriculture, Ecosystems & Environment* **124**: 205–218.

Hobbs RJ, Higgs E, Harris JA. 2009. Novel ecosystems: implications for conservation and restoration. *Trends in Ecology & Evolution* **24**: 599–605.

Hobbs RJ, Higgs ES, Hall C. 2013. *Novel ecosystems: intervening in the new ecological world order*. John Wiley & Sons: Oxford.

Hutchings MJ, Booth KD. 1996. Studies on the feasibility of re-creating chalk grassland vegetation on ex-arable land .1. The potential roles of the seed bank and the seed rain. *Journal of Applied Ecology* **33**: 1171–1181.

Kiehl K, Pfadenhauer J. 2007. Establishment and persistence of target species in newly created calcareous grasslands on former arable fields. *Plant Ecology* **189**: 31–48.

Levine JM, Pachepsky E, Kendall BE, Yelenik SG, Lambers JHR. 2006. Plant–soil feedbacks and invasive spread. *Ecology Letters* **9**: 1005–1014.

Lima-Ribeiro MS, Felizola Diniz-Filho JA. 2013. American megafaunal extinctions and human arrival: Improved evaluation using a meta-analytical approach. *Quaternary International* **299**: 38–52.

Mafra AF, Guedes-Filho SFF, Klauberg O, Santos CP, Almeida JA, DallaRosa J. 2008. Carbono orgânico e atributos químicos do solo em áreas florestais. *Revista Árvore* **32**: 217–224.

Maia FC, Maia MdS, Bekker RM, Berton RP, Caetano LS. 2008. *Lolium multiflorum* seeds in the soil: I. Soil seed bank dynamics in a no till system. *Revista Brasileira de Sementes* **30**: 100–110.

Maraschin GE, Corrêa FL. 1994. Crescimento e desaparecimento de uma pastagem nativa sob diferentes níveis de oferta de forragem. *Pesquisa Agropecuaria Brasileira* **29**: 1617–1623.

Marshall VM, Lewis MM, Ostendorf B. 2012. Buffel grass (*Cenchrus ciliaris*) as an invader and threat to biodiversity in arid environments: A review. *Journal of Arid* Environments **78**: 1–12.

Milchunas D, Sala OE, Lauenroth WK. 1988. A generalized model of the effects of grazing by large herbivores on grassland community structure. *American Naturalist* **132**: 87–106.

Moojen EL, Maraschin GE. 2002. Potencial produtivo de uma pastagem nativa do RS submetida a níveis de oferta de forragem. *Ciência Rural* **32**: 127–132.

Nabinger C, Ferreira E, Freitas A, Carvalho P, Sant'Anna D. 2009. Produção animal com base no campo nativo: aplicações de resultados de pesquisa. Pillar VP, Muller SC, Castilhos. ZMS: 175–198.

Nabinger C, Moraes A, Marschin GE. 2000. Campos in Southern Brazil. In *Grassland Ecophysiology and Grazing Ecology*, Lemaire G, Hodgson JG, Moraes A, Nabinger C, Carvalho PCF (eds). CABI: Wallingford; 355–376.

Neary DG, Klopatek CC, DeBano LF, Folliott PF. 1999. Fire effects on belowground sustainability: a review and synthesis. *Forest Ecology and Management* **122**: 51–71.

Oliveira JM, Pillar VDP. 2004. Vegetation dynamics on mosaics of Campos and Araucaria forest between 1974 and 1999 in Southern Brazil. *Community Ecology* **5**: 197–202.

Overbeck GE, Müller SC, Pillar VDP, Pfadenhauer J. 2005. Fine-scale post-fire dynamics in southern Brazilian subtropical grassland. *Journal of Vegetation Science* **16**: 655–664.

Overbeck GE, Müller SC, Fidelis A, Pfadenhauer J, Pillar VDP, Blanco CC, Boldrini II, Both R, Forneck ED. 2007. Brazil's neglected biome: The South Brazilian Campos. *Perspectives in Plant Ecology Evolution and Systematics* **9**: 101–116.

Overbeck GE, Hermann J-M, Andrade BO, Boldrini II, Kiehl K, *et al.* 2013. Restoration Ecology in Brazil – Time to Step Out of the Forest. *Brazilian Journal of Nature Conservation* **11**: 92–95.

Pallarés OR, Berretta EJ, Maraschin GE. 2005. The south american campos ecosystem. In *Grasslands of the world*, Suttie J, Reynolds SG, Batello C (eds). FAO: Rome; 171–179.

Perin E, Ceretta C, Klamt E. 2003. Tempo de uso agrícola e propriedades químicas de dois Latossolos do Planalto Médio do RS. *Revista Brasileira de Ciência do Solo* **27**: 665–674.

Pillar V, Tornquist C, Bayer C. 2012. The southern Brazilian grassland biome: soil carbon stocks, fluxes of greenhouse gases and some options for mitigation. *Brazilian Journal of Biology* **72**: 673–681.

Pillar VDP, Vélez E. 2010. Extinção dos Campos Sulinos em unidades de conservação: um fenômeno natural ou um problema ético? *Natureza e Conservação* **8**: 84–86.

Pinto CE, Fontoura Júnior JASd, Frizzo A, Freitas TMS, Nabinger C, Carvalho PCdF. 2008. Produções primária e secundária de uma pastagem natural da Depressão Central do RS submetida a diversas ofertas de fitomassa aérea total. *Revista Brasileira de Zootecnia* **37**: 1737–1741.

Reed MS, Buenemann M, Atlhopheng J, Akhtar-Schuster M, Bachmann F, *et al.* 2011. Cross-scale monitoring and assessment of land degradation and sustainable land management: A methodological framework for knowledge management. *Land Degradation & Development* **22**: 261–271.

Rheinheimer DS, Kaminski J, Lupatini GC, Santos EJS. 1998. *Modificações em atributos químicos de solo arenoso sob sistema plantio direto*. Sociedade Brasileira de Ciência do Solo: Campinas.

Sala OE, Paruelo JM. 1997. Ecosystem services in grasslands. In *Nature`s service: Societal dependence on natural ecosystems*, Daily GC (ed). Island Press: Washington.

Sala OE, Stuart Chapin F, III, Armesto JJ, Berlow E, Bloomfield J, *et al.* 2000. Global Biodiversity Scenarios for the Year 2100. *Science* **287**: 1770–1774.

Santos DTd, F. CPCd, C. N, Carassai IJ, Gomes LK. 2008. Bioeconomic efficiency of native pasture fertilization in southern Brazilian. *Ciência Rural* **38**: 437–444.

Schumacher MV, Viera M, Witschoreck R. 2008. Litter production and nutrients transfer in a second rotation area with *Pinus taeda* L. forest in Cambará do Sul, RS. *Ciência Florestal* **18**: 471–480.

Souza AF, Ramos NP, Pizo MA, Hübel I, Crossetti LO. 2013. Afforestation effects on vegetation structure and diversity of grasslands in southern Brazil: The first years. *Journal for Nature Conservation* **21**: 56–62.

Stocking M, Murnaghan N. 2001. *Handbook for the field assessment of land degradation*. Earthscan Publications: London.

Suding KN, Gross KL, Houseman GR. 2004 Alternative states and positive feedbacks in restoration ecology. *Trends in Ecology & Evolution* **19**: 46–53.

Suding KN, Stanley Harpole W, Fukami T, Kulmatiski A, MacDougall AS, Stein C, van der Putten WH. 2013. Consequences of plant–soil feedbacks in invasion. *Journal of Ecology* **101**: 298–308.

Wiesmeier M, Dick DP, Rumpel C, Dalmolin RSD, Hilscher A, Knicker H. 2009. Depletion of soil organic carbon and nitrogen under Pinus taeda plantations in Southern Brazilian grasslands (Campos). *European Journal of Soil Science* **60**: 347–359.

Wilson JB, Peet RK, Dengler J, Pärtel M. 2012. Plant species richness: the world records. *Journal of Vegetation Science* **23**: 796–802.

Zaloumis NP, Bond WJ. 2011. Grassland restoration after afforestation: No direction home? *Austral Ecology* **36**: 357–366.

Zinn YL, Resck DVS, da Silva JE. 2002. Soil organic carbon as affected by afforestation with Eucalyptus and Pinus in the Cerrado region of Brazil. *Forest Ecology and Management* **166**: 285–294.

CONSIDERAÇÕES FINAIS

Por mais que seja conhecida a relação entre solo e vegetação, com base nos resultados acima expostos, foi possível observar especificidades quanto às escalas ecológica e espacial utilizadas. Ao nível local, características físicas do solo relacionadas à disponibilidade de água às plantas, condicionam uma maior variação da vegetação (**capítulo II**), inclusive a dentro de uma mesma espécie (**capítulo I**). Já ao nível regional, as características químicas do solo, que evidenciam variações no clima e material de origem, explicam uma porcentagem significativa da variabilidade da vegetação (**capítulo III**).

De forma geral, a compreensão da relação solo-vegetação pode nos fornecer importantes indicativos do grau de degradação, capacidade de resiliência, bem como a identificação de certos filtros ambientais que devem ser sobrepostos para efetiva recuperação de áreas campestres com alto grau de degradaçao (**capítulo IV**). É imprescindível a compreensão da relação solo-vegetação quando se visa a proposição de praticas de manejo alternativas com vistas à conservação, políticas públicas e práticas de recuperação de áreas degradadas.

Este estudo é somente um dos primeiros estudos nessa linha de pesquisa para o os campos sulinos, ainda existe muito por ser feito para a compreensão do ambiente estudado. Com base no conhecimento adquirido no decorrer do desenvolvimento deste estudo, reitero que alguns cuidados devem ser tomados em estudos futuros e faço a sugestão de temas de pesquisa:

- Ao nível local, sobre condições bastante contrastantes (ex. áreas com diferentes graus de impacto), a variação intraespecífica de atributos pode representar uma porcentagem representativa da variação da vegetação (**capítulo I**). Geralmente, em estudos de comunidades vegetais, a variabilidade intraespecífica é considerada muito inferior à interespecífica e então desconsiderada, entretanto cuidados devem ser tomados para não negligenciá-la, especialmente tratando-se de áreas de estudo com grande variabilidade ambiental natural ou em decorrência de distúrbio. O uso de atributos oriundos de banco de dados também deve ser usado de forma criteriosa. Existem estudos que sugerem o uso de modelos que consideram tanto a variação inter- e intraespecífica nas análises empregadas, e esta pode ser uma alternativa.
- Ao nível local, características físicas do solo apresentam maior correlação com a variabilidade da vegetação em comparação com características químicas. Para pesquisadores que se interessam nesta linha de pesquisa, sugiro o maior investimento em análises físicas do solo ao

nível local, se o objetivo for identificar filtros ambientais atuantes nos padrões de vegetação. O uso de características hidráulicas em associação a características texturais do solo deve ser considerado. Esta indicação não descarta o uso de características químicas ao nível local, visto que são de grande importância para confirmação da classificação dos solos e podem ser importantes filtros em áreas alteradas por ação do manejo (ex. fertilização), contaminação (ex. metais pesados), entre outros.

- Ao nível regional (variação entre biomas) foi observada a grande importância do efeito do teor de alumínio no solo na composição de espécies, somente 20% das espécies amostradas é comum aos dois biomas, no caso deste estudo (**capítulo III**). Sugiro estudos das características genéticas e mecanismos que conferem essa maior capacidade de adaptação aos altos teores de aluminio solo em uma mesma espécie (ex. espécies comuns aos dois biomas: Mata Atlântica versus Pampa). Acredito que essa mesma diferença entre biomas possa também afetar na capacidade de germinação das espécies (ex. espécie proveniente do bioma Pampa e plantada em solos com alto teor de Al no bioma Mata Atlântica), informação de grande importância para futuros estudos de recuperação de áreas campestres. Por isso, importante conhecer e conservar a vegetação local.
- Por fim, ao revisar estudos sobre degradação e recuperação de campos, é evidente a carência de estudos sobre o assunto. Ano após ano os campos estão sendo convertidos principalmente para produção florestal e agrícola. Poucos estudos abordam técnicas de recuperação, e os poucos que o fazem sugerem recuperar campos naturais com espécies arbóreas exóticas, o que é preocupante. Em diversos países da Europa, por exemplo, diversas técnicas de recuperação vêm sendo implementadas e resultados positivos tem sido obtidos. O que nos falta é adaptar para a nossa realidade estas técnicas e testar sua viabilidade.