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**ECOLOGIA HISTÓRICA, INTERAÇÕES BIÓTICAS E A  
EXPANSÃO DA FLORESTA COM ARAUCÁRIA SOBRE OS  
CAMPOS SUBTROPICAIS DE ALTITUDE NO SUL DO BRASIL**

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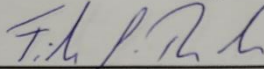
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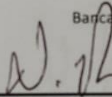
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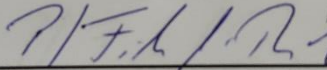


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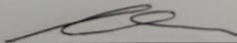


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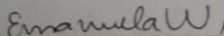


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Dedico esta tese  
a minha mãe Ieda Elena Scarton  
e a meu pai Carlos Alberto Sühs (*in memoriam*).

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“Nos locais mais elevados, como (...) São Joaquim, de 1.200 metros de altitude ou mais, (...) o pinheiro parece tender a expandir-se por sobre os campos limpos e enxutos (...)”  
(Klein, 1984)

“O soberbo pinheiro, não podendo ajudar-se (...) na disseminação de suas pesadas sementes, torna-se humilde servindo de alimento a tantos animais e mesmo ao homem. Neste seu sacrifício está a sua glória: faz amigos que se encarregam de propagar sua espécie”  
(Reitz, 1966)

“(...) I'll take your part  
When darkness comes  
And pain is all around  
Like a bridge over troubled water  
I Will lay me down (...)”  
(Paul Simon, 1970)

## Resumo

Muitos fenômenos ecológicos são impossíveis de serem totalmente compreendidos quando processos agindo por décadas ou séculos são ignorados. Atividades humanas vêm moldando a aparência e função de regiões, paisagens e ecossistemas por milênios. A ecologia histórica pode ser utilizada como ferramenta para compreender padrões gerados por processos complexos, onde além de fatores abióticos e interações bióticas, humanos estão inseridos. A floresta com araucária se expandiu sobre os campos subtropicais de altitude há cerca de 4.000 anos A.P., em virtude de uma mudança no clima, e há cerca de 2.500 anos, em virtude da presença humana. A floresta com araucária e os campos subtropicais de altitude são paisagens influenciadas por milênios por povos pré-Colombianos. Hoje, gestores locais utilizam técnicas similares, por exemplo o fogo, como ferramenta para promover o rebrote dos campos para o pastoreio do gado. A dinâmica floresta-campo envolvida nesses ecossistemas configura um cenário ideal para estudos integrativos, dentro da ampla perspectiva da ecologia histórica. Desta forma, considerando que o clima, as pessoas e a própria araucária são importantes agentes no processo de expansão da floresta sobre o campo e responsáveis pela formação de padrões na estrutura de comunidades, a presente tese teve por objetivo geral avaliar os efeitos das práticas tradicionais de manejo na paisagem e na biodiversidade e verificar os efeitos da microbiota do solo na araucária. O primeiro capítulo objetivou avaliar o tipo de manejo utilizado por gestores locais e verificar os efeitos desse manejo nos campos subtropicais de altitude. Nossa hipótese foi de que a dinâmica da vegetação é determinada pelo tipo de manejo utilizado (com manejo tradicional, e sem manejo tradicional). Especificamente, locais sem manejo tradicional possivelmente são afetados pelo adensamento de arbustos ao longo do tempo. A taxa de adensamento de arbustos é ainda pouco conhecida para os campos subtropicais de altitude no Brasil. Além disso, a dinâmica da vegetação nunca foi examinada comparativamente entre áreas com e sem manejo tradicional. Nossos resultados demonstraram que a criação de gado e o fogo fazem parte do manejo empregado por gestores locais. Demonstramos que alguns anos após a remoção do

fogo e do gado, as taxas de adensamento de arbustos nos campos aumentaram exponencialmente. Os campos foram substituídos por densos arbustais, de forma geral dominados pela espécie *Baccharis uncinella*, uma espécie nativa e comum nas regiões altas do planalto. A taxa de transformação do campo em arbustais foi tão expressiva que a extrapolação sugere que bastam 30 anos sem manejo tradicional para que 99% dos campos tornem-se arbustais. Enquanto isso, os campos manejados por gestores locais, através de práticas tradicionais de manejo (i.e., com utilização do gado e do fogo), permaneceram praticamente inalterados nos últimos 40 anos. Tais componentes, além de manterem os campos subtropicais de altitude, também previnem incêndios florestais em larga escala, que podem ser catastróficos para a biodiversidade. Além do manejo, outros processos estruturam a comunidade biológica nestes ecossistemas, como a facilitação, que tem importante papel na dinâmica da expansão da floresta sobre os campos. Assim, objetivamos com o segundo capítulo investigar como o manejo, as interações bióticas e fatores abióticos afetam a riqueza, a abundância e a composição de espécies florestais. Buscamos entender como que os campos são mantidos pelas práticas de manejo, se as araucárias contribuem para a expansão florestal e o papel das interações bióticas e fatores abióticos (por exemplo, rochas) envolvidos nesse processo. Nossas hipóteses foram: (1) o manejo reduz a riqueza e a abundância e muda a composição de espécies florestais, (2) araucárias aumentam a riqueza e abundância e mudam a composição de espécies florestais, e (3) o efeito interativo entre o manejo e a araucária é responsável por moldar padrões de riqueza, abundância e composição das espécies florestais. Nossos resultados indicaram que a abundância e a composição de espécies florestais são afetadas pela forma de manejo e influência da copa das araucárias. Os maiores valores de abundância foram encontrados sob as copas das araucárias em áreas sem o manejo tradicional. A composição das espécies mudou em todas as combinações avaliadas de manejo e influência da copa. Nosso estudo demonstrou os papéis principais do manejo e da facilitação na estruturação de comunidades, além dos efeitos da cobertura de rochas e gramíneas. Sabendo do importante

papel das interações bióticas no processo de expansão da floresta sobre os campos, no terceiro capítulo avaliamos experimentalmente os efeitos do tipo de solo e de comunidades microbianas do solo nos estágios iniciais de desenvolvimento da araucária. O efeito da comunidade microbiana do solo na germinação e no crescimento inicial de plantas permanece pouco conhecido. Sendo a araucária uma espécie-chave na dinâmica sucessional e na expansão da floresta sobre o campo, nossa hipótese foi de que as comunidades microbianas do solo e tipos de solos comumente associados a essa espécie são benéficas para sua germinação e crescimento inicial, ao passo que solos e comunidades microbianas de campos e arbustais seriam prejudiciais. Contrariando nossas expectativas, a germinação da araucária não foi afetada pelo tipo de solo nem pelos tratamentos. Por outro lado, as comunidades microbianas do solo afetaram negativamente o crescimento inicial da araucária, enquanto as diferenças entre os solos, por não terem afetado as plântulas, podem permitir um ritmo mais rápido de expansão da floresta sobre os campos. As práticas de manejo tradicional secularmente utilizadas por gestores locais, principalmente através da utilização do fogo e do gado, podem contribuir para a manutenção dos campos ao conter o adensamento de arbustos e a expansão da floresta. O processo de facilitação promovido pela araucária contribui para a estruturação de comunidades de espécies florestais e aceleração do processo de expansão. A microbiota do solo, por sua vez, afeta negativamente o estabelecimento da araucária, podendo ser um elemento de contenção da expansão. Para a conservação desses ecossistemas e de seus processos naturais, é preciso reconhecer a importância dos campos como paisagens culturais e do fogo como elemento natural nos campos subtropicais de altitude. A valorização das práticas seculares de manejo tradicional nos campos se faz necessária para estabelecer políticas públicas efetivas de conservação e manejo. Nossos resultados esclareceram padrões e processos que emergem nos campos de altitude, como a conversão de campos em arbustais, a facilitação promovida pela araucária e a influência negativa da microbiota do solo na araucária. Desta forma, reforçamos que a

perda de paisagens culturais pode se tornar iminente quando as principais ações de manejo tradicional são excluídas.

**Palavras-chave:** Adensamento de arbustos; Araucária; Campos subtropicais de altitude; Conservação; Construção de nicho; Ecologia histórica; Estruturação de comunidades; Floresta Ombrófila Mista; Fogo; Interações; Manejo; Mata Atlântica; Microbiota do solo; Paisagem cultural.

# Abstract

Many ecological phenomena are impossible to be fully understood when decades or centuries-long processes are ignored. Human activities have shaped the appearance and function of regions, landscapes and ecosystems for millennia. Historical ecology can be used as a tool to understand patterns generated by complex processes in which humans are inserted, in addition to abiotic factors and biotic interactions. The araucaria forest has expanded over subtropical highland grasslands around 4,000 years ago due to a change in climate, and about 2,500 years ago, due to human actions. Araucaria forest and grasslands have been influenced by pre-Columbian societies for millennia. Today, local managers use similar techniques, for example fire, as a tool to promote grassland regrowth for livestock grazing. The forest-grassland dynamics involved in these ecosystems constitute an ideal scenario for integrative studies within the broad perspective of historical ecology. Thus, considering that climate, people and araucaria are important components in the process of forest expansion over grasslands and are responsible for creating and shaping patterns in the community structure and landscapes, this thesis aimed to evaluate the effects of traditional management practices on the landscape and biodiversity and to verify the effects of soil microorganisms on araucaria seeds and seedlings. In the first chapter I evaluated the type of management used by local managers and I verified the effects of land management in subtropical highland grasslands. The hypothesis was that the vegetation dynamics is determined by the type of management employed (areas with traditional management, and areas without traditional management). Specifically, areas without traditional management are possibly affected by shrub encroachment over time. The rate of shrub encroachment is still unknown for subtropical highland grasslands in Brazil. In addition, vegetation dynamics have never been examined comparatively between areas with and without traditional management. Our results showed that cattle and fire are part of the management used by local managers. We have shown that within a few years after fire and cattle suppression, the rates of shrub encroachment in grasslands increased exponentially.

Grasslands were replaced by shrublands, generally dominated by *Baccharis uncinella*, a native and common shrub species in the highlands. The rate of change of grasslands into shrublands was so intense that our extrapolation suggests that within 30 years, 99% of the areas without traditional management can become occupied by shrubs. Meanwhile, grasslands managed by local managers through traditional management practices (i.e., with cattle and fire) have largely remained unchanged for the past 40 years. Such components (fire and cattle), in addition to maintaining grasslands, also prevent large-scale forest fires, which can be catastrophic for biodiversity. Besides the type of management, other processes structure the biological community in these ecosystems, such as facilitation, which plays an important role in the dynamics of forest expansion over grasslands. Thus, our goal in the second chapter was to investigate how land management, biotic interactions and abiotic factors affect saplings species richness, abundance and composition. We hypothesized that (1) land management would decrease sapling richness and abundance and change sapling composition, (2) nurse araucaria trees would increase species richness and abundance and change sapling composition, and (3) the interactive effect between land management and nurse araucaria trees would shape sapling richness, abundance and composition. Our results indicated that abundance and species composition are affected by land management and araucaria crown influence. The highest values of sapling abundance were found beneath crowns in unmanaged areas. Species composition changed between all assessed combinations of land management and crown influence. Our study demonstrated the major roles of land management and facilitation in structuring communities, despite the effects of rock and grass cover. As araucaria is a keystone species in the successional dynamics and expansion of forest over grasslands, in the third chapter, early stage performance of araucaria was assessed in soils found across a forest-grassland gradient and with varying soil microbial composition. Soil type and microbiota were sampled from grassland, shrubland, forest edge, and forest interior. Microbial composition was either kept unchanged (unsterilized), dismantled (sterilized) or inoculated (sterilized and re-

added with soil extract). Early stage performance on experimental treatments was inferred by both germination rates and early seedling growth, the latter by means of three belowground and two aboveground traits. Contradicting our expectations, soil microbial communities seemed neutral to araucaria germination and detrimental to the early growth of araucaria seedlings. Such net negative effect of soil microbial communities was found across the forest-grassland gradient. Seedling performance was generally worse when grown in forest interior soils where adult trees of araucaria are abundant. Conversely, dismantling soil microbial communities resulted in improved both belowground and aboveground seedling growth and biomass uptake. Such results show that soils differences alone could allow a faster rate of forest expansion over grasslands. I conclude that management practices traditionally employed by local managers, using mainly fire and cattle, can contribute to the maintenance of grasslands by hindering shrub encroachment and forest expansion. The facilitation process promoted by araucaria trees contributes for structuring communities of forest species and accelerating the expansion process. The soil microbiota, in turn, negatively affects the establishment of araucaria, thus also hindering forest expansion. Therefore, there is a need to recognize the importance of grasslands as cultural landscapes and fire as a natural element in grasslands in order to improve the conservation of these threatened ecosystems and their natural processes. Also, it is necessary to recognize traditional management practices employed in grasslands to establish effective public policies of conservation and management. Our results clarified patterns and processes that emerge in highland grasslands, such as the conversion grasslands into shrublands, the facilitation process promoted by araucaria, and the negative influence of soil microbiota on araucaria. In this sense, we reinforce that the loss of cultural landscapes may become imminent when the main traditional management actions are excluded.

**Keywords:** Araucaria; Atlantic Forest; Communities structuring; Conservation; Cultural landscapes; Fire; Historical ecology; Interactions; Management; Mixed rainforest; Niche construction;



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# 1. Introdução Geral

## 1.1 Ecologia histórica

Muitos fenômenos ecológicos são impossíveis de serem totalmente compreendidos quando processos agindo por décadas ou séculos são ignorados (VELLEND et al., 2013). Atividades humanas vêm moldando a aparência e função de regiões, paisagens e ecossistemas por milênios (BÜRGI; GIMMI, 2007). A ecologia histórica é uma disciplina que se dedica ao estudo das interações entre humanos e o ambiente e de suas consequências na formação de culturas e paisagens contemporâneas e passadas (BALÉE, 2006). Assim, a ecologia histórica é uma abordagem interdisciplinar (*e.g.*, abrangendo aspectos da antropologia, geografia, genética, biologia e ecologia), que tem como elemento central a paisagem histórica (BALEE; ERICKSON, 2006; CRUMLEY, 1996). A paisagem histórica é uma entidade física multidimensional, que possui atributos espaciais e temporais, que foi e que tem sido modificada por atividades humanas, tanto de maneira pouco perceptível como de forma dramática (BALEE; ERICKSON, 2006). Desta forma, a ecologia histórica assemelha-se à ecologia de paisagem, a qual estuda a relação da estrutura da paisagem com a função ecológica (IVERSON NASSAUER, 1995). Porém, a principal diferença entre elas é de que algumas definições de ecologia da paisagem fazem uma distinção entre paisagens “sem influência humana” e paisagens “com influência humana”, sendo esta última geralmente postulada como degradada ou simplificada (BALEE; ERICKSON, 2006; ERICKSON, 2006; IVERSON NASSAUER, 1995). No entanto, paisagens sem influência humana são consideradas utópicas, uma vez que praticamente não existe qualquer local no planeta onde humanos não tenham pisado (CLEMENT; JUNQUEIRA, 2010; DENEVAN, 1992; VITOUSEK et al., 1997). A ecologia histórica vê o humano como uma espécie-chave, agindo por exemplo, através da construção de nichos, domesticação de espécies e paisagens e do distúrbio, dessa forma integrando a dinâmica ambiental, podendo inclusive aumentar a riqueza de espécies e diversidade de paisagens (BALEE; ERICKSON, 2006; CLEMENT et al., 2015; VELLEND, 2017; ZEDER, 2016). Portanto, a

ecologia histórica representa uma nova perspectiva de compreensão das complexas relações históricas entre humanos e a biosfera.

## 1.2 A construção de nichos e a domesticação de paisagens

A construção de nicho é o mecanismo pelo qual os organismos, através de seu metabolismo, suas atividades e suas escolhas modificam seus próprios nichos (ODLING-SMEE et al., 2013). A alteração das condições físicas e químicas, construção de tocas e ninhos, sombreamento, alterações na velocidade do vento e a alteração do ciclo de nutrientes pelas plantas são alguns exemplos desse mecanismo. Quando essas modificações alteram as pressões de seleção natural, a evolução pela construção de nicho é um resultado possível (LALAND; MATTHEWS; FELDMAN, 2016). A domesticação de animais e plantas e a modificação do solo podem resultar em paisagens domesticadas com altos níveis de produtividade e maior diversidade de nichos do que paisagens sem ações humanas (CLEMENT et al., 2015; ZEDER, 2016). A domesticação pode ser definida em uma perspectiva ecológica como sendo mutualismos coevolucionários que se desenvolvem através da construção ativa de nichos por seres humanos e plantas ou animais (ZEDER, 2016). Na América do Sul, por exemplo, paisagens domesticadas por sociedades pré-Colombianas podem ser encontradas em uma ampla gama de ambientes, tanto tropicais (CLEMENT et al., 2015; DENEVAN, 2003; ERICKSON, 2006; LEVIS et al., 2017), como subtropicais (*e.g.*, REIS; LADIO; PERONI, 2014). Embora exista uma interdependência entre o manejo cultural humano, interações entre espécies e o ambiente, ainda compreendemos pouco como que estes elementos afetam a diversidade, composição e a distribuição de espécies (BOIVIN et al., 2016; SEDREZ DOS REIS; LADIO; PERONI, 2014). Assim, para a compreensão da dinâmica atual da paisagem, torna-se crucial reconhecer que eventos do passado, muitas vezes milenares, tenham moldado ecossistemas ao redor do mundo (SEDREZ DOS REIS; LADIO; PERONI, 2014; SWETNAM; ALLEN; BETANCOURT, 1999). A construção de nicho por meio da domesticação de animais e plantas (ZEDER, 2006, 2015, 2016) é um exemplo de como atividades humanas podem afetar a estrutura de comunidades (BOIVIN et al., 2016).

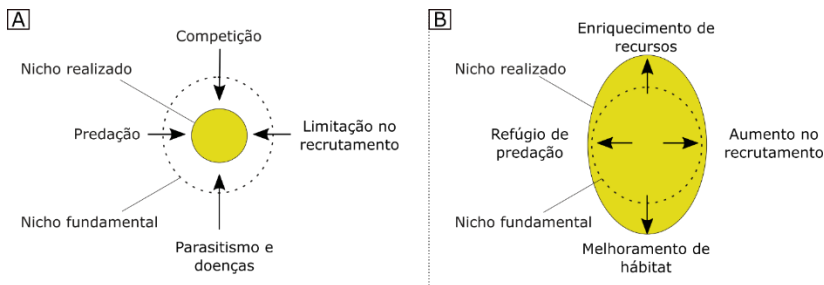
### 1.3 Estruturação de comunidades

Compreender a dinâmica que gera padrões na estrutura das comunidades é uma das grandes prioridades na ecologia (SUTHERLAND et al., 2013). Comunidades vegetais, de maneira geral, são estruturadas por condições abióticas locais, interações bióticas diretas e indiretas entre plantas, interações diretas com outros organismos e processos estocásticos (LORTIE et al., 2004; STACHOWICZ, 2001). Interações bióticas podem promover mudanças em padrões de grande escala na distribuição de espécies, afetando processos mais amplos como a migração, especiação e extinções (WISZ et al., 2013). A combinação de interações negativas (como competição, predação e herbivoria) e fatores abióticos (como fatores relacionados ao clima, solos) configuram os principais componentes da maioria dos modelos de estrutura de comunidades e teorias ecológicas, como por exemplo, a teoria de nicho (BRUNO; STACHOWICZ; BERTNESS, 2003; STACHOWICZ, 2012). Atualmente, a teoria do nicho é uma das principais bases de compreensão da estruturação de comunidades animais e vegetais (SILVERTOWN, 2004). O nicho de uma espécie é formado pelo nicho fundamental e realizado de todos os seus indivíduos (HUTCHINSON, 1957). Figura 1A. O nicho fundamental é a amplitude de condições ambientais na qual uma espécie é fisiologicamente capaz de se desenvolver na ausência de interações. O nicho realizado é a amplitude na qual a espécie é efetivamente encontrada, após a ação de competidores e predadores. Desta forma, espécies circunvizinhas agem de forma negativa (predação, competição, parasitismo) umas nas outras, causando uma restrição no nicho realizado (SILVERTOWN, 2004). Esta ideia está baseada no princípio da exclusão competitiva, a qual prediz que duas espécies não podem ocupar o mesmo nicho (BRUNO; STACHOWICZ; BERTNESS, 2003). No entanto, interações positivas, como a facilitação, também são importantes influenciadores da composição de espécies, diversidade e dinâmica das comunidades (CALLAWAY, 1995; STACHOWICZ, 2001).

## 1.4 Facilitação

A facilitação é um mecanismo importante na estruturação de comunidades terrestres e aquáticas (BROOKER et al., 2008; BRUNO; STACHOWICZ; BERTNESS, 2003; CALLAWAY, 2007; HE; BERTNESS; ALTIERI, 2013). Entende-se por facilitação qualquer relação entre organismos que, preponderantemente beneficie ao menos um dos participantes, sem causar dano ao(s) outro(s) (BRUNO; STACHOWICZ; BERTNESS, 2003). O caráter destas interações pode ser obrigatório (resultado de uma coevolução mutualística) ou facultativo entre os organismos envolvidos. Estas interações positivas são consideradas tão importantes quanto interações negativas (*e.g.*, competição, predação) no que se refere em determinar a dinâmica de comunidades e processos ecossistêmicos (TRAVIS; BROOKER; DYTHAM, 2005). Ao incorporar interações positivas nas teorias ecológicas clássicas, novos paradoxos podem emergir. Por exemplo, ao incluir a facilitação (interação positiva) na teoria do nicho, a extensão espacial do nicho realizado de uma espécie pode ultrapassar os limites espaciais preditos pelo nicho fundamental (BRUNO; STACHOWICZ; BERTNESS, 2003). Figura 1B. Em outras palavras, como o nicho é um princípio fundamental na ecologia, estas mudanças (inclusão de interações positivas) podem culminar em um efeito cascata dentro do panorama geral da ecologia teórica (BRUNO; STACHOWICZ; BERTNESS, 2003). Há evidências de que a facilitação tende a aumentar com a severidade física do ambiente (hábitats com elevado estresse abiótico ou alta pressão de herbívoros) (BOIVIN et al., 2016; CALLAWAY, 1995). Desta forma, em hábitats como marismas, desertos, tundra ártica, sistemas alpinos e ambientes de elevadas altitudes (ambientes montanos e alto-montanos), a facilitação pode ser mais importante do que as interações negativas na estruturação de comunidades (BROOKER et al., 2008; TRAVIS; BROOKER; DYTHAM, 2005). Estes hábitats, inclusive, tendem a ser sensíveis às mudanças climáticas, e a ausência de espécies facilitadoras nestes locais pode resultar em um retardo nos processos migratórios (tanto de plantas como animais), levando à extinção local de espécies (ANTHELME; CAVIERES; DANGLES, 2014).





*Figura 1. Conceitos de nicho. A: conceito de nicho tipicamente abordada (Nicho “Hutchinsoniano”) em que interações negativas regulam a expansão potencial de uma espécie. E B: ao incorporar facilitação no conceito de nicho, interações positivas podem reduzir os efeitos de constrição de nicho, fazendo com que o nicho realizado seja maior que o fundamental. Adaptada de Bruno et al., (2003).*

De maneira geral, espécies de plantas facilitadoras (plantas-berçário, *nurse plants*) alteram o ambiente e podem mitigar estressores potencialmente limitantes, criando habitats adequados para outras espécies (STACHOWICZ, 2012). Embora não se enquadrem dentro do conceito de facilitação, objetos, como rochas e troncos, podem modificar condições microclimáticas e/ou proteger contra o fogo e/ou danos físicos promovidos por animais (BORCHERT et al., 2003; CARLUCCI; DUARTE; PILLAR, 2011; PYATT et al., 2016). Estas alterações ambientais promovidas tanto por estruturas abióticas quanto por entidades bióticas beneficiam outras espécies, que passam a ter oportunidade de se estabelecer em locais antes desfavoráveis. Por exemplo, a facilitação promovida por arbustos e árvores pode aumentar a frequência de polinizadores em um determinado local, oferecer proteção contra herbivoria, reduzir a temperatura do ar, aumentar a umidade do solo, oxigenação e disponibilidade de nutrientes, assim melhorando as condições ambientais sob sua copa (CALLAWAY; PUGNAIRE, 2007; KORNDÖRFER; DILLENBURG; DUARTE, 2015; MORO et al., 1997). Essas mudanças em escala local, induzidas direta ou indiretamente por estas espécies, podem culminar na determinação de padrões e

processos mais amplos, superando fatores de escala regional (BEKKER, 2005). Determinar a amplitude e a importância dessas interações indiretas nas comunidades ecológicas está dentre os grandes objetivos na ecologia contemporânea (SUTHERLAND et al., 2013).

#### 1.4.1 Facilitação e a microbiota do solo

Compreender como a biodiversidade abaixo do solo afeta a biodiversidade acima do solo também é uma das grandes questões contemporâneas da ecologia (SUTHERLAND et al., 2013). Recentemente, têm-se demonstrado que a microbiota do solo, além de influenciar fortemente na performance individual de plantas, também pode estruturar comunidades vegetais, atuando no processo de facilitação planta-planta. Por exemplo, a microbiota do solo influenciada ou cultivada pela planta facilitadora pode ter um importante papel nas interações positivas entre espécies de plantas, através de processos independentes da influência direta da espécie facilitadora (RODRÍGUEZ-ECHEVERRÍA et al., 2013). Neste mesmo contexto, manchas de plantas podem mitigar os efeitos de condições extremas em comunidades associadas de plantas e microbiota, promovendo aumento da biodiversidade do solo e melhorando o funcionamento do ecossistema (HORTAL et al., 2015). À medida em que a espécie facilitadora cresce, sua comunidade microbiana do solo pode mudar quanto à composição, biomassa e atividade (HORTAL et al., 2013). Desta forma, o balanço entre a facilitação e a interferência é influenciado diretamente pelo nível de estresse abiótico (GREENLEE; CALLAWAY, 1996), mas também por diferentes estágios ontogenéticos, tamanho, densidade das espécies que interagem, entre outros fatores (CALLAWAY; WALKER, 1997). Estes exemplos demonstram a existência de interações complexas entre comunidades acima e abaixo do solo, revelando que a comunidade microbiana pode influenciar na diversidade, composição e abundância, crescimento e produtividade, disponibilidade de nutrientes do solo e até mesmo no funcionamento de ecossistemas e na construção de nichos (ODLING-SMEE et al., 2013; RODRÍGUEZ-ECHEVERRÍA et al., 2013).

## 1.5 A floresta com araucária e os campos

### 1.5.1 A floresta com araucária

A floresta com araucária (Floresta Ombrófila Mista) tem seus maiores maciços ocorrendo no planalto Sul-brasileiro (VELOSO; RANGEL FILHO; LIMA, 1991), em elevações variando de 500 a 1800 m acima do nível do mar (a.n.m.) (REITZ; KLEIN, 1966). Nestas florestas, a árvore *Araucaria angustifolia* (Bertol.) Kuntze (a partir de agora, “araucária”) geralmente é abundante e domina o estrato arbóreo (KLEIN, 1984). Nas porções elevadas do planalto, especialmente acima dos 900 m, a floresta com araucária frequentemente associa-se aos campos de altitude e arbustais, formando mosaicos (KLEIN, 1984) (Figura 2). A presença e grande abundância da araucária e outras espécies associadas em meio às florestas subtropicais passou a intrigar muitos naturalistas. Por exemplo, KLEIN (1960), levantou questões sobre uma possível expansão destas florestas sobre os campos. Isto de fato ocorreu a partir de 4.000 anos antes do presente (A.P.), quando o clima, antes mais seco e mais frio (LEDRU; SALGADO-LABOURIAU; LORSCHETTER, 1998), tornou-se mais úmido, favorecendo espécies arbóreas a se expandirem sobre os campos, de maneira lenta e gradual (BEHLING, 1995, 1998; BEHLING et al., 2004; SILVA; ANAND, 2011). No entanto, entre 2.500 e 1.000 anos A.P., a expansão da floresta com araucária sobre os campos passou a ocorrer de forma mais acelerada e o fogo tornou-se mais frequente, apesar de o clima permanecer o mesmo (BEHLING et al., 2004). Assim, mecanismos de *feedback* bióticos também parecem ter tido um importante papel para a expansão e persistência das florestas a longo prazo (SILVA; ANAND, 2011). Esta súbita expansão da floresta com araucária sobre os campos corresponde à chegada de diferentes tradições de sociedades agricultoras que foram se sedentarizando (Tupi e Macro-Jê) no sul do Brasil (BITENCOURT; KRAUSPENHAR, 2006; LAUTERJUNG et al., 2018; REIS; LADIO; PERONI, 2014), e foi intensa a ponto de que poderia ter substituído a totalidade dos campos dentro de um período de apenas 100 anos (IRIARTE; BEHLING, 2007). Durante milênios estes povos utilizaram e manejaram os recursos naturais das paisagens, incluindo a araucária, a qual foi domesticada, e assim contribuíram para a expansão da

floresta com Araucária (LAUTERJUNG et al., 2018; SEDREZ DOS REIS; LADIO; PERONI, 2014; ZECHINI et al., 2018). Assim, torna-se fundamental a inclusão de aspectos relacionados ao manejo histórico na compreensão da dinâmica envolvendo paisagens campestres-florestais.



*Figura 2. Os campos subtropicais de altitude e floresta com araucária no sul do Brasil. Estes ecossistemas geralmente ocorrem associados a partir de 900 m de elevação. Foto do autor, a 1650 m de altitude.*

### 1.5.2 A Domesticação de paisagens no sul do Brasil: passado e presente

No Sul do Brasil, sociedades humanas caçadoras-coletoras chegaram por volta de 12.000 anos A.P., e muitos povos sucessivamente ocuparam as terras altas, dentro dos domínios da floresta com araucária (BUENO; DIAS; STEELE, 2013; NOELLI, 2000). Estas sociedades usavam diversos recursos florestais, como madeira, frutos e sementes, atuando na dispersão de espécies (NOELLI, 2000), inclusive da própria araucária, ao se alimentaram das sementes-*pinhões*- (BITENCOURT; KRAUSPENHAR, 2006; LAUTERJUNG et al., 2018; SEDREZ DOS REIS; LADIO; PERONI, 2014). O uso e manejo de recursos florestais se intensificou há cerca de 2.500 anos A.P., com a

chegada de diferentes tradições de sociedades agricultoras sedentárias (Tupi e Macro-Jê) (NOELLI, 2000, 2008), e o fogo passou a ficar mais frequente nessas paisagens (BEHLING et al., 2004). A intensidade de uso e manejo dos recursos e das paisagens por estes povos durante longos períodos culminou na domesticação destas paisagens (SEDREZ DOS REIS; LADIO; PERONI, 2014). Após a chegada dos colonizadores europeus por volta de 500 anos A.P., as guerras e doenças dizimaram a maioria das populações tradicionais, assentamentos, culturas e paisagens (NOELLI, 2000, 2008; SEDREZ DOS REIS; LADIO; PERONI, 2014). O colapso desses povos possivelmente resultou em uma drástica redução na frequência de fogo e assim possa ter contribuído para a expansão da floresta (JESKE-PIERUSCHKA et al., 2010). Atualmente, proprietários de áreas com ocorrência natural de campos de altitude, que têm como atividade principal a criação de gado de forma extensiva (de agora em diante: gestores locais), regularmente utilizam o fogo como ferramenta para promover a rebrota dos campos para alimentar o gado (NABINGER; MORAES; MARASCHIN, 2000). Estas práticas de manejo (fogo e gado) podem reduzir a expansão natural da floresta e contribuir para a manutenção dos campos (OVERBECK et al., 2007; PILLAR, 2003), e, juntamente com áreas protegidas, podem garantir uma alta diversidade nas paisagens dessas regiões (SÜHS; GIEHL; PERONI, 2018).

O processo de expansão da floresta com araucária ocorre a partir da borda de florestas contínuas e a partir da formação de núcleos isolados (mecanismo sucessional conhecido por nucleação) (e.g., CARLUCCI et al., 2011; DUARTE et al., 2006; KLEIN, 1960; OLIVEIRA; PILLAR, 2004; SILVA; ANAND, 2011). A expansão da floresta com araucária através da formação de núcleos isolados foi inicialmente proposta por KLEIN (1960), destacando que espécies pioneiras seriam capazes de colonizar os campos e assim promover o estabelecimento de outras espécies florestais sob suas copas. Este conceito foi recentemente refinado por alguns estudos, que demonstraram que os núcleos se estabelecem em áreas de campo a partir de espécies facilitadoras e também rochas (e.g., CARLUCCI; DUARTE; PILLAR, 2011; DUARTE et al., 2006). A expansão a partir das

bordas de florestas também parece ser mediada pela facilitação, uma vez que esta expansão depende de espécies de árvores facilitadoras que se estabelecem em áreas de campo próximas às bordas (CARLUCCI et al., 2011). A araucária é considerada uma das principais espécies no processo da expansão, sendo este um dos motivos pela qual é considerada uma espécie-chave nos planaltos do sul do Brasil. Figura 3. O aumento de propágulos de espécies florestais sob sua copa ocorre através do efeito poleiro, onde aves frugívoras dispersam sementes de espécies florestais ao utilizar a araucária como local de pouso (DOS SANTOS et al., 2011; DUARTE et al., 2006). O caráter facilitador da araucária gera melhorias nas condições ambientais sob suas copas ao atenuar altas temperaturas no verão, reduzir a perda de água de outras espécies e aumentar a disponibilidade de nutrientes no solo (KORNDÖRFER; DILLENBURG; DUARTE, 2015). Estas características fazem com que a espécie seja uma espécie nucleadora (*sensu* YARRANTON; MORRISON, 1974) e facilitadora (*sensu* BERTNESS; CALLAWAY, 1994) nestas regiões. Desta forma, sua presença promove um aumento gradual da riqueza e densidade de outras espécies sob sua copa, contribuindo para a expansão da floresta sobre o campo (DUARTE et al., 2006). Estudos avaliando o caráter facilitador da araucária em regiões mais altas, onde o estresse abiótico (como maiores incidências de ventos, geadas e temperaturas mais baixas e maior amplitude térmica) tende a ser maior (afetando o processo de facilitação - BERTNESS; CALLAWAY, 1994), são fundamentais para a compreensão do processo de expansão da floresta sobre os campos.



*Figura 3. Os campos subtropicais de altitude e floresta com araucária no sul do Brasil. Note os afloramentos de rocha, que também podem contribuir para a expansão da floresta, na parte superior; e as araucárias, que aceleram o processo de expansão, na porção inferior, próximas à borda da floresta. Foto do autor, a 1650 m de altitude.*

### 1.5.3 Os campos

Os Campos Sulinos (OVERBECK et al., 2007), de agora em diante: “campos”, são ecossistemas que ocorrem na região sul do Brasil, compreendendo os campos temperados de baixas altitudes que pertencem a ecorregião Pampa e os campos subtropicais de altitude (Campos de Cima da Serra) que pertencem à ecorregião Mata Atlântica (BARROS et al., 2015; DE PATTA PILLAR; VÉLEZ, 2010). Os campos de altitude do sul do Brasil (campos subtropicais de altitude) compõem uma formação vegetal frágil e peculiar pois possuem altos níveis de endemismos, baixa similaridade com outras formações campestres do sul e sudeste do Brasil e ocorrem frequentemente isolados em manchas, cercados por formações vegetais distintas (BARROS et al., 2015; IGANCI et al., 2011). O elevado número de espécies endêmicas comprova que esses campos existem há tempo suficiente para especiação e, portanto, não representam um simples estágio sucessional de florestas perturbadas (BARROS et al., 2015). KLEIN, (1984) considerava esses campos como relictos de um clima pretérito, possivelmente

mantidos através do fogo e/ou atividades humanas. Os campos estão muito mais sujeitos à transformação para outros usos da terra do que as florestas (OVERBECK et al., 2018). Figura 4.

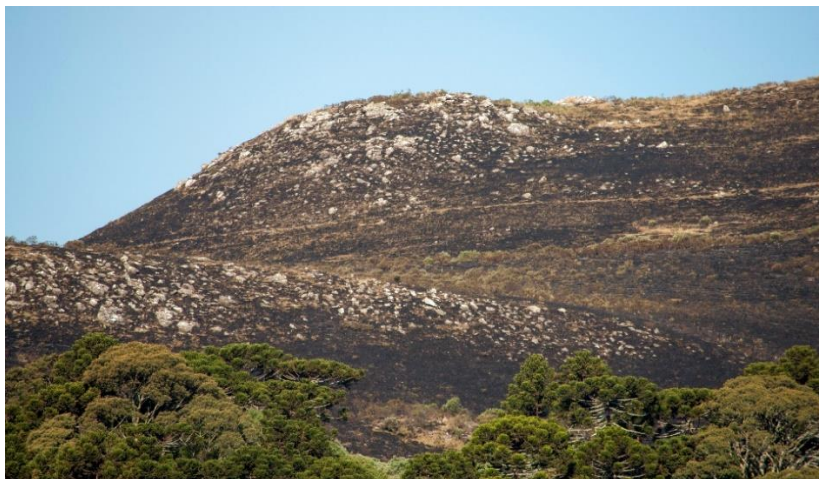


*Figura 4. Os campos subtropicais de altitude. No centro da imagem a presença de pastadores de grande porte (gado). Ao fundo a floresta com araucária. Foto do autor, a 1650 m de altitude, em 27/07/2014.*

#### *1.5.3.1 O uso dos campos*

A ocorrência de distúrbios, como a herbivoria por grandes pastadores (no passado através de herbívoros da extinta megafauna e, atualmente, o gado) e o fogo, faz parte da dinâmica natural dos campos (DE PATTA PILLAR; VÉLEZ, 2010; OVERBECK et al., 2007). Os gestores locais utilizam o fogo como ferramenta para promover o surgimento de vegetação campestre e controlar o adensamento de arbustos, impedindo assim a expansão da floresta (OVERBECK et al., 2007; SÜHS; GIEHL; PERONI, 2018). O fogo é provavelmente o fator mais importante controlando a dinâmica dos mosaicos campestres-florestais e o responsável pela formação das bordas abruptas entre esses dois ecossistemas (JESKE-PIERUSCHKA et al., 2010). Figura 5.





*Figura 5. A queima dos campos. O fogo é utilizado por gestores locais em suas áreas como ferramenta para promover a rebrota dos campos e servir como alimento para o gado. Foto do autor, a 1550 m de elevação em 14/09/2014.*

O uso de fogo na gestão de áreas de campos, tanto em áreas privadas como em áreas protegidas, ainda é controverso (MISTRY et al., 2018). Sistemas pastoris onde o fogo é utilizado ainda são subvalorizados por agências ambientais e gestores de áreas protegidas (ELOY et al., 2018). Essas políticas de “fogo-zero” são adotadas por vários países - incluindo o Brasil - para evitar e controlar o fogo em ecossistemas propensos ao fogo (MISTRY et al., 2018). Desta forma, os efeitos dessas práticas de manejo tornam-se pouco conhecidos na paisagem e na biodiversidade. Com a retirada do fogo, esses ecossistemas passam a enfrentar um risco crescente de incêndios catastróficos devido ao acúmulo de biomassa inflamável. Incêndios catastróficos têm efeitos devastadores sobre a biodiversidade (BEHLING; PILLAR, 2007), bem-estar humano e valores culturais paisagísticos (SÜHS; GIEHL; PERONI, 2018). Quando o fogo e o pastoreio são evitados, touceiras e arbustos tendem a substituir pequenas ervas e gramíneas (BOLDRINI; EGGERS, 1996; NABINGER; MORAES; MARASCHIN, 2000), levando as áreas não-

queimadas e não-pastoreadas ao adensamento de arbustos ao longo do tempo (BEHLING; PILLAR, 2007; OLIVEIRA; PILLAR, 2004; OVERBECK et al., 2007). É importante ressaltar que a conservação da dinâmica campestre-florestal não fique restrita apenas à esses dois estados alternativos estáveis, mas que também vise conservar estados transientes, como os arbustais, que possivelmente servem de abrigo/refúgio para a fauna (CARLUCCI et al., 2016). Embora seja difícil de prever o destino dos campos onde o adensamento de arbustos ocorre (AUKEN, 2000), áreas adensadas por arbustos podem se desenvolver em florestas (e.g., DUARTE et al., 2006) ou permanecer em um estado estável de arbustos, onde a floresta nunca se desenvolve (DECHOUM; PERONI; PUGNAIRE, 2018). Assim, o tipo de manejo utilizado nos campos pode conduzir a dinâmicas de vegetação distintas. Portanto, torna-se fundamental quantificar as taxas de mudança na paisagem ao longo do tempo tanto em áreas com o manejo tradicional quanto em áreas sem o manejo tradicional.

#### 1.5.4 A conservação dos campos e da floresta com araucária

Tanto a floresta com araucária como os campos subtropicais de altitude e seus ecossistemas associados estão seriamente ameaçados (CARLUCCI et al., 2016). A floresta com araucária encontra-se altamente fragmentada e ocupa hoje cerca de 12% de sua cobertura original onde apenas cerca de 3% encontra-se protegida (RIBEIRO et al., 2009). A própria araucária, principalmente devido à perda de habitat e à forte exploração madeireira está criticamente ameaçada de extinção (GUERRA et al., 2002; THOMAS, 2013). Já os campos, além de negligenciados por políticas de conservação (OVERBECK et al., 2007), estão ameaçados por uma série de fatores (ANDRADE et al., 2019; IGANCI et al., 2011), que incluem: a invasão por espécies exóticas (e.g., ZALBA; VILLAMIL, 2002), más práticas de gestão (e.g., OVERBECK et al., 2007) e mudanças climáticas (e.g., ASSIS; DE MATTOS, 2016), sendo estes dois últimos responsáveis pelo aumento no adensamento de arbustos – *shrub encroachment* (e.g., BEHLING; PILLAR, 2007; DECHOUM; PERONI; PUGNAIRE, 2018; OLIVEIRA; PILLAR, 2004). Apesar da grande diversidade, dos altos níveis de endemismo e dos incontáveis serviços ecossistêmicos, o grau de proteção dos campos

permanece muito baixo, pois apenas 0,33% desses ecossistemas estão protegidos em unidades de conservação integral no Brasil (DE PATTA PILLAR; VÉLEZ, 2010; OVERBECK et al., 2007). Esse número tende a ser ainda mais reduzido ao considerarmos apenas os campos subtropicais de altitude (que estão associados às florestas com Araucária nos planaltos). Além disso, quando em unidades de conservação, os campos geralmente não têm manejo adequado, uma vez que distúrbios importantes, como a herbivoria por grandes pastadores e o fogo, tendem a ser suprimidos (OVERBECK et al., 2018). Desta forma, unidades de conservação de uso sustentável, com manejo adequado, possivelmente são alternativas mais eficientes para a conservação dos campos (CARLUCCI et al., 2016). Dentre as más práticas de gestão, incluem-se a substituição dos campos por pastagens exóticas ou monoculturas (como *Pinus spp.*), o sobrepastoreio e o uso excessivo do fogo (BARROS et al., 2015). Por isso, conhecer como os campos subtropicais de altitude são utilizados e os efeitos de sua utilização na paisagem ao longo do tempo, são aspectos fundamentais para embasar a tomada de decisões relacionadas às políticas públicas de conservação.

## 1.6 Objetivo Geral

Considerando que o clima, as pessoas e a própria araucária são importantes agentes no processo de expansão da floresta sobre o campo e responsáveis pela formação de padrões na estrutura de comunidades, o objetivo geral da presente tese foi de avaliar os efeitos da construção de nicho na paisagem, na biodiversidade e no estabelecimento de uma espécie-chave envolvida na expansão da floresta sobre os campos. A tese tem por objetivos específicos:

- 1) avaliar o tipo de manejo utilizado na paisagem por gestores/proprietários e verificar seus efeitos nos campos subtropicais de altitude;
- 2) investigar o papel da facilitação, do manejo tradicional, interações bióticas e fatores abióticos na estruturação de comunidades; e

- 3) avaliar os efeitos de comunidades microbianas do solo no desenvolvimento de uma espécie-chave envolvida na expansão da floresta sobre os campos.

Cada objetivo específico foi desenvolvido em formato de artigo científico, os quais foram organizados em capítulos para a presente tese.

## 2. Materiais e métodos

### 2.1 Área de estudo

#### 2.1.1 Parque Nacional de São Joaquim

A presente tese foi desenvolvida na região do Parque Nacional de São Joaquim (PNSJ). O PNSJ é uma Unidade de Conservação (UC) Federal, localizado nos municípios de Bom Jardim da Serra, Urubici, Lauro Müller, Grão Pará e Orleans, no estado de Santa Catarina, Brasil (Latitude: 28,19 ° S, Longitude: 49,53 ° O). Esta UC foi criada através do Decreto nº 50.922 de 06 de julho de 1961 / Lei nº 13.273 de 15 de abril de 2016 e possui 49.672,38 hectares (ICMbio, 2019). Figura 6. Desde o ano de 2006 até o ano de 2019, cerca de 14.500 hectares foram desapropriados de diversos proprietários pelo órgão ambiental federal, através do processo de indenização. Desta forma, somando-se às áreas devolutas (cerca de 10.000 hectares), estas áreas regularizadas atualmente compreendem cerca de 46% da área total da UC. As duas ecorregiões existentes no PNSJ são as matas úmidas de Araucária (*Araucaria moist forests*) e as florestas costeiras da Serra do Mar (*Serra do Mar coastal forests*), ambas dentro do bioma das Florestas Tropicais e Subtropicais úmidas (*Tropical & Subtropical Moist Broadleaf Forests*) – (OLSON et al., 2001). Os principais tipos de vegetação na UC incluem campos subtropicais de altitude, floresta subtropical mista (floresta com araucária) e floresta subtropical úmida (Atlântica). O clima, na região serrana, entre 1961 e 2016, registrado pela estação meteorológica mais próxima da UC (cerca de 30 km), caracterizou-se por uma precipitação média anual de 1.626,3 mm.ano<sup>-1</sup>, distribuída igualmente ao longo do ano e uma temperatura média anual de 13,3 ° C. A temperatura mínima média para o mês mais frio (julho) foi de

6,0 ° C e a temperatura média máxima para o mês mais quente (janeiro) foi de 22,9 ° C. A temperatura mínima absoluta registada foi de -9,0 ° C e a temperatura máxima absoluta foi de 31,4 ° C. Durante o inverno, as geadas são comuns e, ocasionalmente, neva. Dados climáticos compilados de ('INMET - Instituto Nacional de Meteorologia', [s.d.]). Na região, especialmente nos campos de altitude, gestores locais utilizam o fogo para promover a brotação da vegetação campestre para atividades relacionadas à pecuária extensiva.

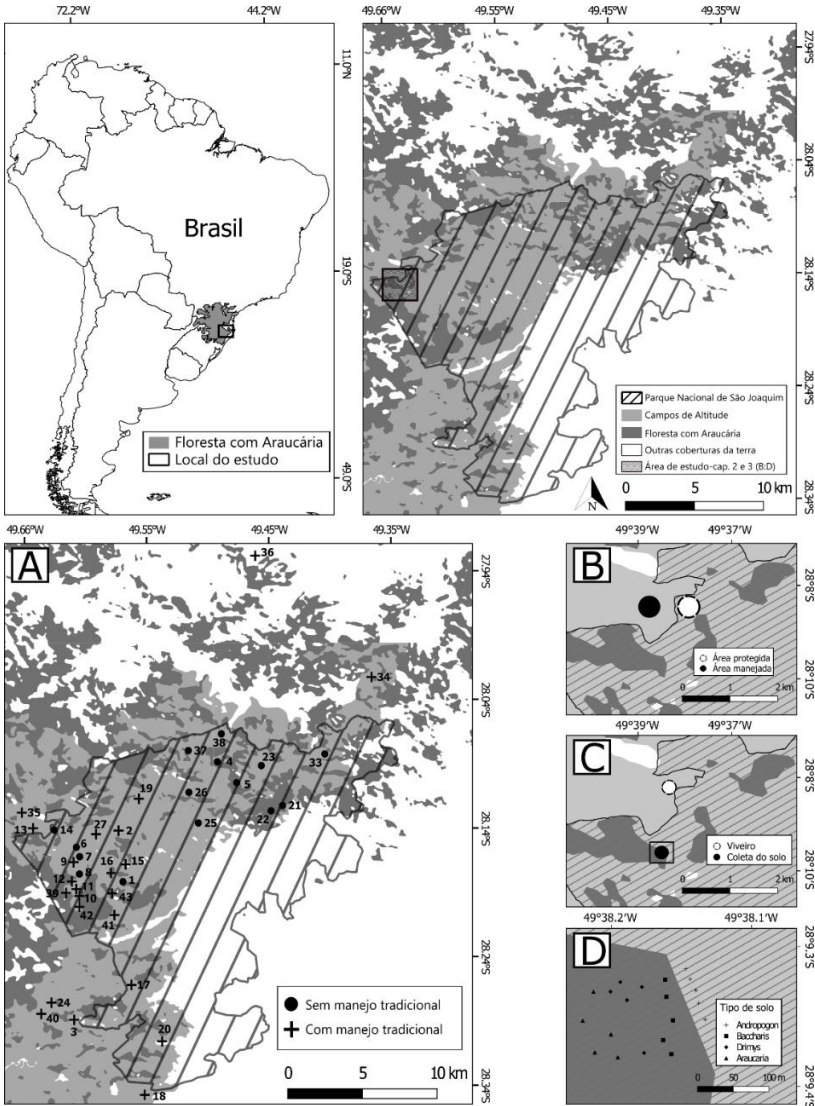


Figura 6. Mapa da área de estudo da tese e dos respectivos capítulos: Capítulo 1: “A”, capítulo 2: “B”, capítulo 3: “C” e “D”.

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## 4. Capítulo 1<sup>†</sup>

### Preventing traditional management can cause grassland loss within 30 years

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## Abstract

Woody encroachment threatens several ecosystems around the world. In general, management of grasslands includes regulation of fire and grazing regimes. Changes in these two types of disturbances are potential drivers of woody encroachment. Here we assessed how the traditional management carried out by local landholders affects a highland grassland ecosystem in southern Brazil. We hypothesized that grasslands converted to protected areas undergo fast woody encroachment. To reconstruct changes in vegetation, we talked to former and current landholders and coupled their knowledge with an analysis of aerial and satellite images. After a few years of fire and cattle removal, rates of woody encroachment in grasslands increased exponentially. Woody encroachment occurred mostly by the replacement of grasslands by shrublands. Meanwhile, grasslands under traditional management remained almost unchanged for the last 40 years. Local farmers seem to manage fire well because it is part of their traditional practices for decades. Such management prevents large-scale wildfires and maintains natural highland grasslands. The quick pace of shrub encroachment in such grasslands threatens its exclusive diversity, human well-being and regional cultural heritage. Thus, conservation policies are needed to regulate and instruct about the use of fire as a management tool in highland grasslands of southern Brazil.

## Introduction

Extensive vegetation change can have important consequences in ecosystem functioning and economy (VENTER; CRAMER; HAWKINS, 2018). For instance, woody encroachment (or woody plant encroachment), which is the increase in density, cover and biomass of shrubs or woody vegetation in grasslands (AUKEN, 2000), has been reported around the world (ELDRIDGE et al., 2011). Woody encroachment is caused by several factors such as shifts in climate and biogeochemical cycles, changes in disturbance regimes (e.g. fire and grazing), or modification in ecological succession by introduction of non-native species or predator removal (ARCHER et al., 2017; AUKEN, 2000; ELDRIDGE et al., 2011; VAN AUKEN, 2009). Woody encroachment alters fundamental ecological processes of ecosystems, including global carbon balances, reduction of water flow or groundwater recharge and the loss of biodiversity (ARCHER et al., 2017; OVERBECK et al., 2007; SCHOLES; ARCHER, 1997). Because woody encroachment tends to negatively affect herbaceous vegetation, it constitutes a major threat to savanna and grassland ecosystems.

Control of woody encroachment is a key concern in rangelands. Woody encroachment reduces forage production, creates habitat for ectoparasites, and hampers animal handling (ARCHER et al., 2017). Woody encroachment concerns land managers and methods for control and eradication include either fire, cutting trees, grazing, or a combination of these three methods (ANGASSA; OBA, 2009; TROLLOPE, 1980). Woody encroachment has been reported all over the world, e.g., southern Ethiopian savanna (ANGASSA; OBA, 2009), North American savanna, shrub-steppe and grasslands ecosystems (BARGER et al., 2011), southeastern South American savanna (BERNARDI et al., 2016; called 'Campos', OVERBECK et al., 2007), and Brazilian savanna (called 'Cerrado', ELOY et al., 2018). In highland grasslands of southern Brazilian, extensive cattle grazing is part of the traditional management employed for centuries (REIS; LADIO; PERONI, 2014). In addition, local ranchers control fire to promote sprouting of grassland vegetation and hinder

forest expansion (OVERBECK et al., 2007; SÜHS; GIEHL; PERONI, 2018). Fire use in the management of rangeland and protected areas is still controversial (MISTRY et al., 2018), with an overlooking of fire and grazing by environmental agencies and protected area managers (ELOY et al., 2018). “Zero-fire” policies are adopted by several countries – including Brazil – to avoid and control fire in fire-prone ecosystems (MISTRY et al., 2018). Despite some benefits of fire removal, such ecosystems face increasing risk of catastrophic fires because of the accumulation of flammable biomass. Catastrophic fires have detrimental effects on biodiversity (BEHLING; PILLAR, 2007), human wellbeing and landscape cultural values (SÜHS; GIEHL; PERONI, 2018). When fire and grazing are prevented, tussock grasses and shrubs tend to replace small grasses and herbs (BOLDRINI; EGGERS, 1996; NABINGER; MORAES; MARASCHIN, 2000), leading unburned and ungrazed areas to woody encroachment over time (BEHLING; PILLAR, 2007; OLIVEIRA; PILLAR, 2004; OVERBECK et al., 2007). Although the fate of grasslands subjected to shrub encroachment might be difficult to predict (AUKEN, 2000), shrub-encroached areas can either develop into forests (*e.g.* DUARTE et al. 2006) or remain in a stable shrubland state, where forest never develops (DECHOUM; PERONI; PUGNAIRE, 2018). Thus, the type of management used in rangelands can drive distinct vegetation dynamics.

High altitude grasslands ecosystems cover a wide geographical extent in southern Brazilian territory, delivering a huge number of benefits because of the ecosystem services they provide. Grasslands seem to be maintained by either fire or human activities, or both (KLEIN, 1984) and are currently threatened by several factors, including a weak set of conservation policies implemented by the Brazilian government (OVERBECK et al., 2007). In highlands, grasslands are interspersed with Araucaria forests (mixed rainforest; Klein 1984), the latter being also threatened and reduced to ~12% of its original cover (RIBEIRO et al., 2009). Mosaics containing Araucaria forests and highland grasslands integrate a domesticated landscape, which has been shaped by pre-Columbian societies via use and management of resources over millennia (BITENCOURT;

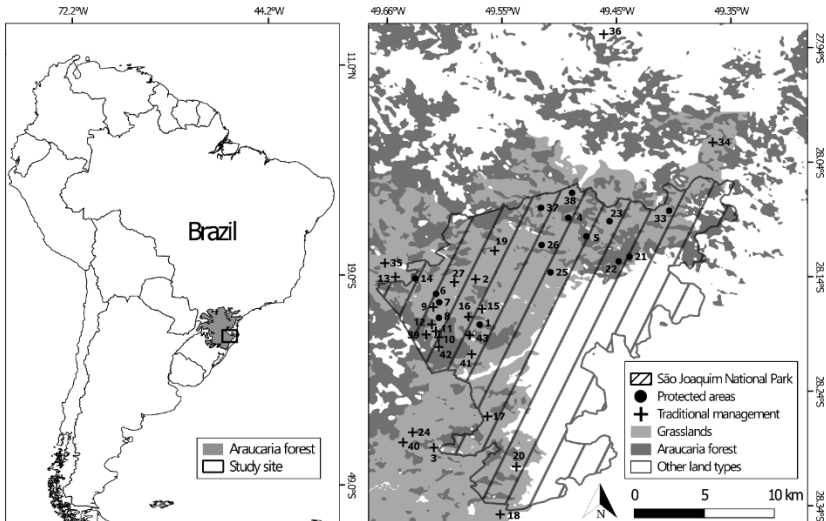
KRAUSPENHAR, 2006; REIS; LADIO; PERONI, 2014). These societies contributed for a fast expansion of Araucaria forests since 1.5 ka BP. Furthermore, it is likely that pre-Columbian societies managed fire (BITENCOURT; KRAUSPENHAR, 2006; KERN, 1998), once it became frequent in that period (BEHLING et al., 2004). Nowadays, fire is frequently used by local ranchers to promote grassland resprouting, which is beneficial for cattle grazing (NABINGER; MORAES; MARASCHIN, 2000). These activities tend to slow down natural forest expansion over grasslands (OVERBECK et al., 2007; PILLAR, 2003) and contribute for the maintenance of high diversity in grasslands and across the landscape (SÜHS; GIEHL; PERONI, 2018).

We carried out a study in a highland grassland ecosystem in southern Brazil to assess which types of management local landholders use and the effects of their traditional management on subtropical highland grasslands. We hypothesize that vegetation dynamics is determined by the type of management. Specifically, grasslands are maintained by either fire or grazing, whereas protected areas should undergo woody encroachment because both types of disturbance are prevented. The rate of woody encroachment is still poorly known to Brazilian highland grasslands (*e.g.* OLIVEIRA; PILLAR, 2004), especially over different management types. Moreover, vegetation dynamics have never been evaluated between areas either with or without traditional management. We interviewed current and former landholders in order to understand which management practices they have been using in their lands. We then analyzed aerial photographs and satellite images to quantify vegetation changes. These two sources of information were used to check whether changes in vegetation depended on the type of management. We expected that traditional management would keep highlands grasslands by hindering woody encroachment, while areas without traditional management (no grazing or fire) would allow fast woody encroachment.

## Material and Methods

### Study Area

The study was conducted in the highlands of southern Brazil, in protected areas and rangelands in the São Joaquim National Park (SJNP) region, state of Santa Catarina, Brazil. Figure 1. This region encompasses one of the highest elevated zones of southern Brazil, reaching up to 1800 m a.s.l. The protected area has 49,300 ha, of which 13,000 ha have been acquired from former landholders by the Brazilian government since 2006. The main ecoregions in these highlands are high-altitude grasslands and mixed rainforest (Araucaria forest). The climate between 1961 and 2016, recorded at the nearest weather station (distant ca. 30 km), was characterized by an annual mean rainfall of 1,626.3 mm.yr<sup>-1</sup>, equally distributed throughout the year, and an annual mean temperature of 13.3 °C. The average minimum temperature for the coldest month (July) was 6.0 °C and the average maximum temperature for the hottest month (January) was 22.9 °C. The minimum absolute temperature recorded was -9.0 °C and the maximum absolute temperature was 31.4 °C. During winter, frosts are common, and snows are occasional. Climate data compiled from (INMET, 2017).



*Figure 1. Location where the study was developed and the evaluated properties in southern Brazilian highlands. More details on evaluated properties can be found on table 1.*

## Data collection

### Interviews

In 2016 and 2017, we talked to owners of existing rangelands and previous owners whose areas were acquired to join the SJNP (Authorization from CEPSPH - Federal University of Santa Catarina: 44039415.6.0000.0121; Number 1.095.964 of 08/06/2015). The interviewees were selected from a list of former and current landholders of the SJNP region provided by the SJNP manager. In addition to the provided list, we also employed snowball sampling to increase the number of participants. Questionnaires contained open-ended questions and followed a semi-structured guide. The questions aimed to get information on the property location and size, the residence and within-family ownership time, the type and frequency of management used in the area and their opinion on the fate of grasslands in the absence of cattle and fire. When cattle grazing was mentioned, we additionally asked how many animals they had. We asked the rangers to locate and delimit their current or past properties on a digital map.

## Imagery

### *Acquisition and georeferencing*

Aerial photographs taken in 1978 were acquired from a local public organ ((Secretaria de Planejamento do Estado de Santa Catarina). These images have  $\sim 5490 \times 5575$  pixels (width x height) and 600 pixels  $\text{inch}^{-1}$  resolution. Very high-resolution satellite imagery (CNES' Pleiades-1A data and Airbus' SPOT series satellite data) taken in 2018 were acquired from Google Earth (GE - <http://earth.google.com>). Both 1978 and 2018 images were georeferenced in an orthorectified mosaic composed by aerial photographs from 2011 (Aerial Survey of the State of Santa Catarina – <http://sigsc.sds.sc.gov.br>). We used the Thin Plate Spline correction for both 1978 and 2018 images. The 2011 orthorectified mosaic was used as reference for ground control points (GCP). At least 20 GCP were established in each image, especially using roads, buildings and rocky outcrops as references. Figure 2.

### *Sampling procedure*

We delimited polygons in which we quantified changes from past to current vegetation cover. Polygons were manually drawn for grasslands (on 1978 images) intersecting with past rangeland delimitation provided by interviewed landholders. Figure 2. Forests were avoided whenever possible, because our primary interest was in changes to grasslands and because preliminary inspection showed little changes to its extent. Polygons were drawn at least 20 m away from buildings and from the edge of continuous forests, because such areas tend to rapidly change after land abandonment (OLIVEIRA; PILLAR, 2004). We also placed polygons 20 m away from property limits, to avoid influence from neighbor areas for which we had no management information. Rangelands located in high distortion zones (*i.e.* in the edges of aerial photographs) or with low grassland cover (*i.e.* less than 2 hectares) were discarded from the analysis.

### *Classification*

We carried out a supervised classification using maximum likelihood algorithm. Because polygons were drawn for 1978 images, when traditional management was in place (data acquired from



interviews), such images were classified in two classes: grassland and forest. In turn, 2018 images were classified in the following classes: grassland, shrubland, water bodies, rocky outcrops, plantations and buildings. Figure 2. Classification accuracy was assessed via Cohen's coefficient ( $\kappa$ ). We only accepted classifications when  $\kappa > 0.8$ , indicating an overall good performance (FLEISS, 1981). Then, the area occupied by each class was calculated for each polygon. Finally, classes only observed in 2018 images (*e.g.* rock outcrops, water bodies, buildings and plantations) were discarded from the total polygon area in both times. We validated the classification outcome by field expeditions. Image classification was carried out in MultiSpec software, version 3.4 (BIEHL; LANDGREBE, 2002). QGIS platform version 2.18.20 (QGIS, 2017) was used for georeferencing, mosaic building and area measurements.

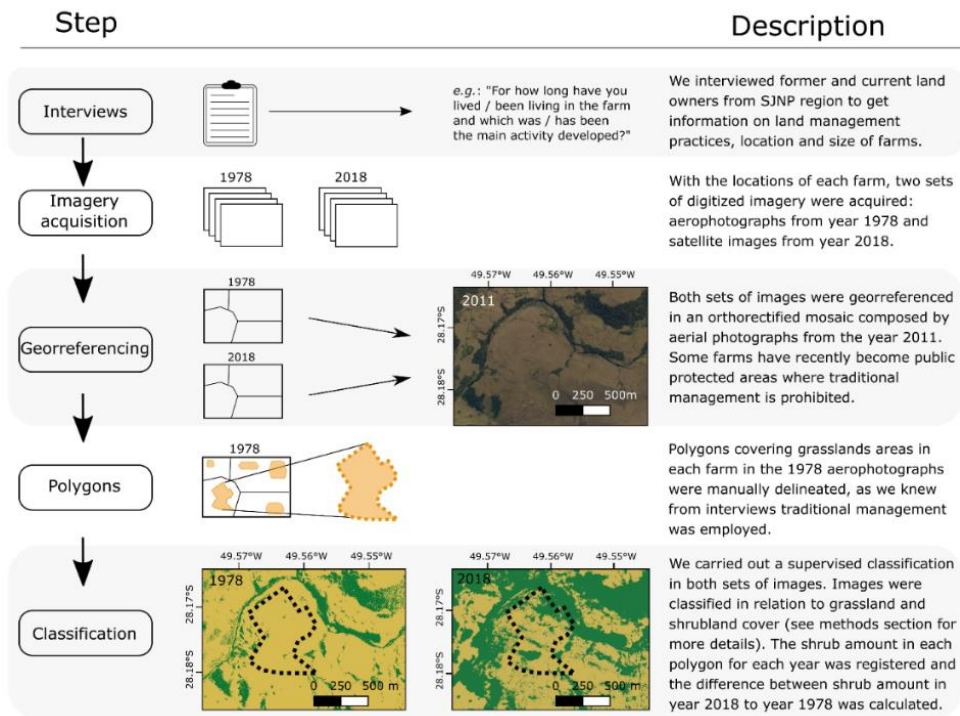


Figure 2. Schematic representation of each step taken before analysis, from interviews to image classification in southern Brazilian highlands.

## Data analysis

Because polygons varied in size depending on property settings, we obtained the proportional extent of every class over the whole polygon extent. We used beta regression models (with a logit link function) to model the rate of shrub encroachment, *i.e.* the increase in shrub area (%) from 1978 to 2018. Beta regression models are suitable to model proportions, because the beta distribution assumes values in the interval (0, 1) (CRIBARI-NETO; ZEILEIS, 2009). As predictors of the rate of shrub encroachment, we used the time since abandonment of traditional management (in years) and elevation. The interaction between both predictors was also tested, because we expected upper areas could change at a slower pace than lower ones within the same time extent. Model selection was based on the Akaike information criterion corrected for small sample sizes (AICc – Zuur et al. 2009) and validated by a graphical analysis of residuals. We constructed 95% confidence intervals for the predicted values based on 1000 bootstrap samples from the original data (OSPINA; CRIBARI-NETO; VASCONCELLOS, 2006).

## Results

### Interviews

We interviewed 36 landholders of 38 past or current rangelands. Five additional rangelands belonging to four more landholders were added because their property location and type of management were freely mentioned by our interviewees, especially by neighbors. Twenty-five properties have been managed with both cattle and fire, whereas 18 properties used to be managed in this way but are currently within the SJNP protected area. Eleven rural properties were left out of our analysis because they did not meet our selection criteria (five properties contained less than two ha of grassland cover; four of them were located in high distortion zones and two properties had no available image from 1978).

Together, all rangelands are larger than 12,000 ha (average  $\pm$  standard deviation =  $284.8 \pm 341.6$  ha) and are located  $\sim 1,500$  m a.s.l ( $1503.8 \pm 146$ ). The average time families own each property was  $125.5 \pm 78.5$  years. Extensive cattle farming is the primary income, with an average density of  $0.41 \pm 0.13$  animals  $\text{ha}^{-1}$ . Fire was or is used by all rangers every  $\sim 2$  years ( $2 \pm 0.5$ ), in the end of the winter (August / September), to accelerate grassland regrowth for cattle. Lacking cattle and fire, 19.4% of interviewees believe grasslands remain stable, 55.6% believe grasslands turn into shrublands, and 11.1% believe grasslands become forest over time. Table 1.

### Imagery

From 1978 images, 73 polygons were drawn in grasslands of selected rangelands, covering an area of 3,191 ha. The extent of grasslands within polygons was 3,118.1 ha in 1978, decreasing 12% (2,746.2 ha) in 2018. The average time rangelands were kept since abandonment of traditional management was  $8.4 \pm 1.6$  years.

*Table 1. Detail on the evaluated lands and management practices in subtropical highland grasslands in southern Brazil. L ID = land ID; O ID = Owner ID; Period = Period the property is within the owner / family; WE = Woody encroachment; P = Within protected area (cattle and fire are prevented); YWTM = number of years without traditional management. Grasslands' fate: the main answer of interviewees for the question: "What happens to grasslands if fire and cattle are excluded?". Grassland 1978: grassland area computed in polygons in 1978. Grassland 2018: grassland area computed in polygons in 2018. \* = property was removed from the analysis due to a recent fire in grasslands. \*\* = owners that were not interviewed. \*\*\* land ID 1 has three owners (all interviewed) who use the same management techniques. NA = Data not available.*

L ID	O ID	Elevation (m)	Land area (ha)	Period (years)	Cattle density (animals.ha <sup>-1</sup> )	Fire frequency (years)	Grasslands' fate	Grassland 1978 (ha)	Grassland 2018 (ha)	WE (%)	P	YWTM
1***	a	1571.4	1200	116	0.3	2	"shrubland"	368.8	367.6	0.41	No	0
2	b	1650.0	340	300	0.38	2	"grassland / shrubland"	146.4	145.9	0.38	No	0
3	c	1250.0	105	100	0.57	2	"shrubland"	23.6	23.6	0.38	No	0
4	d	1616.7	319	100	NA	2	"shrubland / forest"	8.5	5.0	35.18	Yes	8
5	e	1650.0	840	30	0.24	2	"forest"	25.8	22.4	14.60	Yes	11
6*	f	1650.0	58.4	80	0.37	2	"shrubland"	27.4	27.2	0.56	Yes	9
7	f	1650.0	68.5	80	0.37	2	"shrubland"	60.8	59.9	1.41	Yes	9
8	f	1625.0	114	80	0.37	2	"shrubland"	37.2	31.8	11.27	Yes	9

9	g	1650.0	17.4	130	0.57	3	"shrubland"	12.1	12.1	0.05	No	0
10	h	1650.0	68.8	200	0.5	2	"forest"	27.1	26.4	2.40	No	0
11	i	1650.0	65	120	0.46	4	"grassland"	17.5	17.5	-0.14	No	0
12	j	1650.0	65	100	0.42	2	"grassland"	19.6	19.5	0.43	No	0
13	k	1525.0	150	80	0.26	2	"forest"	11.5	11.2	2.14	No	0
14	l	1516.7	190	150	0.42	1	"shrubland"	28.1	11.7	63.60	Yes	9
15	m	1400.0	148	100	0.47	2	"shrubland"	45.6	43.2	3.18	No	0
16	n	1450.0	118	60	0.42	2	"shrubland"	8.3	8.3	-0.44	No	0
17	o	1416.7	800	241	0.37	2-3	"grassland"	353.2	322.2	0.15	No	0
18	p	1400.0	800	62	0.31	2	"shrubland"	451.7	448.8	0.03	No	0
19	q	1700.0	550	250	0.33	2	"shrubland"	146.3	145.2	0.41	No	0
20	r	1416.7	700	55	0.26	2-3	"shrubland"	330.5	330.7	0.06	No	0
21	s	1450.0	114	80	0.4	1	"shrubland"	19.4	12.6	27.34	Yes	9
22	s	1450.0	154	80	0.4	1	"shrubland"	9.1	1.1	76.74	Yes	9
23	t	1600.0	300	35	0.33	2	"shrubland" / "forest"	16.5	6.6	45.94	Yes	5
24	u	1300.0	155.8	150	0.51	2	"shrubland"	54.6	50.8	4.15	No	0
25	v	1650.0	1500	150	0.27	2	"shrubland"	380.8	162.8	54.70	Yes	8
26	v	1521.4	1000	150	0.2	2	"shrubland"	37.9	37.0	-0.76	No	0
27	w	1650.0	200	100	0.5	1	"shrubland"	91.9	87.8	4.31	No	0
28	x**	1550.0	169	NA	NA	NA	NA	109.2	104.7	4.51	No	6

29	x**	1450.0	300	NA	NA	NA	NA	132.5	103.6	21.17	Yes	10
30	y**	1450.0	135	NA	NA	NA	NA	42.9	39.6	7.43	No	6
31	z**	1650.0	101	NA	NA	NA	NA	56.5	45.2	19.12	No	9
32	aa**	1500.0	36.8	NA	NA	NA	NA	16.8	14.2	15.12	No	9
33	bb	1220	199	40	NA	2	"grassland"	NA	NA	NA	No	NA
34	cc	1070	218	56	0.28	2-3	"shrubland"	NA	NA	NA	No	NA
35	dd	1300	41.5	80	0.72	NA	NA	NA	NA	NA	No	NA
36	ee	1550	120	38	0.25	2	"shrubland"	NA	NA	NA	No	NA
37	ff	1600	260.5	29	0.69	2	"forest"	NA	NA	NA	Yes	NA
38	gg	1550	120	150	NA	2	NA	NA	NA	NA	Yes	NA
39	hh	1500	65	250	0.38	2	"grassland"	NA	NA	NA	Yes	NA
40	ii	1280	150	150	0.56	3	"grassland"	NA	NA	NA	No	NA
41	jj	1300	60	200	0.67	2-3	"shrubland"	NA	NA	NA	No	NA
42	kk	1540	67	300	0.52	2	"grassland"	NA	NA	NA	No	NA
43	ll	1445	62.5	300	0.48	NA	"shrubland"	NA	NA	NA	No	NA

## Woody encroachment

The rate of shrub encroachment accelerated with time since abandonment of traditional management ( $w_{AIC} = 0.73$ ), being very little affected by elevation. Table 2. The model containing only time since abandonment of traditional management explained 68% of variation in shrub encroachment rate. Figure 3. In areas where traditional management still consists of fire and grazing cattle, the rate of shrub encroachment remained close to 1%. Conversely, areas which became protected, thus excluding cattle and fire, experienced changes increasing exponentially over time, with an average rate of encroachment of 4.8% year<sup>-1</sup> (ranging from 0.03 to 9.1% year<sup>-1</sup>). The time needed to rate of shrub encroachment to reach 50 and 99% was estimated in 12 and 30 years, respectively. Field expeditions confirmed that the main shrub species encroaching in areas without traditional management is the shrub *Baccharis uncinella* DC. (Asteraceae).



*Table 2. Set of produced models for evaluating shrub encroachment rate in relation to elevation and traditional management in southern Brazilian grasslands. Int = Intercept; Elev. = Elevation; YWTM = years without traditional management, df = degrees of freedom, logLik = log-likelihood, AICc = Akaike information criteria corrected for small samples, wAIC = Akaike weight. Interaction between predictors is represented by “x”. Selected model follows a “\*”. Models ordered by increasing values of AICc.*

<b>Int</b>	<b>Elev</b>	<b>YWTM</b>	<b>Elev. × YWTM</b>	<b>df</b>	<b>logLik</b>	<b>AICc</b>	<b>ΔAIC</b>	<b>w<sub>AIC</sub></b>
-2.965	–	0.244	–	3	58.22	-109.52	0.00	0.73
-2.400	-0.0004	0.246	–	4	58.25	-106.90	2.62	0.20
-3.778	0.0005	0.767	-0.0003	5	58.67	-104.84	4.68	0.07
-1.801	–	–	–	2	46.35	-88.27	21.25	0.00

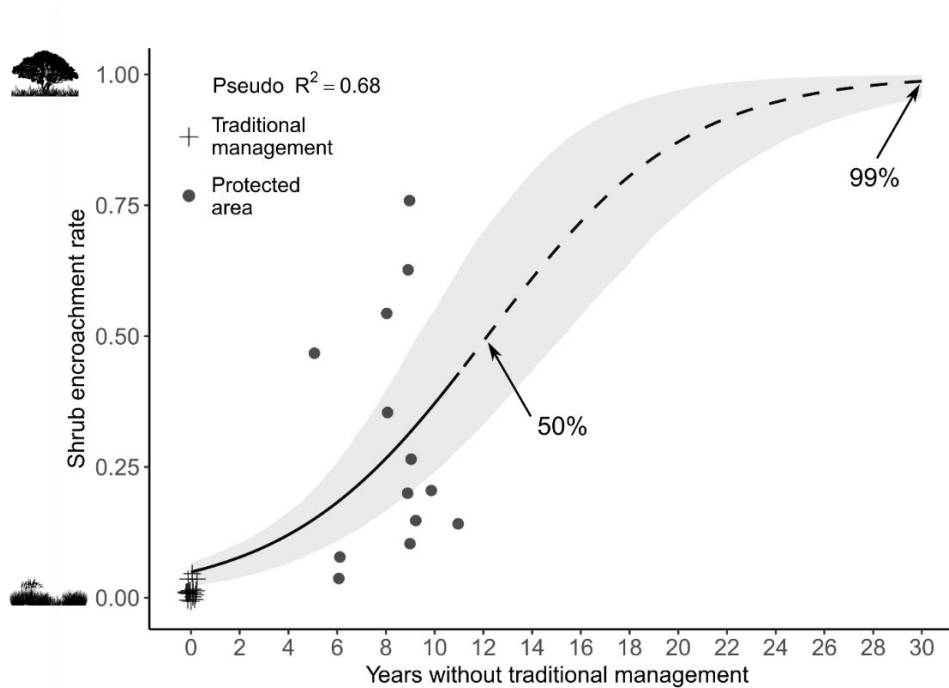


Figure 3. Effect of traditional management on shrub encroachment rate in southern Brazilian highlands. Solid line represents interpolation and dashed line represents extrapolation. Shaded areas represent 95% confidence intervals built from predictions based on 1000 bootstrap replicates of the original data. Arrows indicate 50 and 99 % of shrub encroachment rate (12 and 30 years, respectively).

## Discussion

Changing land management had great impact in vegetation dynamics in our study system. Our results indicate that after grazing cattle and fire withdrawal, grasslands underwent an exponential rate of shrub encroachment over time. Shrub encroachment proceeded in a fast pace in protected areas, replacing large extents of grasslands over short time. Following that pace, shrubs would entirely encroach over grasslands in ~30 years. Conversely, areas keeping traditional management faced little to no shrub encroachment over the last 60 years. These results corroborate our hypothesis and its consequences are discussed below.

Woody encroachment affects open-canopy ecosystems across the world, unleashing a need to understand its causes and consequences. Woody encroachment has been reported on African savannas (ANGASSA; OBA, 2009; VENTER; CRAMER; HAWKINS, 2018), North American savannas and grasslands (BARGER et al., 2011; KNAPP et al., 2008; LETT; KNAPP, 2003) and South American savannas and grasslands (ELOY et al., 2018; OVERBECK et al., 2007), thus posing these ecosystems at risk. Several factors and their interactions cause woody encroachment, even though changes in disturbance regimes, such as fire and grazing, are amongst the most frequent drivers (ARCHER et al., 2017; AUKEN, 2000; ELDRIDGE et al., 2011; VAN AUKEN, 2009). Therefore, the type of land management seems to be of paramount importance for landscape maintenance. For example, grasslands of subalpine ecosystems are being transformed into woodlands, facing shrub encroachment process as agricultural practices are abandoned (NUCHE; ALADOS, 2018). In southeastern South America, the Uruguayan Savanna ecoregion faces a limited forest expansion because of cattle grazing and fire, although tree cover is favored by precipitation (BERNARDI et al., 2016). Our study supports the claim that highland grasslands in southern Brazil are prone to woody encroachment (OLIVEIRA; PILLAR, 2004; OVERBECK et al., 2007), especially in the absence of fire and cattle grazing. Although substantial increases in woody encroachment can occur over decades (ARCHER et al., 2017), we found evidence for an

accelerating rate of shrub encroachment in areas where fire and cattle were excluded. Such management is normally enforced in protected areas. Meanwhile, native grasslands were maintained under traditional management by keeping large herbivores and fire. In North American drylands, for example, rates of encroachment vary from 0.1%–2.3% year<sup>-1</sup>, depending on the ecoregion (ARCHER et al., 2017; BARGER et al., 2011). Here we found rates of shrub encroachment ranging from 0.03% to 9.1% year<sup>-1</sup>. In areas under traditional management, the average rate of shrub encroachment was 1.1% year<sup>-1</sup>, while in areas where traditional management was prevented the average rate was 4.8% year<sup>-1</sup>. Our results indicate a clear effect of the type of management on woody encroachment and highlights the fast pace it may follow.

Replacement of grasslands by woodlands has important consequences at both the community and ecosystem level (AUKEN, 2000; LETT; KNAPP, 2003). In general, chief consequences of woody encroachment are alteration of fundamental ecological processes and loss of biodiversity (ELDRIDGE et al., 2011; LETT; KNAPP, 2003; OVERBECK et al., 2007; SCHOLLES; ARCHER, 1997). In addition, woody encroachment reduces the outcome of cattle production thus affecting human economies (ARCHER et al., 2017; OBA et al., 2000). In the study region, all landholders have been using extensive cattle farming as their primary income for decades. In addition, all landholders manage fire to promote the regrowth of grasslands for cattle foraging in the end of the winter. Thus, it is not surprising that humans seem to shape ecosystems to reach their intended benefits. Over the last centuries, woody vegetation seems to be favored by the suppression of fire and grazing in subtropical ecosystems (BEHLING; PILLAR, 2007) and the dependency of southern Brazilian highland grasslands on fire and grazing has been already reported (*e.g.* CARLUCCI et al., 2016; KLEIN, 1984; LUZA et al. 2014; OLIVEIRA; PILLAR, 2004; OVERBECK et al., 2007; OVERBECK; FERREIRA; PILLAR, 2016). Fire and grazing are key elements of traditional management taken by local landholders in such system (REIS; LADIO; PERONI, 2014; SÜHS; GIEHL; PERONI, 2018). However, use and management of fire is still controversial (MISTRY et al., 2018; OVERBECK et al.,

2007) and rarely described even in fire-managed pastoral systems (ELOY et al., 2018). Furthermore, Brazilian environmental agencies and protected area managers struggle to understand fire-managed pastoral systems (ELOY et al., 2018), which urges the creation of conservation policies that enable the use of fire in fire-prone ecosystems (MISTRY et al., 2018). Fire brings benefits. For example, species richness of abandoned grasslands in southern Brazil can only be maintained by fire (OVERBECK et al., 2007). In mesic grasslands of central United States, the prevention of woody encroachment by frequent burning is the best option (LETT; KNAPP, 2003). In African moist and arid savannas, fire is an effective method for controlling shrub encroachment (TROLLOPE, 1980). However, substantial ecosystem changes following woody encroachment may, in some cases, impair ecosystem recovery even when fire is reintroduced (LETT; KNAPP, 2003).

In general, few woody species tend to become aggressive encroachers or originate the encroachment process (STOKES; ARCHER, 2010). The main shrub species encroaching in the studied grasslands is *Baccharis uncinella*, which is a regionally common but endemic species to southern Brazilian highlands (HEIDEN ,SCHNEIDER, 2015). This species can expand from forest borders toward grasslands (OLIVEIRA; PILLAR, 2004) and facilitate the arrival and development of other shrubs and forest species, including nurse trees, potentially accelerating forest expansion (DOS SANTOS et al., 2011; DUARTE et al., 2006), as well as serve as a native fauna refugee (OVERBECK; FERREIRA; PILLAR, 2016; CARLUCCI et al., 2016). A similar shrub-facilitation system has been reported in subalpine grasslands in Spain, in which a species of shrub that invaded grasslands facilitated the settlement and expansion of another shrub species (NUCHE; ALADOS, 2018). We understand that a native shrubby vegetation, which seems to be a transient alternative state, can be beneficial to some species and enhance diversity and deserves to be protected as well (see CARLUCCI et al., 2016; LUZA et al. 2014; OVERBECK; FERREIRA; PILLAR, 2016). However, larger grassland areas affected by woody encroachment as a result of fire suppression, can alter grassland communities, especially by reducing

native forbs abundance and plant species richness (GUIDO; SALENGUE; DRESSENO, 2017). Besides, encroachment by *B. uncinella* seems to be facilitated by high air temperatures and native grasses, which improve water balance (DECHOUM; PERONI; PUGNAIRE, 2018). At low to moderate densities, encroachment by *B. uncinella* seems to be easily controlled by fire. Under high density of *B. uncinella*, however, landscapes may be threatened by catastrophic fires.

Our findings show that highland grasslands that became protected by law – where both cattle grazing and fire are suppressed – faced woody encroachment. Conversely, highland grasslands under traditional management – with cattle grazing and fire – were maintained. Such management consists of 2-year fire interval and low density of extensive grazing cattle ( $\sim 0.41$  animals  $\text{ha}^{-1}$ ). Most landholders we interviewed believe that, in the absence of cattle and fire, grasslands would turn into shrublands over time. Accumulation of flammable biomass, including that from *B. uncinella* encroachment, also concerns several of the landholders. In addition, landholders reported that unburned grasslands accumulate fuel and that shrubs facilitate fire spread to adjacent forests, because shrubs increase vertical reach of flames. Indeed, flammable biomass accumulates over time in grasslands areas where fire is prevented (BEHLING; PILLAR, 2007; NABINGER; MORAES; MARASCHIN, 2000) and shrubs, which are kept in low abundance in managed systems, establish and increase in abundance mostly from forest edges (OLIVEIRA; PILLAR, 2004). Once inside forests, fire can spread throughout the flammable litter of Araucaria trees. Araucaria is an abundant species and produces great part of the litter in Araucaria forests (BACKES; PRATES; VIOLA, 2005). Also, litter in these forests have a lower decomposition rate compared to other forests (*e.g.*, rainforests) because of the presence of oils and resin in Araucaria litter (BACKES; PRATES; VIOLA, 2005), allowing fire to spread inside forests. Such characteristics may facilitate the occurrence of catastrophic fires, which impose a risk to biodiversity (BEHLING; PILLAR, 2007), human wellbeing and cultural landscapes (SÜHS; GIEHL; PERONI, 2018). Natural grasslands, besides providing a more

reliable carbon sink, can be more resilient to drought and wildfires than forests (DASS et al., 2018; SEDDON et al., 2019). Therefore, policy makers and protected area managers should recognize fire as a natural and critical process in grasslands (MISTRY et al., 2018), as this disturbance is crucial to protect biodiversity-related and cultural aspects of the landscape.

## Conclusion

We found additional evidence for woody encroachment by shrubs in grasslands from southern Brazil highlands. Yet, the most outstanding fact of our results is the pace of vegetation change exponentially accelerating over time. Consequently, large extents of grassland can be replaced by shrublands after just a decade of management change. In addition, we believe that local farmers understand consequences of their management – which includes fire and grazing by cattle – on both the maintenance of grasslands and the prevention of large-scale destructive wildfires. Based on such knowledge and vegetation change analysis, we suggest that the quick pace of shrub encroachment in the region threatens not only grassland ecosystems and its unique biodiversity, but also human wellbeing and cultural heritage of the landscape. Such results highlight the need of grassland conservation policies allowing and instructing on the timing, frequency and extent of prescribed fire regimes as a management tool for both rangelands and protected areas.

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## 5. Capítulo 2<sup>§</sup>

### Interaction of land management and araucaria trees in the maintenance of landscape diversity in the highlands of southern Brazil

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## Abstract

In the southern Brazilian highlands, pre-Columbian societies created domesticated landscapes through the use and management of forests, including nurse *Araucaria angustifolia* trees, a common conifer in these regions. Nowadays, local smallholders still use traditional practices, such as burning, to promote vegetation for cattle grazing in highland grasslands. Even though burning is normally of small extent and low frequency, such management can slow down natural forest expansion and contribute to the maintenance of grasslands, by opposing the facilitative effect of nurse araucaria trees. To comprehend the interplay between human cultural management, species interactions and the environment, it is important to better understand how these relations affect diversity and composition. Our goal was to investigate how land management, biotic interactions and abiotic factors affect saplings species richness, abundance and composition. We hypothesized that (1) land management would decrease sapling richness and abundance and change sapling composition, (2) nurse araucaria trees would increase species richness and abundance and change sapling composition, and (3) the interactive effect between land management and nurse araucaria trees would shape sapling richness, abundance and composition. Data were collected in unmanaged and managed conditions, both beneath araucaria crowns and in nearby treeless areas. Our results indicate that abundance and species composition are affected by land management and araucaria crown influence. The highest values of sapling abundance were found beneath crowns in unmanaged areas. Species composition changed between all assessed combinations of land management and crown influence. Our study demonstrates the major roles of land management and facilitation in structuring communities, despite the effects of rock and grass cover. Moreover, our results clarify patterns and processes that may emerge in natural highland grasslands, such as the conversion of grasslands into forests and the loss of cultural landscapes when the main local management actions are excluded.

**Keywords:** human activities, disturbance, biodiversity, conifers, grasslands, fire, cattle.

## Introduction

Plant communities are structured by local abiotic conditions, direct and indirect biotic interactions among plants, direct interaction with other organisms, stochastic processes, evolution, space and time (LORTIE et al., 2004). Biotic interactions can promote changes in large-scale patterns of species distribution, affecting broader processes such as migration, speciation and extinctions (WISZ et al., 2013). The combination of negative interactions (*e.g.*, competition, predation) and abiotic factors are the main components of most models of community structure and ecological theories (BRUNO; STACHOWICZ; BERTNESS, 2003; STACHOWICZ, 2012). However, positive interactions, such as mutualism and facilitation, are also important in shaping species composition, diversity and community dynamics (*e.g.*, (CALLAWAY, 1995; STACHOWICZ, 2001)). At the same time, past and present human activities help shaping large-scale patterns of species composition and community structure (BOIVIN et al., 2016), having an important role in niche construction through domestication of animals and plants (ZEDER, 2016). Domestication is here defined as co-evolutionary mutualisms that develop through active niche construction by both humans and plants or animals (ZEDER, 2016). Domestication of animals and plants, and soil modification may result in domesticated landscapes with high levels of productivity and greater diversity of niches than landscapes without human actions (CLEMENT et al., 2015; ZEDER, 2016).

Facilitation is a major mechanism in diversity maintenance (BRUNO; STACHOWICZ; BERTNESS, 2003; CALLAWAY, 2007; HE; BERTNESS; ALTIERI, 2013), therefore contributing to niche construction. Facilitator species alter the environment and can mitigate potentially limiting stressors, thereby creating suitable habitats for other species (STACHOWICZ, 2012), whereas the absence of a facilitator species in regions susceptible to climate change may slow down migratory processes, leading to species extinctions (ANTHELME; CAVIERES; DANGLES, 2014). Facilitation tends to increase with physical severity of the environment (*i.e.*, abiotic stress or high herbivore pressure) (CALLAWAY, 1995; HE; BERTNESS;



ALTIERI, 2013). In severe habitats, such as elevated regions (highlands), facilitation may be more important than negative interactions (BROOKER et al., 2008; TRAVIS; BROOKER; DYTHAM, 2005). Facilitation by shrubs and trees usually ameliorates environmental conditions because these plants provide shade and increase soil humidity, oxygenation and nutrient availability beneath their crowns (KORNDÖRFER; DILLENBURG; DUARTE, 2015; MORO et al., 1997). Abiotic structures like rocks can also be beneficial to plant species, for example, by changing microclimatic conditions or protecting against fire (BORCHERT et al., 2003; CARLUCCI; DUARTE; PILLAR, 2011; PYATT et al., 2016). These local scale changes induced by habitat amelioration may determine broader patterns and processes, overcoming regional-scale factors, such as climate (BEKKER, 2005).

Although there is interplay between human cultural management, species interactions and the environment, how these factors affect diversity, composition and species distribution is still poorly understood (BOIVIN et al., 2016; SEDREZ DOS REIS; LADIO; PERONI, 2014). To understand current landscape dynamics, it is crucial to recognize past processes that have shaped terrestrial and aquatic ecosystems around the world for millennia (BOIVIN et al., 2016). In South America, domesticated landscapes created by pre-Columbian societies can be found in tropical (CLEMENT et al., 2015; LEVIS et al., 2017) and subtropical forests and grasslands (SEDREZ DOS REIS; LADIO; PERONI, 2014). In southern Brazil, hunter-gatherer human societies arrived around 12 kyr BP and several societies successively occupied the highlands, within Araucaria forest domains (NOELLI, 2000). These societies used several forest resources, such as wood, fruits and seeds, and probably also contributed to seed dispersal (NOELLI, 2000). The use and management of forest resources, intensified at around 2.5 kyr BP with the arrival of different traditions of sedentary and agricultural societies (Tupi and Macro-Jê) (NOELLI, 2000, 2008). Following the arrival of European settlers around 500 years BP, wars and diseases decimated most traditional populations, settlements, cultures and landscapes (NOELLI, 2000, 2008; SEDREZ DOS REIS; LADIO; PERONI, 2014).

Araucaria forests (mixed rain forests) occur mostly throughout the southern Brazilian plateau (VELOSO; RANGEL FILHO; LIMA, 1991), at altitudes varying from 500 m to 1800 m a.s.l. (REITZ; KLEIN, 1966). In these forests, the dioecious conifer *Araucaria angustifolia* (hereafter “araucaria”) is abundant and dominates the tree stratum (KLEIN, 1984). At high altitudes (above 900 m a.s.l.), forests frequently form mosaics associated with shrubs and grasslands, the latter considered relicts of a past climate and likely maintained by fire and/or human activities (KLEIN, 1984). The presence of araucaria within subtropical forests has raised questions of a possible past expansion of these forests into grasslands (KLEIN, 1984). Indeed, around 4 kyr BP the regional climate became wetter, leading to a slow expansion of Araucaria forests into grasslands (BEHLING, 1995, 1998; BEHLING et al., 2004). Around 1.5 kyr BP, however, the expansion was much faster, and fire became more frequent than before, while the climate remained the same (BEHLING et al., 2004). This sudden expansion matches the arrival of pre-Columbian societies in the region (BITENCOURT; KRAUSPENHAR, 2006; SEDREZ DOS REIS; LADIO; PERONI, 2014). Previous studies have shown that as a consequence, domesticated landscapes were created through forest use and management (ZEDER, 2016), including the management of araucaria trees, whose seeds constituted part of the diet of these peoples (BITENCOURT; KRAUSPENHAR, 2006; SEDREZ DOS REIS; LADIO; PERONI, 2014). Nowadays, local smallholders regularly use fire to promote grassland for cattle grazing (NABINGER; MORAES; MARASCHIN, 2000). Yet, such management may slow down natural forest expansion and contribute to the maintenance of grasslands (OVERBECK et al., 2007; PILLAR, 2003), which are threatened by a series of factors, including forest expansion (OVERBECK et al., 2007). Additionally, grasses can hinder woody plant establishment through light and resource competition (SCHOLES; ARCHER, 1997; ZANDAVALLI; DILLENBURG, 2015). Araucaria trees, on the other hand, promote woody plant establishment because adult trees of this species act as perches for seed dispersers (DOS SANTOS et al., 2011; DUARTE et al., 2006) and ameliorate conditions beneath their crowns by attenuating high

temperatures in summer and increasing soil nutrient availability (KORNDÖRFER; DILLENBURG; DUARTE, 2015).

Conifers play an important role in structuring communities in high-altitude ecosystems across the globe (*e.g.*, (BEKKER, 2005; CHOLER; MICHALET; CALLAWAY, 2009; PYATT et al., 2016)). In Brazil, Araucaria forests and associated highland grasslands are highly threatened. Araucaria forests currently occupy around 12% of their original area (RIBEIRO et al., 2009; SEDREZ DOS REIS; LADIO; PERONI, 2014) and *Araucaria angustifolia* is at risk of extinction (IUCN, 2017). In addition, Brazilian grasslands are often neglected by conservation policies and are threatened by several factors, including invasion by exotic species (ZALBA; VILLAMIL, 2002), mismanagement practices (OVERBECK et al., 2007) and climate change (ASSIS; DE MATTOS, 2016). Subtropical highland grasslands deserve attention not only because of these threats but also due to high levels of endemism, species richness and genetic diversity (BARROS et al., 2015).

We carried out a field study in an upper-montane Araucaria forest-grassland mosaic to investigate how land management, biotic interactions and abiotic factors affect saplings species richness, abundance and composition. By testing the following hypotheses, we tried to understand how grasslands are maintained by management practices, how araucaria trees promote forest expansion, and the role of biotic interactions and abiotic factors involved in these processes. We hypothesized that (1) land management would decrease sapling richness and abundance and change sapling composition, (2) nurse araucaria trees would increase species richness and abundance and change sapling composition, and (3) the interactive effect between land management and nurse araucaria trees would shape sapling species richness, abundance and composition. We expect negative effects of land management because burning and grazing can kill or damage seedlings and saplings (OLIVEIRA; PILLAR, 2004). Conversely, nurse araucaria trees shade grasses, reducing their competitive ability, and favor recolonization by woody species because of a perch effect and facilitation (DUARTE et al., 2006; KORNDÖRFER; DILLENBURG;

DUARTE, 2015). Finally, we expect that the effect of management would override the nurse effect of araucaria trees.

## Materials and methods

### Study site

The study was conducted in the highlands of southern Brazil, in São Joaquim National Park (Lat 28.19°S, Lon 49.53°W), which is located within the municipalities of Bom Jardim da Serra, Urubici, Lauro Müller, Grão Pará and Orleans, in Brazil. This protected area has 49,300 hectares, of which 13,000 have been effectively protected (*i.e.* acquired by the Brazilian government) since 2006. The main vegetation types in the protected area include high-altitude grasslands, mixed rainforest (Araucaria forest) and tropical rainforest (Atlantic rainforest) (VELOSO; RANGEL FILHO; LIMA, 1991). The climate between 1961 and 2016, recorded by the nearest weather station (ca. 30 km), was characterized by an annual mean rainfall of 1,626.3 mm.yr<sup>-1</sup>, equally distributed throughout the year, and an annual mean temperature of 13.3 °C. The average minimum temperature for the coldest month (July) was 6.0 °C and the average maximum temperature for the hottest month (January) was 22.9 °C. The minimum absolute temperature recorded was -9.0 °C and the maximum absolute temperature was 31.4 °C. During winter, frosts are common and it occasionally snows. Climate data compiled from ('INMET - Instituto Nacional de Meteorologia', [s.d.]). In the region, especially in high-altitude grasslands, usual management actions consist of using fire every two or three years and removing shrubs to promote grassland vegetation for extensive livestock farming.

### Sampling methods and data collection

Data was collected in two locations, in 2015 and 2016. One location is situated inside the national park (Lat 28.142°S, Lon 49.631°W) and has not been managed (with fire, cattle and shrub removal) since 2008 (hereafter: "unmanaged"). The second location is a private property situated outside the protected area (Lat 28.142°S, Lon 49.644°W) and is currently managed (hereafter

“managed”). The authorization for developing this study in the protected area and private property was approved by the Brazilian government (SISBio project code 48898-1) and the landholder, respectively. These locations are geographically close to each other (ca. 800 m away) and have the same climate (humid subtropical), soil type, elevation (ca. 1,450 m a.s.l.) and vegetation (mosaic of grasslands and shrubs among araucaria trees), and the terrain has a similar slope and aspect. There are no major environmental differences among the locations (more details in S1 Fig). In these locations, *Baccharis uncinella* DC. is the most common shrub, while *Scleria sellowiana* Kunth. and *Andropogon lateralis* Nees are the most common grasses. The locations were equally managed (fire and cattle grazing) until 2008, when the unmanaged location became protected. In the managed location, the last fire occurred in 2014 and there is an average of 0.15 cattle per hectare. These locations represent well the regional vegetation type with traditional land use history.

Isolated adult (>20 cm DBH) araucaria trees were randomly selected across the two locations, totaling 70 individuals in the unmanaged and 30 individuals in the managed location. This sampling difference is because the managed location has less trees than the unmanaged location. However, to keep the same variation in the environment, we tried to keep the same distance, for both locations, from the first selected tree to the last selected tree. The selection criteria were that trees should be mature and the next tree should be at least 10 meters apart from the previous selected tree (DUARTE et al., 2006). Trees were sampled in one direction (from north to south) in both locations, within the same mosaic type. The areas covered by the surveys correspond to approximately 8 ha in the unmanaged location and five hectares in the managed location. Vegetation and environmental data were collected beneath crowns and near (hereafter “treeless areas”) araucaria trees, resulting in paired samples. Samples in treeless areas were randomly placed (drawn from cardinal points) at two meters away from the limits of the crown of the sampled araucaria tree and had no influence of any other tree species crown. Because we sampled the whole area

beneath the crown of araucaria trees, the sample area in treeless areas was adjusted to match the area sampled beneath the crown. Thus, we also recorded the area of each sample, and the height and crown diameter of each araucaria tree with a digital laser distance meter. We recorded the identity and number of individuals of each sapling that was 30–200 cm tall beneath the crown of each selected araucaria tree and in the paired treeless areas. Species were identified in the field using scientific literature. Species nomenclature and classification follow the Brazilian Flora checklist (JARDIM BOTÂNICO DO RIO DE JANEIRO, 2017).

To assess the role of covariates that can also affect woody seedling establishment, four squares ( $0.5 \times 0.5$  m) subdivided in four quadrats ( $0.25 \times 0.25$  m) were placed towards the geographical cardinal points, both beneath crowns and in the sampled treeless areas. We treated as covariates the cover of rock, shrub (*Baccharis uncinella*) and grasses. In addition, the average height of grasses was measured in each quadrat with a wooden ruler (1 cm precision). Although other graminoids were present and measured, they are hereafter referred to as only “grasses.” Grass volume was estimated as the product of grass cover and average grass height. Data collected from quadrants was averaged for each of the four squares and then averaged for each sample.

## Data Analysis

We built mixed models (GLMM) using either species richness or abundance of saplings, as response variables and generalized linear model for multivariate responses (GLM<sub>mv</sub>) using community composition as response variables. For both types of models, land management, araucaria crown influence, grass volume, rock and shrub cover were treated as fixed effects terms. Models were built considering the hierarchical arrangement of variables, such as land management and araucaria crown influence affecting shrub cover and grass volume. Thus, we considered land management and crown influence as top variables, and therefore the effects of grass volume, rock and shrub cover were nested within the interaction between the

two top variables. Based on this assumption, along with biological relevance and the hypotheses to be tested (ZUUR et al., 2009), a total of eight candidate models plus an intercept-only model (null) were built. To account for sampling pairing (beneath crowns and treeless areas), we treated pairs of samples (blocks) as random effects terms for GLMMs. We specified contrasts of factor levels for each model and adjusted P-values for multiple comparisons via the Holm-Bonferroni method.

For multivariate data (species composition), we also checked which species were significantly affected by land management, araucaria crown influence, grass volume and rock and shrub cover with univariate tests (using GLMs). To control for the paired sampling design, a permutation matrix was generated, where pairs of samples (blocks) were fixed, but blocks and factor levels within blocks (beneath crowns / treeless areas) were randomized. P-values were calculated based on the 10,000 matrices via PIT-trap resampling (adjusted for multiple testing) calculated using a stepdown resampling algorithm (WANG et al., 2015). To visually check for changes in the community composition of sites differing in land management and crown influence, we explored the data with non-metric multidimensional scaling (nMDS). Before running the models, outliers were removed (3 samples) through graphical exploratory data analysis.

For all models, we chose the negative binomial distribution because it visually fit the residuals better compared to Poisson distribution (ZUUR et al., 2009). To account for variations in sample size, we used sampling area as an offset in the models. Sampling area was log-transformed to match the scale of the modeled response (link function is log for negative binomial family). Model selection was based on the Akaike information criterion (AIC) and validated by a graphical analysis of residuals (ZUUR et al., 2009). Finally, we assessed model performance through marginal and conditional  $R^2$  for GLMM (following (NAKAGAWA; SCHIELZETH, 2013)) and pseudo  $R^2$  for  $GLM_{mv}$  (following (SLAVICH et al., 2014)). We checked for spatial autocorrelation through correlograms that test for autocorrelation in

the residuals and there was no clear pattern of decreasing autocorrelation with distance. All analyses were run in the R environment (R CORE TEAM, 2017) using the “vegan” (OKSANEN et al., 2012) package for producing the ordinations, “glmmADMB” (SKAUG et al., 2016) for GLMMs and “mvabund” (WANG et al., 2015) for GLM<sub>mv</sub>. An example of the mixed model built is the following:

$$\begin{aligned} \text{Richness} \sim & (LM \times CI) + LM/CI/Rock + LM/CI/Shrub \\ & + LM/CI/Grass + \text{offset}(\log(\text{Area})) \\ & + (1|Block) \end{aligned}$$

Where LM = land management (managed / unmanaged), CI = araucaria crown influence (beneath crowns / treeless areas), Rock = rock cover, Shrub = shrub cover, Grass = grass volume, area = sampling area, Block = blocks.

## Results

### Sapling Richness

A total of 19 woody native species were found (no alien species were found) (S1 Table). The model best fitting differences in species richness contained land management, crown influence and grass volume as predictors (S2 Table). This model had the lowest AIC from the set of candidate models (Table 1) and residuals were visually adequate. Fixed effects accounted for 33% of the differences in species richness (marginal R<sup>2</sup>), reaching 62% when considering both fixed and random effects (conditional R<sup>2</sup>).



Table 1. Set of produced models for evaluating sapling species richness, abundance and composition in an upper-montane Araucaria forest, southern Brazil. Int = Intercept; CI = Araucaria crown influence; LM = land management; Rock = rock cover; Shrub = shrub cover; Grass = grass volume; df = degrees of freedom; logLik = log likelihood; “+” sign stands for inclusion in the referred model. For species composition models, logLik and AIC values are expressed as the sum of these parameters from the species univariate GLM. Interaction between predictors is represented by “x”.

Model	Model ID	Int	CI × LM	CI × LM × Rock	CI × LM × Shrub	CI × LM × Grass	df	logLik	AIC	Delta AIC	AIC Weights
<b>Sapling species richness</b>	ric.7	-3.25	+			+	10	-351.6	723.2	0.00	0.588
	ric.2	-3.65	+	+		+	14	-348.8	725.6	2.42	0.175
	ric.8	-3.04	+				6	-356.9	725.7	2.57	0.162
	ric.5	-3.26	+	+			10	-354.6	729.1	5.98	0.030
	ric.3	-3.26	+			+	14	-350.7	729.5	6.32	0.025
	ric.1	-3.71	+	+	+	+	18	-347.6	731.1	7.98	0.011
	ric.6	-3.05	+			+	10	-355.9	731.9	8.73	0.007
	ric.4	-3.32	+	+	+		14	-353.4	734.9	11.69	0.002
	null	0.80					3	-394.7	795.4	72.2	0.000
	Variable Weight			1.00	0.22	0.04	0.80				

<b>Sapling abundance</b>	abu.2	-3.43	+	+		+	14	-506.3	1040.5	0.00	0.594	
	abu.1	-3.54	+	+	+	+	18	-502.9	1041.8	1.3	0.310	
	abu.5	-3.00	+	+				10	-513.0	1046.0	5.51	0.038
	abu.7	-2.58	+				+	10	-513.4	1046.8	6.28	0.026
	abu.4	-3.07	+	+	+			14	-509.6	1047.1	6.58	0.022
	abu.3	-2.58	+			+	+	14	-510.6	1049.3	8.73	0.008
	abu.8	-2.44	+					6	-520.2	1052.4	11.83	0.002
	abu.6	-2.42	+			+		10	-516.9	1053.7	13.17	0.001
	null	1.70						3	-565.6	1137.2	96.65	0.000
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	Variable Weight		1.00	0.96	0.34	0.94						
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<b>Sapling Composition</b>	com.8	+				5	-1676.5	3363.1	0.00	0.999
	com.7	+			+	9	-1692.9	3403.7	40.67	<.001
	com.6	+		+		9	-1710.4	3438.7	75.66	<.001
	com.5	+	+			9	-1716.3	3450.6	87.49	<.001
	com.2	+	+		+	13	-1726.7	3479.4	116.28	<.001
	com.3	+		+	+	13	-1736.1	3498.3	135.22	<.001
	com.4	+	+	+		13	-1750.3	3526.6	163.57	<.001
	com.1	+	+	+	+	17	-1766.5	3567.0	203.90	<.001
	null					2	-1798.3	3600.6	237.55	<.001
Variable Weight	1.00	<.001	<.001	<.001						

Beneath crowns, sapling species richness was three times higher in unmanaged than in managed areas (Estimate = -1.09, Std. Error = 0.31,  $z = -3.54$ ,  $P_{(adj.)} = 0.002$ ; Fig 1, Table 2). Grass volume correlated negatively with species richness in treeless areas (Estimate = -19.91, Std. Error = 7.79,  $z = -2.55$ ,  $P = 0.0107$ ), but was uncorrelated with species richness beneath crowns (S2 Table).

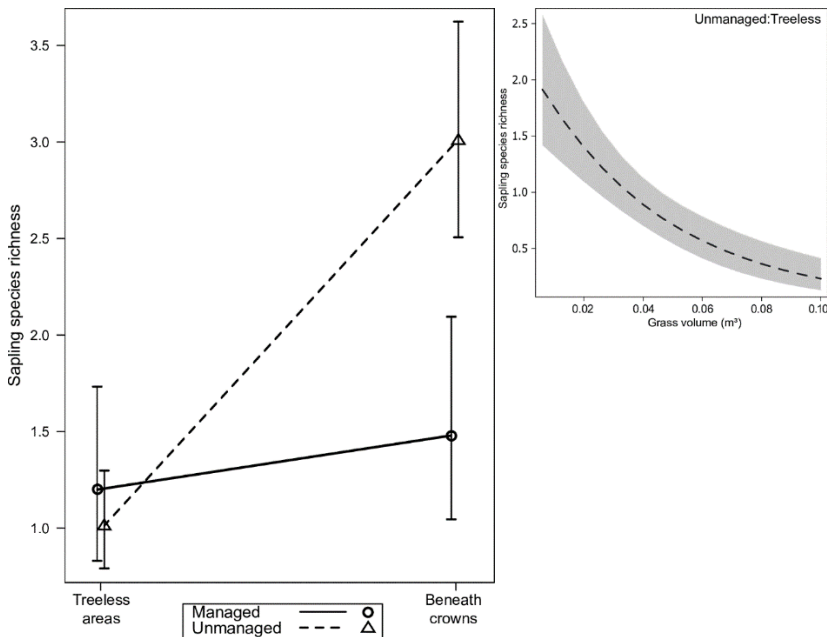


Figure 1. Species richness of saplings (species per m<sup>2</sup>) in relation to land management, araucaria crown influence and grass volume in an upper-montane *Araucaria* forest, southern Brazil. Shaded areas represent 95% confidence intervals. Effects of land management and araucaria crown influence on richness were plotted without the effects of grass volume. Effect of grass volume on richness was plotted from a GLM.

Table 2. Comparisons among groups of land management and araucaria crown influence of selected models for evaluating saplings species richness and abundance in an upper-montane Araucaria forest, southern Brazil. These results are represented in figures 1 and 2.

Model	Model ID	Coefficient comparisons			Estimate	Std.Error	z	P <sub>(adj.)</sub>
Sapling species richness	ric.7	Unman × Crowns	vs.	Unman × Treeless	-0.458	0.324	-1.41	0.628
		Unman × Crowns	vs.	Man × Treeless	0.973	0.524	1.86	0.316
		Unman × Crowns	vs.	Man × Crowns	-1.420	0.387	-3.67	<b>0.001</b>
		Unman × Treeless	vs.	Man × Crowns	-0.973	0.524	-1.86	0.316
		Unman × Treeless	vs.	Man × Treeless	-0.447	0.456	-0.98	0.654
		Man × Crowns	vs.	Man × Treeless	0.515	0.412	1.25	0.634
Sapling abundance	abu.2	Unman × Crowns	vs.	Unman × Treeless	-1.122	0.382	-2.94	<b>0.013</b>
		Unman × Crowns	vs.	Man × Treeless	2.085	0.616	3.39	<b>0.004</b>
		Unman × Crowns	vs.	Man × Crowns	-2.393	0.497	-4.81	<b>&lt;.0001</b>
		Unman × Treeless	vs.	Man × Crowns	-2.085	0.616	-3.39	<b>0.004</b>
		Unman × Treeless	vs.	Man × Treeless	-0.308	0.551	-0.56	1.000
		Man × Crowns	vs.	Man × Treeless	0.962	0.483	1.99	0.139

Significant P-values after adjustment for multiple comparisons are in bold. Covariates were included in these models but are presented in a separate table. Unman = unmanaged sites; Man = managed sites; Crowns = beneath araucaria crowns; Treeless = treeless areas. Interaction between predictors is represented by “×”.

## Sapling Abundance

A total of 1,300 sapling individuals were sampled (S1 Table). The model best fitting differences in abundance of saplings included land management, araucaria crown influence, grass volume and rock cover as predictors (S2 Table). This model had the lowest AIC from the set of candidate models (Table 1) and residuals were visually adequate. Fixed effects accounted for 50% of the differences in abundance (marginal  $R^2$ ), reaching 89% when considering both fixed and random effects (conditional  $R^2$ ).

Sapling abundance was 12 times higher in the unmanaged-beneath crowns condition than in the managed-beneath crowns condition (Estimate = -2.39, Std. Error = 0.49,  $z = -4.81$ ,  $P_{(adj.)} < 0.001$ ), 5 times higher than managed-treeless areas (Estimate = 2.08, Std. Error = 0.61,  $z = 3.39$ ,  $P_{(adj.)} = 0.004$ ), and 3 times higher than unmanaged-treeless areas (Estimate = -1.12, Std. Error = 0.38,  $z = -2.94$ ,  $P_{(adj.)} = 0.013$ ). In unmanaged-treeless areas, sapling abundance was 3.5 times higher than in the managed-beneath crowns condition (Estimate = -2.08, Std. Error = 0.61,  $z = -3.39$ ,  $P_{(adj.)} = 0.004$ ; Fig 2, Table 2). In unmanaged conditions, grass volume correlated negatively with sapling abundance in treeless areas (Estimate = -19.02, Std. Error = 8.83,  $z = -2.15$ ,  $P = 0.031$ ), but not beneath crowns. In managed conditions, rock cover correlated positively with sapling abundance beneath crowns (Estimate = 18.07, Std. Error = 5.24,  $z = 3.45$ ,  $P < 0.001$ ), but was uncorrelated with abundance in treeless areas. No association of rock and shrub cover with abundance was found in unmanaged areas (S2 Table).

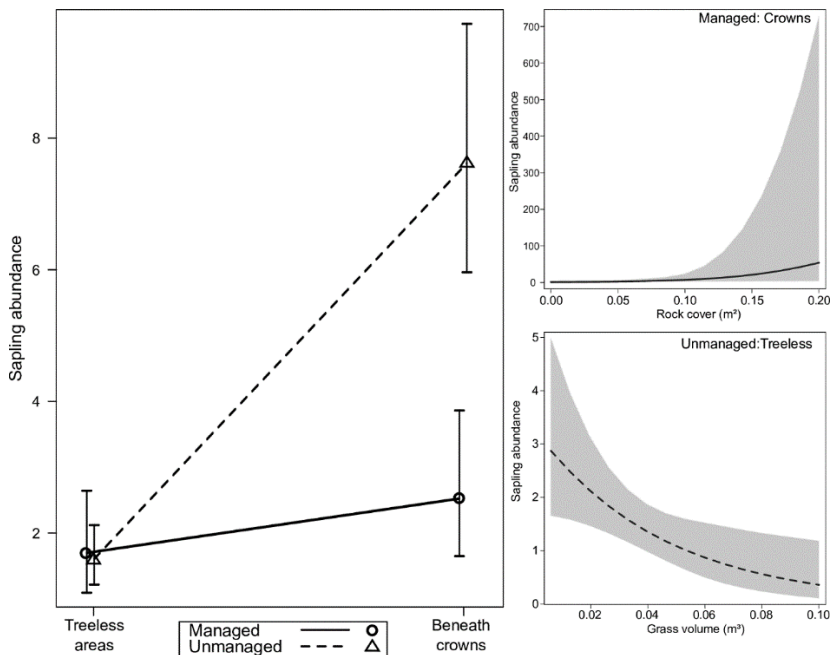


Figure 2. Abundance of saplings (individuals per  $m^2$ ) in relation to land management, araucaria crown influence and covariates (grass volume and rock cover) in an upper-montane *Araucaria* forest, southern Brazil. Shaded areas represent 95% confidence intervals. Effects of land management and araucaria crown influence on abundance were plotted without the effects of covariates (rock cover and grass volume). Effects of rock cover and grass volume on abundance were plotted from a GLM.

## Sapling Composition

Land management and araucaria crown influence were the main predictors of sapling composition. The model containing these two variables had the lowest AIC from the set of candidate models (Table 1) and residuals were visually adequate. The model explained 15% of the variation in species composition (pseudo  $R^2$  for  $GLM_{mv}$ ). Species composition beneath crowns differed from treeless areas in both unmanaged (likelihood ratio test,  $LR = 214.20$ ,  $P_{adj.} < 0.001$ ) and managed conditions ( $LR = 31.40$ ,  $P_{adj.} = 0.001$ ). In treeless areas, species composition differed in both unmanaged and managed

conditions (LR = 21.03,  $P_{\text{adj.}} = 0.04$ ). Similarly, beneath crowns, species composition differed between unmanaged and managed conditions (LR = 73.70,  $P_{\text{adj.}} < 0.001$ ). Finally, species composition was distinct between unmanaged-treeless areas and managed-beneath crowns conditions (LR = 31.40,  $P_{\text{adj.}} = 0.001$ ) and between managed-beneath crowns and managed-treeless areas conditions (LR = 36.69,  $P_{\text{adj.}} < 0.001$ ) (Table 3).



Table 3. Pairwise comparisons of species composition under different land management and araucaria crown influence in an upper-montane *Araucaria* forest, southern Brazil.

Model	Model ID	Coefficient comparisons			LR	P <sub>(adj.)</sub>
Sapling composition	com.8	Unman × Crowns	vs.	Unman × Treeless	214.2	<b>0.0006</b>
		Unman × Crowns	vs.	Man × Treeless	31.4	<b>0.001</b>
		Unman × Crowns	vs.	Man × Crowns	73.7	<b>0.0006</b>
		Unman × Treeless	vs.	Man × Crowns	31.4	<b>0.001</b>
		Unman × Treeless	vs.	Man × Treeless	21.0	<b>0.039</b>
		Man × Crowns	vs.	Man × Treeless	36.7	<b>0.0006</b>

LR = likelihood ratio test. Unman = unmanaged sites; Man = managed sites; Crowns = beneath araucaria crowns; Treeless = Treeless areas. Bold values indicate significant P-values after Holm-Bonferroni correction for multiple testing. Interaction between predictors is represented by “×”.

Univariate results for species showed that type of land management and araucaria crown influence were correlated with 11 sapling species (58% of all species – S3 Table). There were fewer araucaria saplings beneath crowns than in treeless areas in both unmanaged and managed conditions (Fig 3, S3 Table).

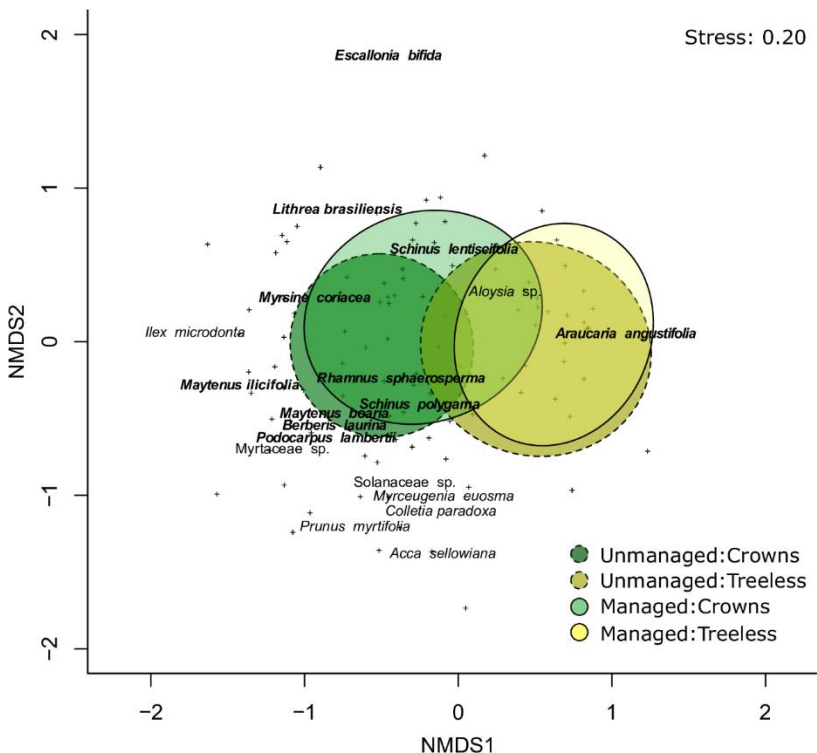


Figure 3. Non-metric multidimensional scaling (nMDS) ordination of saplings in relation to land management and araucaria crown influence in an upper-montane *Araucaria* forest, southern Brazil. Land management (unmanaged = dashed lines; managed = solid lines) and araucaria crown influence (beneath crowns = green tones; treeless areas = yellow tones). “+” represents sites and species in bold were significantly related to management and tree crown in the multivariate analysis. These ordinations are for visualization only; all statistical tests on the effects of land management and araucaria crown influence on community composition were conducted using

*multivariate response GLMs and taking the paired-sampling design into account.*

## Discussion

According to our results, the main consequence of the interactive effect between land management and crown influence was that land management overrode the facilitative effect provided by the nurse tree species. That is, although species richness was not generally affected by crown influence and land management, sapling abundance was normally higher in unmanaged conditions than managed conditions and increased beneath crowns for both management types. In addition, species composition was strongly affected by the interactive effect, differing in all combinations between land management and araucaria crown influence. Rock cover and grass volume affected richness and abundance of saplings in specific circumstances, while no effect of shrubs was found. Remarkably, araucaria saplings were found mostly in treeless areas in both managed and unmanaged conditions, indicating a possible preference for open spaces created by disturbances (SOUZA, 2007). Although several studies had been carried out demonstrating the elements involved in patch formation and forest expansion in southern Brazilian highlands (*e.g.* (DUARTE et al., 2006), (CARLUCCI; DUARTE; PILLAR, 2011), (KORNDÖRFER; DILLENBURG; DUARTE, 2015), (ZANDAVALLI; DILLENBURG, 2015), (MARCILIO-SILVA et al., 2015)), as far as we know, our study is the first one to date that combines both land management and facilitation.

Araucaria forests have been shaped by human societies for millennia, transforming landscapes into biocultural systems (SEDREZ DOS REIS; LADIO; PERONI, 2014). Therefore, it is essential to look at human activities and the relation of such activities with ecosystem structure (BOIVIN et al., 2016). Our results indicate that land management aiming to keep native grasslands hinders the establishment of most species but benefits araucaria seedlings and saplings. Therefore, local smallholders may play an important role in both araucaria protection and grassland maintenance. Local smallholders may also contribute to seed dispersal by voluntarily or

involuntarily planting, since araucaria seeds represent an important source of food and income (SEDREZ DOS REIS; LADIO; PERONI, 2014) and are frequently collected and transported from one place to another. As fire and cattle grazing are common management practices, they further contribute to grassland maintenance and diversity, hindering forest expansion (BEHLING; PILLAR, 2007; OLIVEIRA; PILLAR, 2004). Yet, most saplings were found beneath crowns in both managed and unmanaged conditions. The perch effect of araucaria trees (DOS SANTOS et al., 2011; DUARTE et al., 2006) and the amelioration of environmental conditions beneath crowns (KORNDÖRFER; DILLENBURG; DUARTE, 2015) favor the establishment of woody species in these areas, which later grow into forest patches (DUARTE et al., 2006), when management is absent.

Grass volume and shrub cover are much higher in unmanaged conditions due to the lack of cattle grazing and fire. In southern Brazil, grasslands are dominated by tussock grasses and shrubs when cattle grazing is of low intensity or missing (BOLDRINI, 1996; NABINGER; MORAES; MARASCHIN, 2000). Over time, this vegetation can shift to shrublands and forests (BEHLING; PILLAR, 2007; OLIVEIRA; PILLAR, 2004). However, under such circumstances, high accumulation of flammable biomass increases the risk of catastrophic fires that can strongly reduce biodiversity (BEHLING; PILLAR, 2007) and negatively affect human wellbeing and cultural landscapes. In treeless areas, which had fewer species and individuals than beneath crowns, increasing grass volume further negatively affects sapling richness and abundance. Grasses and saplings may compete for resources, such as soil nutrients, water and light (SCHOLLES; ARCHER, 1997; ZANDAVALLI; DILLENBURG, 2015), and growth in height of grasses may be advantageous in competing for light, but may represent a disadvantage when herbivore pressure is high (SKARPE, 2001). We believe that grasses may succeed over seedlings of woody species and saplings because of the physical barrier provided by their own fast-growing aboveground biomass, which may hinder the establishment of most woody species, such as araucaria (ZANDAVALLI; DILLENBURG, 2015). There was no effect of grass volume on sapling richness and abundance beneath crowns,

possibly because of both facilitation and perch effects that araucaria trees exert on woody species and the interference of shade produced by araucaria crowns on grasses, resulting in lower grass volume.

The influence of abiotic factors, such as non-living objects (also called nurse objects, *e.g.*, tree stumps and rocks), are still poorly explored (MUNGUÍA-ROSAS; SOSA, 2008). In southern Brazilian grasslands, it has been shown that rocky outcrops favor the establishment of woody plants, contributing to patch formation and forest expansion (CARLUCCI; DUARTE; PILLAR, 2011). Our results indicate that rocks may influence sapling abundance only under specific conditions. We found a very weak effect or no effect at all of rocks in treeless areas in managed and unmanaged conditions and beneath crowns in unmanaged areas. We believe that treeless areas in managed conditions are too exposed to harsh climate conditions, cattle herbivory and trampling and management fires, hindering the role of rocks in benefitting woody plants. Conversely, rocks positively affected sapling abundance beneath crowns in managed conditions. In this case, the combined positive effects of araucaria trees and rocks may have been responsible for this pattern. Rocks can improve plant germination, establishment and fitness by ameliorating conditions (such as shade, accumulation of soil and water), reducing grazing, protecting against mechanical and fire damage (BORCHERT et al., 2003; CALLAWAY, 2007; CARLUCCI; DUARTE; PILLAR, 2011), and by never competing with its nurse (MUNGUÍA-ROSAS; SOSA, 2008). Furthermore, rocks can also act as perches for frugivorous birds, refuge for small rodents and as a place for countermarking and a latrine for medium-sized omnivores (CARLUCCI; DUARTE; PILLAR, 2011).

In this study, the highest richness and abundance of saplings and the differences in community composition found beneath crowns in relation to treeless areas and to managed conditions further corroborate the role of araucaria trees in favoring forest species (DUARTE et al., 2006). Conversely, low richness and abundance of saplings in managed conditions further reinforces the role of local smallholders in grassland maintenance. Although sapling

richness decreases when cattle and fire are present, richness of grasses may increase when these components are appropriately managed (BOLDRINI, 1996). Moreover, because araucaria saplings were found mostly in treeless areas in both unmanaged and managed conditions, it seems disturbances create gaps that benefit *A. angustifolia* (SOUZA, 2007).

Among other woody species, *Araucaria angustifolia* itself seemed to be negatively affected by its own crown in both managed and unmanaged conditions. Thus, land management allowing fire and cattle grazing seems to be neutral or even favorable for araucaria trees, at least when the fire interval is long enough to allow seedling development. Disturbances that create gaps, such as storms and fires, have been suggested as beneficial for *A. angustifolia*, at least within small spatial extents where seed rain is not disrupted (SOUZA, 2007; SOUZA et al., 2008). Our results also indicate the role gaps created or maintained by fire have, together with additional management actions, on persistence and abundance of araucaria trees. Remarkably, similar land management actions were formerly made by pre-Columbian societies who managed forests and used fire (BITENCOURT; KRAUSPENHAR, 2006). These actions are now carried out by local smallholders (SEDREZ DOS REIS; LADIO; PERONI, 2014), who contribute to niche construction (ZEDER, 2016) and promote the maintenance of a higher landscape diversity than expected without management.

Despite the small extent of the study site and geographical proximity of the locations, our sampling design allowed for reducing the effect of confounding factors, such as climate, soil type and depth, and topography. Moreover, both locations have a similar use history, since the land management employed in both areas in the past was the same. We also encourage future researches to include the relationships of humans with the landscape, since several species and their interactions can be affected by landscape domestication. To further understand the effects of land management along with ecological interactions, we recommend future studies to quantify land management, for example, by measuring grazing pressure and

fire intensity. Understanding such relationships will allow for a better comprehension of ecologically relevant patterns and processes.

## Conclusions

Our study supports both the role araucaria trees have in promoting forest expansion and the importance of local smallholders in maintaining grasslands. On the one hand, araucaria trees contribute to increasing sapling species richness and abundance, and in changing community composition beneath their crowns. On the other hand, land management actions taken by local smallholders help in maintaining natural grasslands by hindering forest expansion through fire and cattle grazing. We demonstrate the key roles these two major components (land management actions and araucaria trees) have in plant community assemblage, contributing to landscape diversity maintenance in upper-montane regions. Disturbances promoted by land management can favor the maintenance of threatened landscapes and trees, such as highland grasslands and araucaria trees. Our results also clarify patterns and processes that may emerge in natural highland grasslands, such as the conversion of grasslands into forests and modification of cultural landscapes when the most significant management actions (grazing and fire) are excluded. Consequently, maximal diversity can be achieved by a balanced set of both protected areas and maintenance of traditional management practices.

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## Supporting information

*S1 Table. Summary of main variables measured under distinct land management and araucaria crown influence and covariates (grass volume, rock and shrub cover) in an upper-montane Araucaria forest, southern Brazil. Except for sampling area, richness and abundance, other variables were mean  $\pm$  standard deviation.*

<b>Araucaria crown influence</b>	<b>Variables</b>	<b>Managed</b>	<b>Unmanaged</b>	<b>Total</b>
<b>Beneath crowns</b>	Sampling area (m <sup>2</sup> )	1065.3	2809.1	3874.4
	Grass volume (m <sup>3</sup> )	0.013 $\pm$ 0.009	0.021 $\pm$ 0.016	0.019 $\pm$ 0.015
	Shrub cover (m <sup>2</sup> )	0.0018 $\pm$ 0.006	0.064 $\pm$ 0.061	0.046 $\pm$ 0.059
	Rock cover (m <sup>2</sup> )	0.025 $\pm$ 0.036	0.031 $\pm$ 0.042	0.029 $\pm$ 0.041
	Total richness	10	15	15
	Total abundance	186	884	1070
<b>Treeless areas</b>	Sampling area (m <sup>2</sup> )	1065.3	2809.1	3874.4
	Grass volume (m <sup>3</sup> )	0.013 $\pm$ 0.013	0.036 $\pm$ 0.018	0.029 $\pm$ 0.020
	Shrub cover (m <sup>2</sup> )	0.0009 $\pm$ 0.003	0.052 $\pm$ 0.051	0.037 $\pm$ 0.049
	Rock cover (m <sup>2</sup> )	0.030 $\pm$ 0.037	0.014 $\pm$ 0.025	0.019 $\pm$ 0.030
	Total richness	10	12	14
	Total abundance	92	138	230

<b>Total</b>	Sampling area (m <sup>2</sup> )	2130.6	5618.2	7748.8
	Grass volume (m <sup>3</sup> )	0.013±0.011	0.028±0.019	0.024±0.018
	Shrub cover (m <sup>2</sup> )	0.001±0.004	0.058±0.019	0.041±0.054
	Rock cover (m <sup>2</sup> )	0.028 ±0.037	0.022±0.036	0.024±0.036
	Total richness	13	17	19
	Total abundance	278	1022	1300

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*S2 Table. Complete GLMMs for evaluating species richness and abundance of saplings in relation to land management, araucaria crown influence and covariates (grass volume, rock and shrub cover) in an upper-montane Araucaria forest, southern Brazil. Significant P-values are in bold. Unman = unmanaged sites; Man = managed sites; Crowns = beneath araucaria crowns; Treeless = treeless areas; Grass = grass volume; Rock = rock cover.*

Model	Model ID	Coefficients	Estimate	Std. Error	z	P
Sapling species richness	ric.7	(Intercept)	-3.245	0.274	-11.86	<b>&lt; 0.0001</b>
		Treeless	0.185	0.311	0.59	0.5530
		Unman	1.098	0.310	3.54	<b>0.0004</b>
		Unman×Treeless	-0.768	0.421	-1.83	<b>0.0679</b>
		Man×Crowns×Grass	19.232	17.654	1.09	0.2760
		Man×Treeless×Grass	-19.123	18.625	-1.03	0.3045
		Unman×Crowns×Grass	-6.646	6.156	-1.08	0.2803
		Unman×Treeless×Grass	-19.908	7.799	-2.55	<b>0.0107</b>
Sapling abundance	abu.2	(Intercept)	-3.428	0.412	-8.33	<b>&lt; 0.0001</b>
		Treeless	0.962	0.483	1.99	<b>0.0463</b>
		Unman	2.393	0.497	4.81	<b>&lt;0.0001</b>
		Unman×Treeless	-2.085	0.616	-3.39	<b>0.0007</b>
		Man×Crowns×Rock	18.079	5.244	3.45	<b>0.0006</b>
		Man×Treeless×Rock	5.035	5.576	0.9	0.3666



Unman×Crowns×Rock	2.175	3.295	0.66	0.5091
Unman×Treeless×Rock	-6.171	6.735	-0.92	0.3595
Man×Crowns×Grass	34.061	22.142	1.54	0.1240
Man×Treeless×Grass	-41.692	23.562	-1.77	0.0768
Unman×Crowns×Grass	-13.800	9.314	-1.48	0.1384
Unman×Treeless×Grass	-19.026	8.839	-2.15	<b>0.0314</b>

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S3 Table. Univariate results of multivariate tests. This table shows how species abundances were affected by the interaction between land management and araucaria crown influence as well as the direction of the effect. Overall Test Stats = overall effect for each species. LR = likelihood ratio test, UM = Unmanaged conditions, M = Managed conditions, Canopies = beneath crowns, Treeless = Treeless areas. Significant effects are in bold. "+" indicate positive effects and "-" indicate negative effects (from the second level compared to the first).

Species	Overall Test Stats		UM×Crowns x M×Crowns		UM×Crowns x UM×Treeless		UM×Crowns x M×Treeless	
	LR	P	LR	P	LR	P	LR	P
<i>Acca sellowiana</i> (O.Berg) Burret	2.301	0.4147	1.265	0.6429	0.777	0.5756	0.157	0.8854
<i>Aloysia</i> sp.	1.57	0.4147	0.887	0.6429	0.14	0.8572	0	0.9488
<i>Araucaria angustifolia</i> (Bertol.) Kuntze	17.828	<b>0.0023</b>	1.397	0.6429	8.432 <sup>(+)</sup>	<b>0.0035</b>	1.346	0.5938
<i>Berberis laurina</i> Billb.	31.748	<b>0.0001</b>	3.298	0.3288	28.599 <sup>(-)</sup>	<b>0.0001</b>	4.72	0.0764

<i>Colletia paradoxa</i> (Spreng.) Escal.	3.87	0.2063	0	0.6429	0	0.9901	0.001	0.8858
<i>Escallonia bifida</i> Link & Otto	11.727	<b>0.0144</b>	8.002 <sup>(+)</sup>	<b>0.033</b>	0	0.8572	0	0.9488
<i>Ilex microdonta</i> Reissek	2.105	0.4147	0.719	0.6429	1.386	0.2702	0.001	0.8858
<i>Lithrea brasiliensis</i> Marchand	57.18	<b>0.0001</b>	13.686 <sup>(-)</sup>	<b>0.0037</b>	43.806 <sup>(-)</sup>	<b>0.0001</b>	7.252 <sup>(+)</sup>	<b>0.0173</b>
<i>Maytenus boaria</i> Molina	47.019	<b>0.0001</b>	10.491 <sup>(-)</sup>	<b>0.0123</b>	42.887 <sup>(-)</sup>	<b>0.0001</b>	16.687 <sup>(+)</sup>	<b>0.0003</b>
<i>Maytenus ilicifolia</i> Mart. ex Reissek	11.784	<b>0.0141</b>	7.561 <sup>(+)</sup>	<b>0.0384</b>	0	0.9901	0.002	0.8858

<i>Myrceugenia euosma</i> (O.Berg) D.Legrand	3.78	0.2143	0	0.8712	0	0.9901	0.001	0.8858
<i>Myrsine coriacea</i> (Sw.) R.Br. ex Roem. & Schult.	52.587	<b>0.0001</b>	8.356 <sup>(-)</sup>	<b>0.033</b>	36.549 <sup>(-)</sup>	0.0001	0.033	0.8858
Myrtaceae sp.	6.712	0.0861	3.144	0.3288	3.017	0.0559	0	0.9488
<i>Podocarpus lambertii</i> Klotzsch ex Endl.	9.982	<b>0.0264</b>	1.508	0.6429	7.579 <sup>(-)</sup>	<b>0.0035</b>	0	0.9488
<i>Prunus myrtifolia</i> (L.) Urb.	4.211	0.1812	1.439	0.6429	2.772	0.0559	0.001	0.8858
<i>Rhamnus sphaerosperma</i> Sw.	14.796	<b>0.0055</b>	1.681	0.6429	11.443 <sup>(-)</sup>	<b>0.0008</b>	0	0.9488

<i>Schinus lentiscifolia</i> Marchand	15.432	<b>0.0055</b>	3.597	0.3149	11.407 <sup>(-)</sup>	<b>0.0008</b>	1.108	0.5938
<i>Schinus polygama</i> (Cav.) Cabrera	21.543	<b>0.0006</b>	5.953	0.101	14.055 <sup>(-)</sup>	<b>0.0003</b>	0.088	0.8854
Solanaceae sp.	2.105	0.4147	0.719	0.6429	1.386	0.2702	0.001	0.8858

---

S3 Table (cont.)

Species	UM×Treeless x M×Treeless		UM×Treeless x M×Crowns		M×Crowns x M×Treeless	
	LR	P	LR	P	LR	P
<i>Acca sellowiana</i> (O.Berg) Burret	0.295	0.9763	0.157	0.8854	0.004	0.9376
<i>Aloysia</i> sp.	0.589	0.9201	0	0.9488	0	1
<i>Araucaria angustifolia</i> (Bertol.) Kuntze	0.118	0.9827	1.346	0.5938	9.867 <sup>(+)</sup>	<b>0.0086</b>
<i>Berberis laurina</i> Billb.	1.683	0.8067	4.72	0.0764	1.63	0.5355
<i>Colletia paradoxa</i> (Spreng.) Escal.	2.471	0.6041	0.001	0.8858	1.43	0.5355

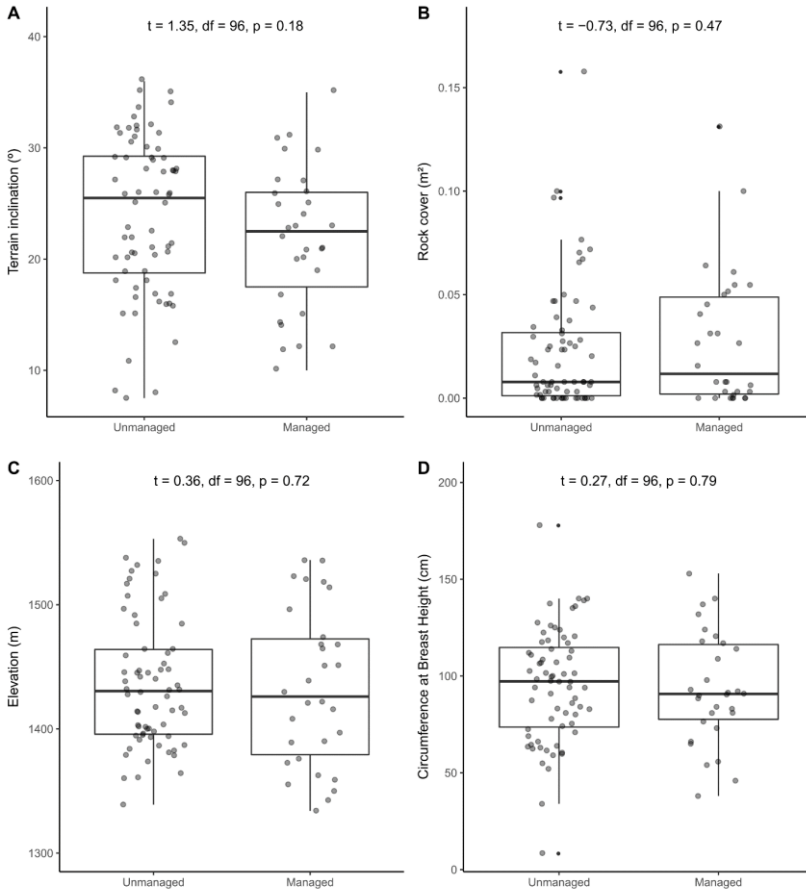
<i>Escallonia bifida</i> Link & Otto	2.409	0.6201	0	0.9488	1.85	0.5355
<i>Ilex microdonta</i> Reissek	0	0.9998	0.001	0.8858	0	0.9376
<i>Lithrea brasiliensis</i> Marchand	0.013	0.9827	7.252 <sup>(-)</sup>	<b>0.0173</b>	1.644	0.5355
<i>Maytenus boaria</i> Molina	8.125 <sup>(+)</sup>	<b>0.0431</b>	16.687 <sup>(-)</sup>	<b>0.0003</b>	0.058	0.9376
<i>Maytenus ilicifolia</i> Mart. ex Reissek	0	0.9998	0.002	0.8858	4.383	0.1127
<i>Myrceugenia euosma</i> (O.Berg) D.Legrand	2.394	0.624	0.001	0.8858	1.386	0.5355

<i>Myrsine coriacea</i> (Sw.) R.Br. ex Roem. & Schult.	1.527	0.8067	0.033	0.8858	9.248 <sup>(-)</sup>	<b>0.0104</b>
Myrtaceae sp.	0.632	0.9108	0	0.9488	0	1
<i>Podocarpus lambertii</i> Klotzsch ex Endl.	0	0.9827	0	0.9488	1.085	0.5355
<i>Prunus myrtifolia</i> (L.) Urb.	0	0.9827	0.001	0.8858	0	1
<i>Rhamnus sphaerosperma</i> Sw.	0	0.9998	0	0.9488	2.098	0.4708
<i>Schinus lentiscifolia</i> Marchand	0.061	0.9827	1.108	0.5938	0.694	0.644



<i>Schinus polygama</i> (Cav.) Cabrera	0.712	0.891	0.088	0.8854	1.312	0.5355
Solanaceae sp.	0	0.9998	0.001	0.8858	0	1

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*S1 Fig. Differences among measured variables for unmanaged and managed locations in an upper-montane Araucaria forest, southern Brazil. A = terrain inclination (°); B = rock cover (m<sup>2</sup>); C = elevation (m); D = Circumference at Breast Height (CBH) of sampled araucaria trees (cm). Circles represent samples (average of the sampled pair – except for CBH).*

**S1 File. Data set used for analyses.**

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## 6. Capítulo 3<sup>††</sup>

# No benefit from soil microbiota on performance of nurse tree seedlings: consequences for forest-grassland dynamics

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## Abstract

1. Seed germination and seedling growth rates curb under extreme abiotic conditions and strong negative interactions, whereas facilitation aids on such rates. Yet, positive effects of facilitation can be mediated by soil microbial communities, adding a component to be understood in vegetation dynamics. To tackle this issue, we scrutinized the effects of soil types and microbiota from a grassland-forest gradient on plant performance. Such effects were checked for germination and seedling growth of a key nurse tree species involved in grassland replacement by forests, expecting a beneficial effect of its associated soil microbiota and forest soils.
2. By means of an experiment, early stage performance of a nurse tree species was assessed in soils found across a forest-grassland gradient and with varying soil microbial composition. Soil type and microbiota were sampled from grassland, shrubland, forest edge, and forest interior. Microbial composition was either kept unchanged (unsterilized), dismantled (sterilized) or inoculated (sterilized and re-added with soil extract). Early stage performance on experimental treatments was inferred by both germination rates and early seedling growth, the latter by means of three belowground and two aboveground traits.
3. Contradicting our expectations, soil microbial communities seemed neutral to araucaria germination and detrimental to the early growth of araucaria seedlings. Such net negative effect of soil microbial communities was found across the forest-grassland gradient. Seedling performance was generally worse when grown in forest interior soils where adult trees of araucaria are abundant. Conversely, dismantling soil microbial communities resulted in improved both belowground and aboveground seedling growth and biomass uptake.
4. *Synthesis*. Soil microbial communities reduced the performance of araucaria seedlings – a nurse tree known to

accelerate the replacement of grasslands by forests in the highlands of southern Brazil. Such reduction was found in soils from across the landscape, although stronger in soils where adult araucarias grow. While positive for the maintenance of threatened subtropical highland grasslands, such results suggest the slow pace of forest expansion over grasslands can be mediated by soil microbiota, confirming an important but not necessarily positive effect of soil organisms in plant communities.

Keywords: araucaria, belowground interactions, facilitation, forest expansion, germination, highland grasslands, nurse trees, soil microbiota

## Resumo

1. Determinar quais fatores afetam a performance das plantas em estágios iniciais de desenvolvimento auxilia na compreensão da colonização, sucessão e na expansão de plantas. Além de fatores abióticos, a competição, predação, herbivoria e alelopatia podem prevenir, reduzir ou retardar taxas de germinação e crescimento, em oposição à facilitação. A potencial importância da comunidade de microrganismos do solo em afetar a performance de plantas em seu estágio inicial pode mudar nossa compreensão de ao menos parte dos benefícios atribuídos a interações planta-planta.
2. Neste estudo, nós avaliamos experimentalmente os efeitos do tipo de solo e comunidades microbianas do solo nos estágios iniciais de desenvolvimento da araucária. A araucária é uma espécie facilitadora e importante impulsionadora da expansão da floresta com araucária sobre os campos nas terras altas do sul do Brasil. Nossa hipótese foi de que as comunidades microbianas do solo e o tipo de solo geralmente associado à araucária seriam benéficos para sua germinação e crescimento inicial. Por outro lado, tipos de

solo e comunidades microbianas onde os campos e arbustais prosperam deveriam ser prejudiciais para a germinação e crescimento inicial da araucária. Em nosso experimento, avaliamos o desempenho do sistema radicular (comprimento da raiz principal, peso seco da raiz e área das raízes laterais) e da parte aérea (comprimento do broto e peso seco do broto) da araucária em solos de diferentes unidades de paisagem do gradiente campestre-florestal, e com comunidades microbianas não esterilizadas, esterilizadas ou inoculadas.

3. Contrariando nossas expectativas, as comunidades microbianas do solo foram neutras à germinação da araucária e prejudiciais ao seu crescimento inicial. Esse efeito negativo das comunidades microbianas do solo foi encontrado ao longo do gradiente avaliado, em todos os solos avaliados: campo, arbustal, borda de floresta e interior de floresta. O desempenho das plântulas foi em geral pior em solos do interior de floresta, onde as árvores adultas da araucária são abundantes. Por outro lado, o desmantelamento das comunidades microbianas do solo resultou na melhoria do crescimento e na absorção de biomassa das plântulas, tanto na parte aérea quanto no sistema radicular.
4. *Síntese.* As comunidades microbianas do solo afetam negativamente o crescimento inicial das plântulas de araucária – uma árvore que acelera a substituição de campos por florestas nas terras altas do sul do Brasil. Este efeito foi encontrado em solos ao longo do gradiente floresta-campo, sendo mais forte em solos onde árvores adultas da araucária são encontradas. Este efeito permite um ritmo lento de expansão da floresta sobre o campo, contribuindo assim para a manutenção dos ameaçados campos de altitude subtropicais.

Palavras-chave: araucaria, expansão da floresta, facilitação, germinação, campos de altitude, interações abaixo do solo, microbiota do solo, planta-berçário.



## Introduction

Large-scale vegetation displacement can deeply change ecosystem functioning. Meanwhile, causes of vegetation dynamics are complex, involving a set of drivers across spatial and temporal scales. For example, distribution of vegetation is normally predicted by climate, with warm and wet areas being generally covered by forests. Grasslands are exceptions, replacing forests under high herbivory intensity, high fire frequency, or both (BOND, 2008; SÜHS; GIEHL; PERONI, 2018). In such situations, if fire is prevented and large herbivores are removed, grasslands tend to undergo shrub encroachment (DECHOUM; PERONI; PUGNAIRE, 2018; OLIVEIRA; PILLAR, 2004) and ultimately develop into forests. Besides such factors, additional drivers operating at fine spatial and temporal scales can shape vegetation dynamics. They include differences in performance during early plant life stages and plant-soil interactions.

Germination and seedling establishment are crucial plant life stages (FARNSWORTH, 2008). Consequently, the assembly and maintenance of plant communities depends on species' differential performances during such stages (KELLOGG; BRIDGHAM; LEICHT, 2003; LECK; PARKER; SIMPSON, 2008). Germination and seedling growth are affected by disturbances, climate, and soil characteristics, such as water retention and nutrient availability (FARNSWORTH, 2008; ISSELSTEIN; TALLOWIN; SMITH, 2002; LECK; PARKER; SIMPSON, 2008; RAYNAUD; LEADLEY, 2004; WESTMAN, 1981). In addition, germination and seedling growth rates are affected by biological interactions. Negative interactions such as competition by interference from neighbouring plants, seed predation and herbivory reduce germination and seedling growth rates (FENNER; THOMPSON, 2005). Moreover, inhibitory interactions such as allelopathy can either prevent, reduce or delay seed germination and seedling growth rates (MORRIS; WOOD, 1989; WALKER; VITOUSEK, 1991). Conversely, positive interactions like facilitation can also play a role by enhancing seedling growth rates (*e.g.* Connell & Slatyer, 1977; Walker & Vitousek, 1991). Therefore, determining factors affecting

performance in early life stages aids understanding plant colonization, succession, and range expansion.

Out of the list of relevant biological interactions, some details about facilitation are incompletely understood. Facilitation is a positive, non-trophic interaction that occurs between physiologically independent plants, mediated by changes promoted by the benefactor on either the abiotic environment, other organisms, or both (BROOKER et al., 2008). Facilitation seems to be a key mechanism in structuring communities and in keeping diversity (BRUNO; STACHOWICZ; BERTNESS, 2003; CALLAWAY, 2007), especially in harsh environments (BROOKER et al., 2008; CALLAWAY, 1995). Benefactor species create suitable habitats for other species through changes in the environment that mitigates potentially limiting stressors (STACHOWICZ, 2001). For example, certain shrubs and trees can ameliorate environmental conditions beneath their canopies through changes either aboveground (e.g. shading), belowground (e.g. nitrogen fixation) or both, thus reducing temperature and increasing soil humidity, oxygenation, and nutrient availability (KORNDÖRFER; DILLENBURG; DUARTE, 2015; MARON; CONNORS, 1996; MORO et al., 1997). Nevertheless, belowground effects are not necessarily tracked down to the benefactor plant but can result from changes to the soil microbial community associated with it (RODRÍGUEZ-ECHEVERRÍA et al., 2013). Despite few empirical assessments in facilitation studies (RODRÍGUEZ-ECHEVERRÍA; LOZANO; BARDGETT, 2016), the potential importance of the soil microbial community can change our understanding of at least part of the benefits attributed to direct plant-plant positive interactions.

Whereas soil microbial communities can drive apparent plant-plant facilitation, feedbacks likely exist. Soil microbial communities can influence plant physiology and development (MENDES; GARBEVA; RAAIJMAKERS, 2013) and individual plant species can be either favoured or limited by microbial diversity (ARAYA et al., 2017). Mycorrhizal fungi can protect seedlings against droughts, pathogenic soil fungi and heavy metals, and increase nutrient uptake (MARSCHNER; DELL, 1994; VAN DER HEIJDEN; HORTON, 2009). In turn, plant patches can mitigate the effects of

extreme abiotic conditions, favouring its associated plant-soil microbiota communities (RODRÍGUEZ-ECHEVERRÍA et al., 2013). In addition, nurse plants can change the composition of soil microbial communities by selecting organisms that promote plant growth and are more effective in nutrient mineralization (HORTAL et al., 2013; RODRÍGUEZ-ECHEVERRÍA; LOZANO; BARDGETT, 2016). The resulting changes to individual plant fitness and soil nutrient availability can affect diversity, species abundance and composition of plant communities, further impacting ecosystem functioning (HORTAL et al., 2013; RODRÍGUEZ-ECHEVERRÍA et al., 2013; RODRÍGUEZ-ECHEVERRÍA; LOZANO; BARDGETT, 2016). Yet, the outcome can be driven either by nurse plants alone, its associated soil microbial community, or both.

In some cases, nurse plants can be locally dominant species, thus displaying paramount contribution to community structure and ecosystem functioning. In the subtropical montane areas of Brazil, the conifer *Araucaria angustifolia* (Bertol.) Kuntze (hereafter “araucaria”) abounds and dominates the tree layer of mixed rainforests (Araucaria forests) (KLEIN, 1984). Araucaria forests occur over 500 m a.s.l. (REITZ; KLEIN, 1966) and frequently form mosaics with highland grasslands beyond 900 m a.s.l. (RAMBO, 1994), with abrupt transitions. It is yet unclear why araucaria is unable to colonize grasslands at higher altitudes (FRANCO et al., 2005). Nevertheless, since around 4 kyr BP, raising temperatures and rainfall increase have favoured forest expansion (BEHLING, 1995, 1998; BEHLING et al., 2004), which is likely to accelerate because of global warming. Furthermore, land management by local smallholders, which includes cattle raising and fire, hinders forest expansion (BEHLING; PILLAR, 2007; OLIVEIRA; PILLAR, 2004; SÜHS; GIEHL; PERONI, 2018). Without herbivory and fire, grasslands tend to be colonized by Araucaria forests. This process starts with the arrival of araucarias, because the species is a pioneer and colonizes even stressful environments (FRANCO et al., 2005), and then speeds up because araucarias act as nurse plants for other forest species (DOS SANTOS et al., 2011; DUARTE et al., 2006; KORNDÖRFER; DILLENBURG; DUARTE, 2015; OLIVEIRA; PILLAR, 2004; SÜHS; GIEHL; PERONI, 2018).

Araucaria forests replace grasslands expanding both from forest edges (OLIVEIRA; PILLAR, 2004) and by nucleation following the establishment of even a single araucaria amid grasslands (DUARTE et al., 2006; KLEIN, 1960). In some cases, however, grasslands persist, or vegetation dynamics may end up in a shrubland stage where forest development may never happen (DECHOUM; PERONI; PUGNAIRE, 2018). Therefore, climate or disturbances alone may be insufficient to explain the vegetation mosaic in the region. Whether there is a contribution of small-scale differences in soil and soil microbial communities to either keep the vegetation mosaic or explain where and why araucaria is likely to succeed is underexplored.

Soil microbial community can affect araucaria germination and early establishment in either positive (MOREIRA-SOUZA; CARDOSO, 2003; ZANDAVALLI; DILLENBURG; DE SOUZA, 2004) or negative ways (DALMAS et al., 2013), whereas the net effect of the entire soil community is still unknown. Here we assessed experimentally the effects of soil type and soil microbial communities on early life stages of the nurse tree araucaria. We hypothesize soil microbial communities and soil type associated with this nurse species are beneficial to the germination and early growth of its seedlings. Conversely, soil types and microbial communities where grasslands and shrublands thrive should be detrimental to araucaria germination and seedling growth. In our experiment, we assessed performance on soils from vegetation found on different units of the forest-grassland gradient and with either unsterilized, sterilized, or inoculated soil microbial communities. Contradicting our expectation, soil microbial communities seemed detrimental to the early growth of araucaria seedlings, while soil differences alone could allow a faster pace of forest expansion over grasslands.

## **Materials and methods**

### **STUDY AREA**

We conducted the study in the highlands of southern Brazil, in the São Joaquim National Park (elevation: 1350 m; coordinates: 28.10°S; 49.39°W – 28.08°S; 49.37°W), state of Santa Catarina (Supporting

Information: Fig. S1). This protected area has 49,300 ha out of which ~14,000 ha has been enacted since 2006. The most common vegetation types found in these highlands are high-altitude grasslands and Araucaria forests (a type of mixed rainforest). The region has an annual mean rainfall of 1,626 mm yr<sup>-1</sup>, being well-distributed throughout the year, and an annual mean temperature of 13.3 °C. The average minimum temperature of the coldest month (July) is 6.0 °C and the average maximum temperature of the hottest month (January) is 22.9 °C. During winter, frost occurs every year and snow is occasional (data from the nearest weather station, located ~30 km from the study site and compiled from 1961 to 2016; source INMET, 2017). Local ranchers raise extensive livestock and use fire on grasslands for regrowth of pastures (SÜHS; GIEHL; PERONI, 2018). Inside the protected area, fire and cattle grazing are prevented.

## STUDY SPECIES

We carried out experiments about the germination and early growth of seedlings of *Araucaria angustifolia*, a common and dominant tree species in southern Brazilian highlands. Araucaria is considered a key species in forest succession and forest expansion over grasslands in southern Brazilian highlands (*e.g.* Duarte et al., 2006; Klein, 1960) and is an important source of food and income for local smallholders (SEDREZ DOS REIS; LADIO; PERONI, 2014).

## DATA COLLECTION

### **Seeds**

Araucaria seeds were acquired from local sellers in early August 2016. Before planting, seeds were subjected to disinfection and to a flotation test, in which seeds were poured in a container with water. Seeds that didn't sink to the bottom were discarded. Disinfection consisted of keeping the seeds in water with 10% bleach for 10 min. Floating seeds were discarded to reduce the amount of inviable seeds. Seeds were planted in pots containing 1.7 L of soil.

### ***Soil-plant systems and soil sampling***

Soil was collected across a forest-grassland ecotone inside the protected area (elevation: 1610 m; coordinates: 28°09'S, 49°38'W; Supporting Information: Fig. S1). Aiming at standardization, top-soil up to 15 cm was extracted nearby the same set of plant species representative of the units of the forest-grassland gradient. Such soil-plant systems characterized grasslands, shrublands, forest edges, and forest interior. The selected species were: *Andropogon lateralis* Nees (grasslands), *Baccharis uncinella* DC. (shrublands), *Drymis angustifolia* Miers (forest edge) and araucaria (forest interior) Fig. 1. *Andropogon lateralis* is a common and dominant grass species found in highland grasslands. *Baccharis uncinella* is a dominant shrub that occurs in open areas associated to grasslands and in forest edges and was also suggested to facilitate the establishment of trees in the region (KORNDÖRFER; DILLENBURG; DUARTE, 2015). *Drymis angustifolia* is an abundant pioneer tree species usually occurring nearby forest edges in high-montane forests. *Araucaria angustifolia* is a dominant and abundant pioneer tree species that colonizes stressful environments (FRANCO et al., 2005), and act as nurse plants for other forest species (DOS SANTOS et al., 2011; DUARTE et al., 2006; KORNDÖRFER; DILLENBURG; DUARTE, 2015; OLIVEIRA; PILLAR, 2004; SÜHS; GIEHL; PERONI, 2018). Araucaria trees can be found together with all other species and across landscape units.

We collected 400 kg of soil under five plants out of each soil-plant system. Plants were chosen to be at least 10 m apart, but in the same region, thus reducing variation in soil physical and chemical properties both within and between soil-plant systems. Plant replicates under which soils were collected were mature and similar in size. Soils collected within the same soil-plant system were pooled forming a composite sample. Out of each composite sample, we sterilized 2/3 of it by means of two heat-treatments in an autoclave, each treatment taking 1 h. The remaining 1/3 of the soil was kept unsterilized to maintain its original microbial community. A small soil sample of each soil-plant system (unsterilized and sterilized) was

destined for chemical and physical analysis before and after the experiment described below.

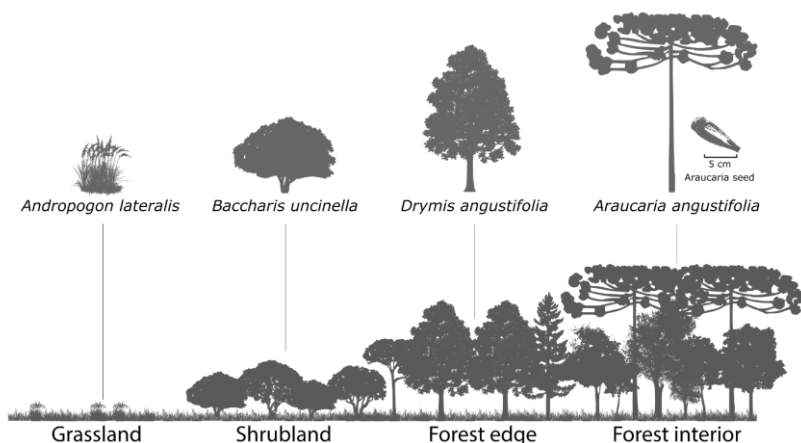


Figure 1. Depiction of the studied units of the forest-grassland gradient and their respective representative species selected for soil extraction in the highlands of southern Brazil (not to scale).

### ***Inocula preparation***

Soil inocula was prepared in watering days by forming a composite soil sample collected nearby at least three different plants from each soil-plant system (as described in the previous section). The pooled, unsterilized soil samples were used to prepare soil inocula for each of the four soil-plant systems (following Rodríguez-Echeverría et al., 2013). Soil was collected, sieved through a 5 mm sieve and stirred in distilled water in a proportion of 1 : 2 (v : v). The liquid was filtered through a 0.5 mm sieve allowing through only fungal spores and hyphae, bacteria and microfauna. The volume of soil extract : distilled water added to each experimental pot was adjusted to have an inoculum density of 20% (v : v). Because different soil substrates have different densities, the exact volume of inoculated soil extract : distilled water slightly varied among soil types.

### ***Treatments and microcosmos experiment***

An experiment was set up to test whether the germination and early growth of araucaria seedlings differed in soils from different landscape units and with distinct composition of soil microbial communities. We monthly watered the experimental pots using either distilled water or soil extract to inoculate the soil microbial community from distinct soil-plant systems, totalling five inoculations per treatment. Five araucaria seeds were selected at random and sown in 90 pots for every soil-plant system. Of these, 30 pots contained unsterilized soil (“unsterilized treatment”), 30 pots contained sterilized soil (“sterilized treatment”), and 30 pots contained sterilized soil and were inoculated with soil extract (“inoculated treatment”), totalling 90 pots per soil-plant system. Soils in sterilized and unsterilized treatments were watered with distilled water. We did not carry out a fully crossed design by inoculating soil extracts among soil-plant systems. Rather, we focused on the effects each of the microbial community of each soil-plant systems had on the germination and early growth of araucaria seedlings. In this way, we assessed the short-term effects of soil microbial communities from distinct soil-plant systems on early araucaria performance instead of a longer-term effect of potentially microbial communities shaped on distinct soils by araucaria itself. The assessed effects are of great importance because they can clarify whether distinct soil types or their specific microbial communities influence the establishment of araucaria across the landscape.

Seeds were planted in a seedling nursery with open-sides on 03 August 2016 in pots and overseen until 06 January 2017, totalling 156 days. On top, the nursery was screened with a 40% shade cloth. The seedling nursery was built ~1.5 km away from the soil collection sites, maintaining the same climate of the landscape of interest. Pots were kept in groups of 6 pots per treatment (experimental blocks) and randomly displaced at 1.5 m above the ground on wooden benches to avoid seed predation. During the experiment, a rain gauge was used to record daily rainfall and air temperature was hourly measured with three I-buttons located near the nursery. Mean daily



temperatures ranged from 3.9 to 22.9 °C and total rainfall amount was 1067.6 mm (Supporting Information: Fig. S2). Such values are expected for the region in such time of the year and thus should not interfere with our ability to extrapolate the experimental results.

In the germination experiment, we recorded the number of germinated seeds per pot (%; N = 150 seeds per treatment, except for sterilized and inoculated grassland soils, where N = 145 seeds). We considered the seed as germinated whenever it presented a visible radicle. Germination was checked twice for early (30 days after sowing) and late germination (60 days). For the assessment of seedling early growth, one of the germinated seeds per plot was selected at random and left growing for another 60 days (N = 30 seedlings per treatment, except for sterilized and inoculated grassland soils, where N = 29 seedlings). At the end of the experiment, seedlings were carefully removed from pots along with their clod and submerged in water to delicately separate soil particles from roots. Next, seedlings and seeds were photographed and put in individual paper bags. We checked seedling growth by measuring taproot and shoot length, root and shoot dry mass, and lateral root area, totalling three belowground and two aboveground traits. Before obtaining dry mass, seedlings were oven-dried at 70 °C during 72 h. Photographs of seedlings and seeds were corrected for lens distortion and then analysed in ImageJ, version 1.50i (NIH, <http://imagej.nih.gov/ij>). After calibration using a scale added to each photograph, we drew both the taproot and shoot length, and the area (proxy for size) of the endosperm of each seed. For measuring root surface, we encoded distortion-free photographs in HSB (hue-saturation-brightness) colour space, allowing for better shadow detection, and then counted pixels using the triangle thresholding algorithm (TAJIMA; KATO, 2011). To improve detail accuracy, the saturation mode was used to calculate thresholds, with levels being manually adjusted for each image (levels were kept between minimal values of 34-45 and a maximal value fixed at 255). Lateral root area was measured by summing the area of the entire root system and discounting the taproot area.

## DATA ANALYSIS

### ***Soil characteristics***

Soil parameters were assessed and interpreted following the standards of the Brazilian Soil Science Society (SOCIEDADE BRASILEIRA DE CIÊNCIA DO SOLO. COMISSAO DE QUÍMICA E FERTILIDADE DO SOLO, 2004). We checked for soil changes contrasting soil chemical and physical parameters before and after the experiment using linear models for multivariate data (LMmv). Soil parameters were standardized before running the LMmv to meet distributional assumptions and to avoid distortions resulting from distinct measurement scales. A principal component analysis was used for visualization of multivariate data.

### ***Germination***

We used generalized linear mixed models assuming a binomial distribution (with a logit link function) to contrast germination rates (%) among treatments (unsterilized, sterilized, or inoculated) and soil type (grassland, shrubland, forest edge, or forest interior). In addition, we tested for differences in early and late germination rates (30 and 60 days after sowing). Experimental blocks were included as random effects. Mixed models were formulated as below:

$$\textit{Germination} \sim \textit{Treatment} * \textit{Time} + (1 \mid \textit{Block})$$

Early growth of seedlings was assessed based on both taproot and shoot length, root and shoot dry mass, and lateral root area. Differences among treatments and soil-plant systems were analysed with generalized linear mixed models assuming a Gamma distribution (with a log link function). We used the log of endosperm area of each seed as an offset term in the models because the endosperm nourishes the seedling and any variation remaining after randomizing seeds to treatments could have affected initial seedling development (especially the development of the taproot; Moreira-Souza, Trufem, Gomes-Da-Costa, & Cardoso, 2003 – Supporting Information: Fig. S3). Experimental blocks were again used as random

effects. Models were constructed for each of the five response variables as follows:

$$Taproot \sim Treatment + offset(\log(Endosperm\ area)) \\ + (1 | Block)$$

For both contrasting germination and early growth rates, we only assessed differences between treatments within soil-plant systems and soil-plant systems for similar treatments by manually setting a matrix of contrasts. To account for multiple testing, *P*-values were adjusted with the Holm-Bonferroni method. In addition, we lowered our significance level to  $\alpha = 0.01$  because we carried out the same set of contrasts for five seedling traits.

All analyses were carried out in the R environment (R CORE TEAM, 2017). Package “vegan” (OKSANEN et al., 2012) was used for ordination, “glmmADMB” (SKAUG et al., 2016), “mvabund” (WANG et al., 2015) for model building, “multcomp” (HOTHORN; BRETZ; WESTFALL, 2008) for performing multiple comparisons and “visreg” (BREHENY; BURCHETT, 2013) for visualization and prediction of model outputs.

## Results

### SOIL CHARACTERISTICS BEFORE AND AFTER THE EXPERIMENT

Soils had in general a low clay content (~20%), high acidity (average pH = 4.3), high organic matter content (~13%) and very high cation-exchange capacity (CEC > 40 cmol<sub>c</sub>/dm<sup>3</sup>). In addition, soils had high K, medium P, and low Ca and Mg content. Most soil parameters remained unchanged during the experiment, except for K (F = 23.76, P = 0.001) and organic matter (F = 16.30, P = 0.008) that declined from before to after the experiment (Supporting Information: Tables S4 to S6, Fig. S7).

## GERMINATION RATE

Germination rates were unaffected by either soil types or soil microbiota composition (treatments) but increased with time (Fig. 2; Supporting Information Table S8). Early germination (30 days) was observed on ~42% of the seeds, reaching ~87% later (60 days). Regarding soil microbiota, germination rates on unsterilized soil raised from 40% (early) to 84% (late). For inoculated soil, germination rates increased from 43% (early) to 77% (late) and raised from 44% (early) to 76% (late) on sterilized soil raised. Regarding soil types, on average, 46% of the seeds germinated early, totalling 85% at late observation for grassland soil. For shrubland soil, 40% of the seeds germinated early, reaching 87% later. For forest edge soil, early germination was 40% and increased later to 86%. For forest interior soil, 44% of the seeds germinated early and increased to 87% later.

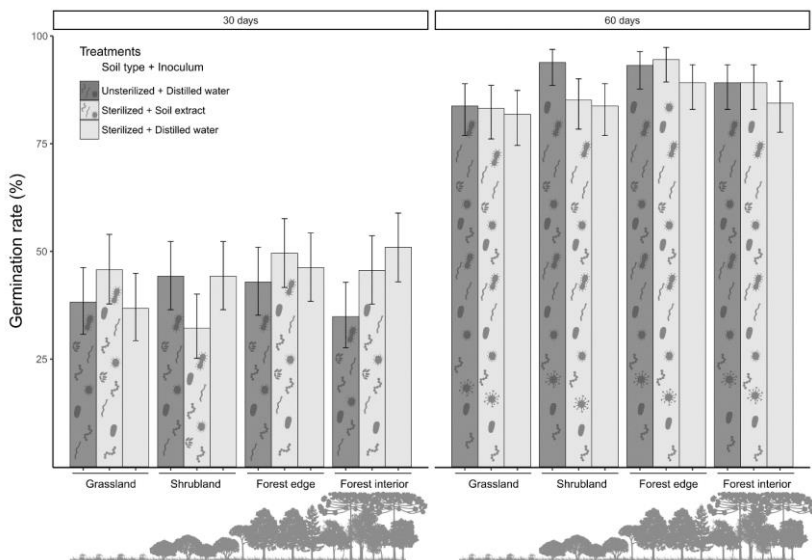


Figure 2. Germination rates of *araucaria* seeds for different treatments and soil-plant systems.  $N = 150$  seeds per treatment, except for sterilized and inoculated grassland soils, where  $N = 145$  seeds. Bars represent 95% confidence intervals.

## EARLY SEEDLING GROWTH RATE

### *Overview*

In no case seedlings growing in soils with unsterilized or inoculated soil outperformed seedlings growing in sterilized soils, thus pointing out a net negative effect of soil microbiota (Table 1). When contrasting unsterilized to sterilized soils, we found eight cases in which there was a difference in size or mass of either belowground or aboveground traits. Of these, seedlings always grew smaller or accumulated less biomass in unsterilized soils. Although the effects of soil microbial communities could be expected to manifest more directly on belowground traits, larger differences were found on aboveground traits. In most cases, statistical conclusions about growth of belowground and aboveground traits were similar or, otherwise, showed weak differences for belowground traits. Of the significant differences found either within soil types or treatments, 18 (20%) were observed for growth of belowground –results from taproot length, root dry mass, and lateral root area– and 33 (55%) for growth of aboveground traits – based on shoot length and shoot dry mass.

For soils containing soil microbial communities – unsterilized and inoculated treatments– seedlings grew larger and heavier on shrubland and grassland soils than elsewhere. Such pattern was clearer for three traits: lateral root area, shoot length, and shoot dry mass (Table 1). For these three traits, growth further decreased from shrubland and grassland soils toward forest edge and forest interior soils. For shoot dry mass, growth in unsterilized shrubland soil even outperformed grassland soil, even though this effect was unobserved for inoculated soil. Sterilized soils were a remarkable exception to such pattern, when seedling growth tended to be larger in forest interior soils than on other soil types. In addition, shoot length and shoot dry mass was found to be larger on shrubland soils than on forest edge or grassland soils.

Our results also indicated sterilization has not changed soil properties. Differences in growth observed between inoculated and

sterilized soil were like those between unsterilized and sterilized soil (Table 1). In 15 cases, we found the same effect – in terms of statistical significance and direction of effects – contrasting either unsterilized or inoculated to sterilized soil, and just five cases of distinct effects. In addition, there was only one case where we found contrasting growth between unsterilized and inoculated soils: root mass was found to be larger in the former for shrubland soil.

*Table 1. Contrasts between treatments within soil-plant systems or between soil types within treatments to assess differences in seedling growth. Treatments (soil type + inoculum): Sterilized = sterilized soil + distilled water, Unsterilized = unsterilized soil + distilled water and Inoculated = Sterilized soil + soil extract. Shown are Z-values resulting from the indicated pairwise contrasts so that signs indicated the direction of effects: a positive value indicates the first treatment had larger values for a given descriptor of seedling growth. The larger the Z-values, regardless of sign, the larger the statistical differences. Z-values followed by “\*\*\*\*” and “\*\*\*” had P-values below 0.001 and 0.01, respectively, after correcting for multiple comparisons with the Holm-Bonferroni method. The belowground traits were TL: taproot length; RM: root dry mass; and LRA: lateral root area; the aboveground traits, SL: shoot length; and, SM: shoot dry mass.*

Within	Contrast	Belowground			Aboveground	
		TL	RM	LRA	SL	SM
Forest interior	Unsterilized × Sterilized	-8.25***	-2.42	-8.48***	-8.7***	-10.04***
	Inoculated × Sterilized	-9.29***	-3.49	-10.72***	-11.75***	-13.02***
	Unsterilized × Inoculated	1.03	1.06	1.98	2.95	2.95
Forest edge	Unsterilized × Sterilized	-1.19	-0.37	-2.22	-3.19	-3.82**
	Inoculated × Sterilized	-3.64**	-0.37	-4.54***	-4.42***	-5.49***
	Unsterilized × Inoculated	2.42	< 0.01	2.29	1.2	1.62
Shrubland	Unsterilized × Sterilized	-1.63	-1.45	-2.69	-4.72***	-4.48***
	Inoculated × Sterilized	-3.33	-5.41***	-3.24	-4.81***	-5.69***
	Unsterilized × Inoculated	1.7	3.96**	0.55	0.1	1.21
Grassland	Unsterilized × Sterilized	1.35	0.77	-2.49	-3.07	-3.96**
	Inoculated × Sterilized	-1.69	-1.89	-3.2	-0.56	-0.61

	Unsterilized × Inoculated	3.04	2.67	0.78	-2.49	-3.31
Unsterilized	Forest interior × Forest edge	-1.74	0.73	-3.74**	-1.72	-4.22***
	Forest interior × Shrubland	-1.22	-1.52	-6.03***	-5.2***	-7.14***
	Forest interior × Grassland	-3.48	-1.51	-5.55***	-2.05	-3.41**
	Forest edge × Shrubland	0.53	-2.26	-2.36	-3.51**	-2.89
	Forest edge × Grassland	-1.73	-2.24	-1.82	-0.32	0.84
	Shrubland × Grassland	-2.28	0.02	0.53	3.21	3.76**
Inoculated	Forest interior × Forest edge	-0.37	-0.34	-3.61**	-3.53**	-5.62***
	Forest interior × Shrubland	-0.57	1.33	-7.68***	-8.13***	-8.92***
	Forest interior × Grassland	-1.44	0.12	-6.82***	-7.43***	-9.56***
	Forest edge × Shrubland	-0.2	1.68	-4.13***	-4.65***	-3.33
	Forest edge × Grassland	-1.08	0.45	-3.27	-3.99**	-4.08***
	Shrubland × Grassland	-0.88	-1.2	0.76	0.58	-0.81
Sterilized	Forest interior × Forest edge	5.36***	2.8	2.67	3.88**	1.98
	Forest interior × Shrubland	5.46***	-0.55	-0.15	-1.16	-1.55
	Forest interior × Grassland	6.12***	1.68	0.56	3.65**	2.67
	Forest edge × Shrubland	0.11	-3.36	-2.82	-5.04***	-3.54**
	Forest edge × Grassland	0.81	-1.1	-2.1	-0.21	0.7
	Shrubland × Grassland	0.7	2.22	0.71	4.8***	4.21***



### Detailed belowground effects

Lateral root area was the belowground trait to differ more between experimental contrasts, whereas taproot length and root dry mass differed only for a few contrasts. Taproots in unsterilized and inoculated soils (mean  $\pm$  standard deviation:  $14 \pm 2.7$  cm) grew to half the length than in sterilized soil ( $30.5 \pm 9.3$  cm) within forest interior soils (Fig. 3; Table 1). In addition, for sterilized soils, taproots grew longer in forest interior soils ( $30.5 \pm 9.3$  cm) than in other soil types ( $18.5 \pm 5.4$  cm; Fig. 3). Taproots grew to similar sizes in all other experimental contrasts.

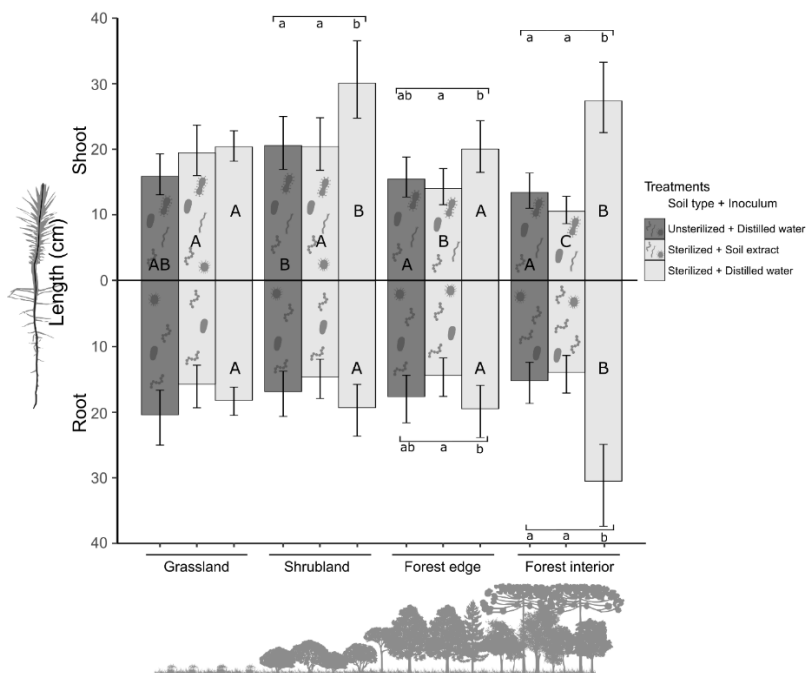


Figure 3. Length of fresh taproot and shoot of araucaria seedlings for different treatments and soil-plant systems.  $N = 30$  seedlings per treatment, except for sterilized and inoculated grassland soils, where  $N = 29$  seedlings. Bars represent 95% confidence intervals. Lowercase letters refer to

significant differences ( $P < 0.01$ ) among treatments of same soil-plant systems. Horizontally aligned uppercase letters refer to significant differences ( $P < 0.01$ ) among different soil-plant systems.

Accumulation of root dry mass gradually reduced from sterilized ( $0.596 \pm 0.142$  g), to unsterilized ( $0.539 \pm 0.139$  g) and inoculated soil ( $0.407 \pm 0.124$  g) within shrubland soils (Fig. 4; Table 1). Biomass accumulation was similar in all other contrasts.

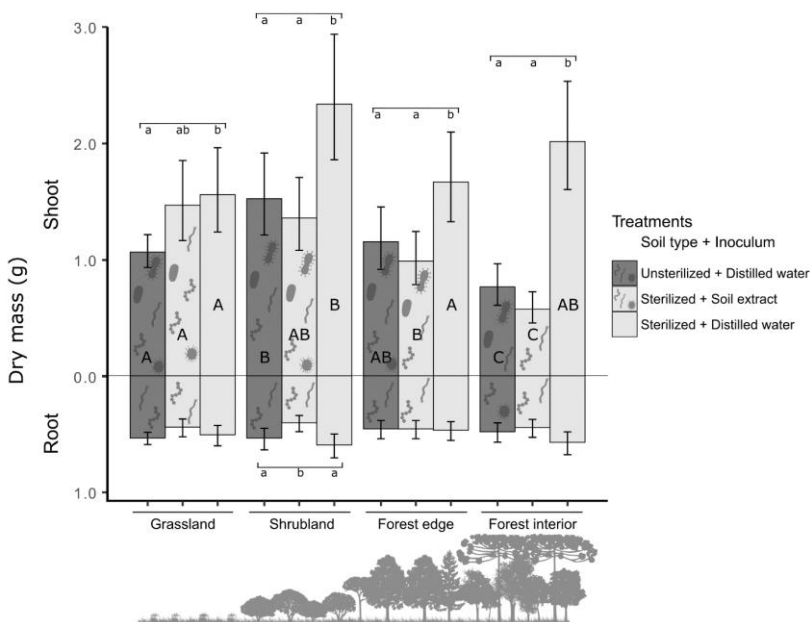


Figure 4. Root and shoot systems' dry mass of araucaria seedlings for different treatments and soil-plant systems.  $N = 30$  seedlings per treatment, except for sterilized and inoculated grassland soils, where  $N = 29$  seedlings. Bars represent 95% confidence intervals. Lowercase letters refer to significant differences ( $P < 0.01$ ) among treatments of same soil-plant systems. Horizontally aligned uppercase letters refer to significant differences ( $P < 0.01$ ) among different soil-plant systems.

Lateral roots grew four times larger in sterilized soil ( $24.9 \pm 7.6 \text{ cm}^2$ ) than in unsterilized and inoculated soils ( $5.7 \pm 4.3 \text{ cm}^2$ ) within forest interior soils (Fig. 5; Table 1). A decrease in lateral root area was found also from sterilized ( $16.3 \pm 6 \text{ cm}^2$ ), to unsterilized ( $11.8 \pm 5.8 \text{ cm}^2$ ) and inoculated soils ( $8.2 \pm 3.8 \text{ cm}^2$ ) within forest edge soils (Fig. 5; Table 1). Moreover, lateral roots developed just half the extent in forest interior ( $6.6 \pm 4.8 \text{ cm}^2$ ) than other soil types ( $14.4 \pm 5.6 \text{ cm}^2$ ) within unsterilized treatments (Fig. 5; Table 1). For inoculated soils, forest interior soil had the smallest lateral root area ( $4.8 \pm 3.8 \text{ cm}^2$ ), followed by forest edge ( $8.2 \pm 3.8 \text{ cm}^2$ ) and then shrubland and grassland soils ( $14.7 \pm 6.3 \text{ cm}^2$ ; Fig. 5; Table 1). Lateral root area grew similar between soil types within sterilized soils ( $21.7 \pm 7.3 \text{ cm}^2$ ; Fig. 5; Table 1).

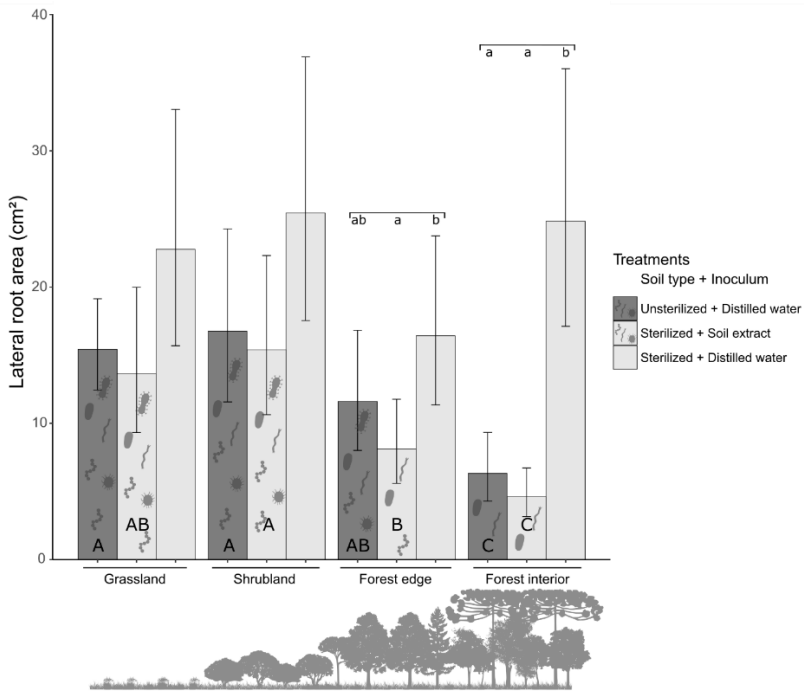


Figure 5. Area of live lateral roots of *araucaria* seedlings for different treatments and soil-plant systems.  $N = 30$  seedlings per treatment, except for sterilized and inoculated grassland soils, where  $N = 29$  seedlings. Bars represent 95% confidence intervals. Lowercase letters refer to significant differences ( $P < 0.01$ ) among treatments of same soil-plant systems. Horizontally aligned uppercase letters refer to significant differences ( $P < 0.01$ ) among different soil-plant systems.

### ***Detailed aboveground effects***

Both aboveground traits – shoot length and shoot dry mass – had a similar number of differences between experimental contrasts, yet with some differences in which contrasts they differ. Shoots grew twice longer in sterilized soil ( $27.1 \pm 4.7$  cm) than unsterilized and inoculated soils ( $11.9 \pm 5$  cm) within forest interior soil (Fig. 3; Table 1). Within shrubland and forest edge soils, a similar pattern was found, except for a decreasing effect size from shrubland to forest edge. For shrubland soils, shoot length was longer for sterilized soil ( $29.5 \pm 4.7$  cm) than unsterilized and inoculated soils ( $20.1 \pm 3.6$  cm; Fig. 3; Table 1). For forest edge soils, shoot length differed only from sterilized ( $19.3 \pm 3.4$  cm) to inoculated soils ( $13.9 \pm 3.6$  cm), with unsterilized soil showing intermediate length ( $15.2 \pm 3.5$  cm; Fig. 3; Table 1). Shoot length was similar for all treatments within grassland soils ( $18.2 \pm 3.4$  cm; Fig. 3; Table 1).

When contrasting between soil types, there were more and larger differences in shoot length within inoculated treatments. Shoot length gradually decreased from shrubland ( $19.8 \pm 4.4$  cm) and grassland ( $19.3 \pm 3.3$  cm) to forest edge ( $13.9 \pm 3.6$  cm) and forest interior soils ( $10.6 \pm 5.2$  cm) within inoculated treatments (Fig. 3; Table 1). For unsterilized soil, shrubland ( $20.4 \pm 2.6$  cm) and forest interior ( $13.4 \pm 4.5$  cm) soils differed, with grassland ( $15.6 \pm 2.6$  cm) and forest edge ( $15.2 \pm 3.5$  cm) developing intermediate shoot lengths. Results differed for sterilized soils, where shoots grew longer for shrubland and forest interior soils ( $28.3 \pm 4.8$  cm) than for grassland ( $20 \pm 3.6$  cm) and forest edge soils ( $19.7 \pm 3.4$  cm; Fig. 3; Table 1).

Shoots took up three-times more biomass in sterilized soil ( $2.029 \pm 0.561$  g) than in unsterilized and inoculated soils ( $0.685 \pm 0.413$  g) within forest interior soil (Fig. 4; Table 1). A similar distinction was found within shrubland soils, yet with a smaller difference between sterilized soil ( $2.348 \pm 0.655$  g) and the other treatments ( $1.436 \pm 0.391$  g; Fig. 4; Table 1). Gain of shoot biomass was also larger in sterilized ( $1.636 \pm 0.489$  g) than unsterilized and inoculated soils ( $1.074 \pm 0.39$  g) within forest edge soil, with an even reduced

effect size (Fig. 4; Table 1). For grassland soils, the only exception was that sterilized and inoculated treatments accumulated larger shoot biomass ( $1.504 \pm 0.422$  g) than in the unsterilized treatment ( $1.061 \pm 0.26$  g; Fig. 4; Table 1).

Between soil types, the larger effect sizes were found for shoot biomass within inoculated treatments, mostly mirroring the results found for shoot length. Shoot biomass was found smaller for forest interior ( $0.591 \pm 0.34$  g) and gradually increased from forest edge ( $0.99 \pm 0.336$  g) toward shrubland ( $1.358 \pm 0.461$  g) and grassland soils ( $1.469 \pm 0.398$  g) within the inoculated treatment (Fig. 4; Table 1). Within the unsterilized treatment, again forest interior soil accumulated less biomass ( $0.783 \pm 0.47$  g) and gradually increased for grassland ( $1.061 \pm 0.26$  g) to forest edge ( $1.162 \pm 0.43$  g) and shrubland soils ( $1.516 \pm 0.296$  g; Fig. 4; Table 1). Results again differed for sterilized soils, with more shoot biomass being accumulated in shrubland soil ( $2.348 \pm 0.655$  g) and less in grassland soil ( $1.525 \pm 0.428$  g) and forest edge ( $1.636 \pm 0.489$  g), while forest interior ( $2.029 \pm 0.561$  g) showed intermediate values (Fig. 4; Table 1).

## Discussion

The net effect of soil microbial communities was detrimental to the early growth of araucaria seedlings, while germination was unaffected, thus contradicting our initial hypothesis. Such net effect was so strong as to overcome soil type preferences. Seedling performance was generally worse when grown in forest interior soils where adult trees of araucaria are abundant. Conversely, dismantling soil microbial communities resulted in improved both belowground and aboveground seedling growth and biomass uptake. Such net negative effect of soil microbial communities was found across soils from the entire forest-grassland transition. Below, we ponder on consequences of such results to explain vegetation distribution and dynamics.

Germination of araucaria seeds was unrelated to either soil type or soil microbial communities. This indicates soil microbial

communities have no net effect on the germination of araucaria seeds. Germination rates found were also high across soils from the landscape (early rate of ~42%, after 30 days, and late rate of ~87%, after 60 days vs. ~25% and ~60% found on an identical time setting by (MOREIRA-SOUZA; CARDOSO, 2003). We suggest such high germination rates to be the result of large size of araucaria seeds (6 - 7.2 g, reviewed in Reis et al., 2014), further explaining why the species is considered a pioneer even though the species is undemanding regarding light regime (DUARTE; DILLENBURG, 2000). Whereas genotype differences may still play a role (PRANGE, 1963), our results point out to a genotypic potential to successfully germinate in contrasting soil types and with low influence from microbial communities across the landscape.

Although our results suggest araucaria seeds can germinate well across the landscape, differing seedling performance may drive vegetation patterns. Specifically, soil microbial communities curb araucaria seedling development. Fungal pathogens have already been reported to infect araucaria seeds before dispersion and inhibit seedling development (DALMAS et al., 2013). Here we carried out disinfection of seeds prior to sowing, thus suggesting infection from pathogens may also occur directly from the soil. Conversely, araucaria roots are known to build beneficial associations with arbuscular mycorrhizal fungi and rhizobacteria (BREUNINGER et al., 2000; MOREIRA-SOUZA et al., 2003; MOREIRA-SOUZA; CARDOSO, 2003; MOREIRA et al., 2006; ZANDAVALLI; DILLENBURG; DE SOUZA, 2004; ZANDAVALLI; STÜRMER; DILLENBURG, 2008). Nevertheless, mycorrhizae can be demanding to plants and tend to payoff only on poor soils (MARSCHNER; DELL, 1994). Indeed, soils across the landscape are nutrient-poor, except for potassium, have low pH and high aluminium content (which seems to be common for the system, *e.g.* Silva & Anand, 2011). Despite being potentially unfavourable, araucaria seedlings grew well in such soils, except when grown with soil microbial communities. Thus, both pathogens and additional energetic expenditure to maintain even beneficial associations may contribute to the observed net negative effect of soil microbial communities on the development of seedlings.

Despite the above-mentioned differences in seedling development, few seedlings died during the experiment. This further implies that araucaria early establishment differs in terms of growth rather than survival, at least without considering competition with other plants. In soils with intact microbial communities, seedling performance was best in shrubland and grassland soils, decreasing toward soils of forest edge and forest interior. Specifically, lateral root area, shoot length, and shoot dry mass were found to increase in shrubland and grassland soils, likely improving both nutrients and light capture (BROUWER, 1962; THORNLEY, 1972). Conversely, at forest edge and forest interior, soil microbial communities associated with araucaria trees lead to a net detrimental effect on seedling performance. These results suggest araucaria seedlings grow better outside the range of its own adults (*e.g.* SÜHS; GIEHL; PERONI, 2018). Thus, why is the pace of forest expansion over grasslands or shrublands so slow across the landscape?

We believe the ultimate outcome in the field depends on both disturbance, plant-plant competition, and propagule pressure. Araucaria seedlings likely reach their best performance in grassland and shrubland soils only without interspecific competition. Yet, such improved performance can be still small to outcompete fast growing grasses (evidence on grasses shoot competition ability was found by ZANDAVALLI & DILLENBURG, 2015) and shrubs. Still, even if araucaria seedlings could outcompete grasses and shrubs, propagule pressure decreases away from forest interior because of a simultaneous reduction in adult araucaria abundance (IOB; VIEIRA, 2008). Thus, despite being able to reach maximal performance on shrubland and grassland soils, odds can still be low of successful colonization in such vegetation because of seed limitation. Indeed, araucaria seedlings scarcely develop outside forests, especially in highland grasslands (*e.g.* FRANCO et al., 2005; GUGLIELME & GANADE, 2006). Common management strategies involving grazing and frequent fire seem to both promote grasses' competitive ability and reduce araucaria establishment success (DUARTE et al., 2006; NABINGER; MORAES; MARASCHIN, 2000; OVERBECK et al., 2007; PILLAR, 2003; SÜHS; GIEHL; PERONI, 2018). These mechanisms can thus reconcile the



ability of araucaria seedlings to grow faster on soil from shrublands and grasslands with the slow pace of vegetation change. In addition to a detrimental effect of soil microbial communities on araucaria seedlings, hence reducing its overall competitive ability, the probability of successful colonization of grasslands and shrublands by araucaria is also reduced by either disturbance, competition, seed limitation, or both, withholding the pace of forest expansion.

Global warming and changes in land management are likely to alter competition output and accelerate forest expansion. Global warming tends to favour colonization of grasslands by shrubs (e.g. *Baccharis uncinella*, Dechoum et al., 2018) and forests worldwide (BOND, 2008; CARLUCCI et al., 2011; SILVA; ANAND, 2011). Moreover, Brazil adopts “zero-fire” policies in fire-prone ecosystems (MISTRY et al., 2018), which is controversial because it favours woody encroachment of highland grasslands (DECHOUM; PERONI; PUGNAIRE, 2018; OLIVEIRA; PILLAR, 2004). Our results further suggest soil microbial communities can also be affected by climate change (VAN DER PUTTEN, 2012) and, depending on the changes to such communities, araucaria may increase or decrease its colonization ability. If araucaria colonization success increases because changes to climate lessen negative effects of soil microbiota, forests will likely replace grasslands in a faster pace, resulting from araucaria trees’ facilitation effects on other forest species (DUARTE et al., 2006; KORNDÖRFER; DILLENBURG; DUARTE, 2015; SÜHS; GIEHL; PERONI, 2018). Conversely, if araucaria colonization success decreases, upper montane araucaria forests will be unable to migrate uphill in a pace that prevents a replacement by tropical forests now found in lower montane areas. (FEELEY et al., 2011). Thus, it seems likely that either highland grasslands or araucaria forests will become further threatened, both along with the evolutionary history and cultural heritage they carry and the ecosystem functioning they provide.

Our study demonstrates a strong detrimental effect of soil microbial communities on araucaria seedlings – a nurse tree involved in the replacement of grasslands by forests in the highlands of

southern Brazil. These detrimental effects were strongest for the soil microbial communities found beneath adult araucarias, although such detrimental effect was found in soils across the forest-grassland gradient. Such effect can be one of the factors responsible for the slow pace of forest expansion over grasslands in the region, thus also contributing for the maintenance of threatened subtropical highland grasslands. Remarkably, it seems that changes in soil microbial communities can alter the results of competitive interactions between araucaria and dominant plant species found in distinct units of the forest-grassland gradient. Consequently, as a result of climate change, vegetation patterns can be reshaped because of both direct effects on species performance and indirect effects mediated by changes to soil microbial communities. We thus confirm an important but not necessarily positive effect of soil organisms in plant communities.

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## Authors' contributions

RBS and NP conceived the ideas and RBS designed the methodology; RBS collected the data; RBS and ELHG analysed the data; RBS and ELHG wrote the manuscript and NP reviewed the manuscript. All

authors contributed critically to the drafts and gave final approval for publication.

## Data accessibility

Data available from the Dryad Digital Repository <https://>

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## Supplementary material

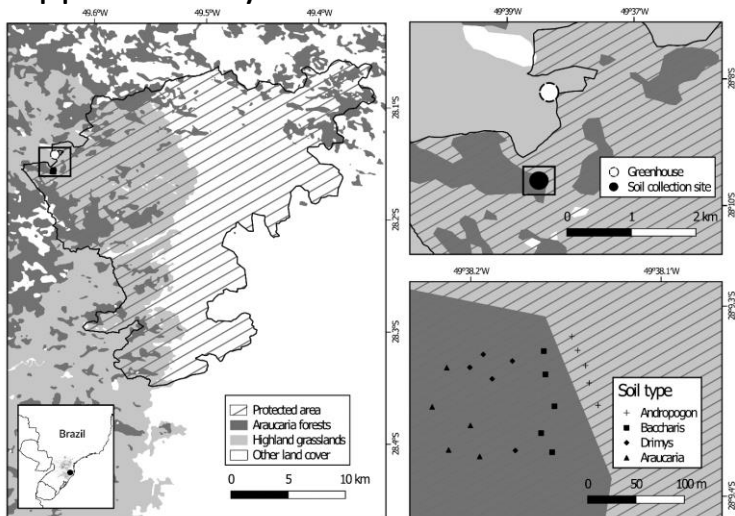


Figure S1. Location where the study was developed and where soil samples were collected in southern Brazilian highlands.

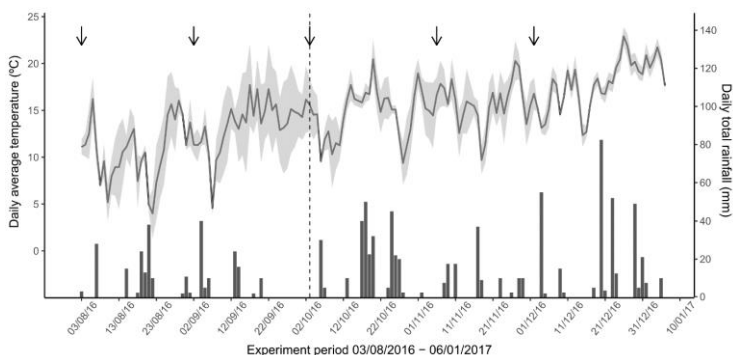


Figure S2. Daily average temperature and total rainfall recorded during the experiment developed in southern Brazilian highlands. Grey band represent standard deviation; dashed line indicates the end of the germination experiment and beginning of initial growth experiment. Arrows indicate when water/inocula was added to the experimental pots.

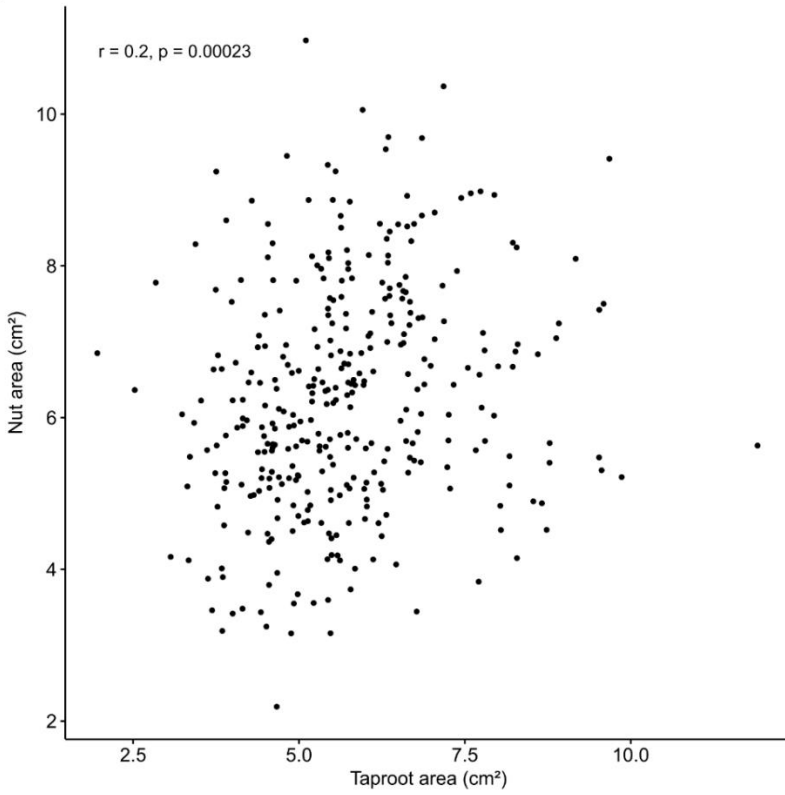


Figure S3. Correlation between area occupied by the nut in araucaria seed (Nut area) and araucaria taproot area.

*Table S4. Simplified table of average  $\pm$  standard deviation (Std. Dev.) of soil parameters evaluated before (T<sub>0</sub>) and after (T<sub>F</sub>) the experiment in southern Brazilian highlands.*

<b>Parameter</b>	<b>Time point</b>	<b>Average</b>	<b>Std. Dev.</b>
Clay (%)	T <sub>0</sub>	20.3	3.9
	T <sub>F</sub>	20	5.7
pH	T <sub>0</sub>	4.3	0.4
	T <sub>F</sub>	4.6	0.4
Phosphorous (mg/dm <sup>3</sup> )	T <sub>0</sub>	16.1	10.3
	T <sub>F</sub>	9.0	5.0
Potassium (mg/dm <sup>3</sup> )	T <sub>0</sub>	153.7	50.4
	T <sub>F</sub>	79.7	15
Organic Matter (%)	T <sub>0</sub>	13.4	1.5
	T <sub>F</sub>	11.2	1.1
Aluminium (cmol <sub>c</sub> /dm <sup>3</sup> )	T <sub>0</sub>	4.1	1.5
	T <sub>F</sub>	5.7	1.4
Calcium (cmol <sub>c</sub> /dm <sup>3</sup> )	T <sub>0</sub>	1.2	0.7
	T <sub>F</sub>	1.2	0.5
Magnesium (cmol <sub>c</sub> /dm <sup>3</sup> )	T <sub>0</sub>	0.4	0.2
	T <sub>F</sub>	0.3	0.1
Cation Exchange Capacity (cmol <sub>c</sub> /dm <sup>3</sup> )	T <sub>0</sub>	42.6	17.0
	T <sub>F</sub>	34.3	14.5
Sum of Bases	T <sub>0</sub>	2.0	0.9
	T <sub>F</sub>	1.7	0.6

Table S5. Results from linear models for multivariate data (ImMV) to test whether soil parameters differed from before (T<sub>0</sub>) to after (T<sub>F</sub>) the experiment in southern Brazilian highlands. Significant P-values are in bold.

Soil Parameter	T <sub>0</sub> (Intercept)			T <sub>F</sub>		
	Coefficient	F	P	Coefficient	F	P
Sum of bases	0.184	0.401	0.817	-0.367	0.801	0.649
pH	-0.428	2.601	0.412	0.856	5.202	0.138
Phosphorous	0.408	2.313	0.422	-0.816	4.625	0.162
Potassium	0.705	11.883	0.019	-1.411	23.767	<b>0.001</b>
Organic Matter	0.639	8.151	0.064	-1.277	16.301	<b>0.008</b>
Aluminium	-0.486	3.599	0.304	0.972	7.199	0.068
Calcium	0.021	0.005	0.991	-0.043	0.011	0.983
Magnesium	0.234	0.669	0.789	-0.469	1.338	0.537
Cation Exchange Capacity	0.26	0.832	0.789	-0.519	1.663	0.532
Clay	0.035	0.014	0.991	-0.07	0.028	0.983

*Table S6. Results from soil analysis for different soil parameters from before (T<sub>0</sub>) to after (T<sub>F</sub>) the experiment in southern Brazilian highlands. Tr = treatment, where ST = sterilized, IN = Inoculated and UST = Unsterilized.*

Within	Tr	Clay (%)		pH		Phosphorous (mg/dm <sup>3</sup> )		Potassium (mg/dm <sup>3</sup> )		Organic Matter (%)	
		T <sub>0</sub>	T <sub>F</sub>	T <sub>0</sub>	T <sub>F</sub>	T <sub>0</sub>	T <sub>F</sub>	T <sub>0</sub>	T <sub>F</sub>	T <sub>0</sub>	T <sub>F</sub>
Forest interior	ST	24	16	3.7	4.9	33.3	7.9	136	66	11.4	11
	IN	10	15	4.6	4.7	11.5	9.4	120	74	12.8	11.4
	UST	21	30	4.5	4.3	13.8	9.8	190	74	15.2	12.6
Forest edge	ST	20	18	4.3	4.7	20	14.1	108	62	12.6	12.4
	IN	21	17	4.2	4.8	23.9	12.3	140	80	14.2	12.2
	UST	24	29	3.5	4.1	35.3	21.5	126	72	15.8	11.2
Shrubland	ST	20	18	3.6	5.2	15.6	6.1	136	112	13.4	11.2
	IN	18	14	4.7	5	12.4	6.4	250	84	15.6	10.8
	UST	24	20	4.3	4.6	12.7	7.3	254	78	12	11
Grassland	ST	21	16	4.6	4.7	5.2	4.7	146	84	12.6	8.4
	IN	18	19	4.7	4	4.6	5.9	120	66	12.2	11
	UST	23	28	4.4	4.5	5.2	2.8	118	104	12.6	11.6

Table S6 continuação...

Within	Tr	Aluminium (cmol <sub>c</sub> /dm <sup>3</sup> )		Calcium (cmol <sub>c</sub> /dm <sup>3</sup> )		Magnesium (cmol <sub>c</sub> /dm <sup>3</sup> )		Cation Exchange Capacity (cmol <sub>c</sub> /dm <sup>3</sup> )		Sum of Bases (cmol <sub>c</sub> /dm <sup>3</sup> )	
		T <sub>0</sub>	T <sub>F</sub>	T <sub>0</sub>	T <sub>F</sub>	T <sub>0</sub>	T <sub>F</sub>	T <sub>0</sub>	T <sub>F</sub>	T <sub>0</sub>	T <sub>F</sub>
Forest interior	ST	2.1	4.9	1.5	1.3	0.5	0.2	60.78	28.23	2.38	1.73
	IN	5.2	5.5	1.2	1.1	0.3	0.3	33.57	33.74	1.77	1.54
	UST	4.1	7.2	0.8	0.8	0.3	0.2	38.45	34.09	1.55	1.19
Forest edge	ST	2.1	3.5	2	2.1	0.2	0.5	37.26	46.09	2.46	2.79
	IN	2.3	3.5	2	1.7	0.7	0.5	52.29	39.36	3.09	2.46
	UST	3.7	4.3	2	1.6	0.5	0.4	83.4	73.23	2.8	2.23
Shrubland	ST	7.1	6.2	0.8	1.3	0.3	0.4	53.55	20.07	1.45	1.97
	IN	3.6	5.1	1.6	1.2	0.7	0.3	30.96	19.28	2.96	1.78
	UST	3.7	7.4	1.7	1.4	0.8	0.3	36.02	31.56	3.12	1.86
Grassland	ST	4.6	5.9	0.3	0.7	0.2	0.2	31.94	25.8	0.84	1.1
	IN	5.1	7.7	0.3	0.5	0.1	0.1	24	24.67	0.7	0.77
	UST	5	6.6	0.3	0.5	0.1	0.2	28.73	35.06	0.73	0.96



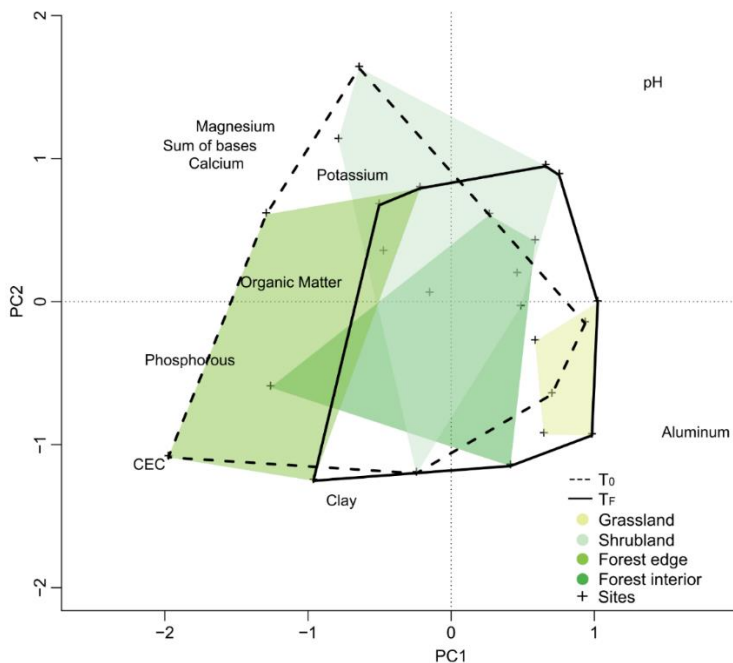


Figure S7. Principal component analysis of evaluated soil parameters before (T<sub>0</sub>) and after (T<sub>F</sub>) the experiment in southern Brazilian highlands and the relationship with each soil type. CEC = cation exchange capacity. This ordination is for visualization only. Soil statistical analysis was conducted via linear models for multivariate data (lmMV).

*Table S8. Contrasts between treatments within soil-plant systems or between soil types within treatments to assess differences in germination rates in different time points. Treatments (soil type + inoculum) are: Sterilized = sterilized soil + distilled water, Unsterilized = unsterilized soil + distilled water and Inoculated = Sterilized soil + soil extract. Subscripted numbers after treatments indicates the respective time point (30 or 60 days after sowing). Shown are Z-values resulting from the indicated pairwise contrasts so that signs indicated the direction of effects: a positive value indicates the first treatment had larger values for a given descriptor of seedling growth. The larger the Z-values, regardless of sign, the larger the statistical differences. Z-values followed by “\*\*\*” had P-values below 0.001, after correcting for multiple comparisons with the Holm-Bonferroni method.*

<b>Within</b>	<b>Contrast</b>	<b>Z</b>
Forest interior	Unsterilized <sub>30</sub> × Sterilized <sub>30</sub>	-2.788
	Inoculated <sub>30</sub> × Sterilized <sub>30</sub>	-0.924
	Unsterilized <sub>30</sub> × Inoculated <sub>30</sub>	-1.881
Forest edge	Unsterilized <sub>30</sub> × Sterilized <sub>30</sub>	-0.581
	Inoculated <sub>30</sub> × Sterilized <sub>30</sub>	0.578
	Unsterilized <sub>30</sub> × Inoculated <sub>30</sub>	-1.158
Shrubland	Unsterilized <sub>30</sub> × Sterilized <sub>30</sub>	<0.001
	Inoculated <sub>30</sub> × Sterilized <sub>30</sub>	-2.134
	Unsterilized <sub>30</sub> × Inoculated <sub>30</sub>	2.134
Grassland	Unsterilized <sub>30</sub> × Sterilized <sub>30</sub>	0.257
	Inoculated <sub>30</sub> × Sterilized <sub>30</sub>	1.549
	Unsterilized <sub>30</sub> × Inoculated <sub>30</sub>	-1.308
Unsterilized	Forest interior <sub>30</sub> × Forest edge <sub>30</sub>	0.808
	Forest interior <sub>30</sub> × Shrubland <sub>30</sub>	1.155
	Forest interior <sub>30</sub> × Grassland <sub>30</sub>	2.434
	Forest edge <sub>30</sub> × Shrubland <sub>30</sub>	0.348

	Forest edge <sub>30</sub> × Grassland <sub>30</sub>	1.644
	Shrubland <sub>30</sub> × Grassland <sub>30</sub>	1.302
Inoculated	Forest interior <sub>30</sub> × Forest edge <sub>30</sub>	-0.694
	Forest interior <sub>30</sub> × Shrubland <sub>30</sub>	2.362
	Forest interior <sub>30</sub> × Grassland <sub>30</sub>	-0.032
	Forest edge <sub>30</sub> × Shrubland <sub>30</sub>	3.037
	Forest edge <sub>30</sub> × Grassland <sub>30</sub>	0.656
	Shrubland <sub>30</sub> × Grassland <sub>30</sub>	-2.374
Sterilized	Forest interior <sub>30</sub> × Forest edge <sub>30</sub>	-1.421
	Forest interior <sub>30</sub> × Shrubland <sub>30</sub>	-1.652
	Forest interior <sub>30</sub> × Grassland <sub>30</sub>	-0.6
	Forest edge <sub>30</sub> × Shrubland <sub>30</sub>	-0.233
	Forest edge <sub>30</sub> × Grassland <sub>30</sub>	0.823
	Shrubland <sub>30</sub> × Grassland <sub>30</sub>	1.056
<hr/>		
Forest interior	Unsterilized <sub>60</sub> × Sterilized <sub>60</sub>	1.172
	Inoculated <sub>60</sub> × Sterilized <sub>60</sub>	1.172
	Unsterilized <sub>60</sub> × Inoculated <sub>60</sub>	<0.001
Forest edge	Unsterilized <sub>60</sub> × Sterilized <sub>60</sub>	1.182
	Inoculated <sub>60</sub> × Sterilized <sub>60</sub>	1.616
	Unsterilized <sub>60</sub> × Inoculated <sub>60</sub>	-0.462
Shrubland	Unsterilized <sub>60</sub> × Sterilized <sub>60</sub>	2.614
	Inoculated <sub>60</sub> × Sterilized <sub>60</sub>	0.315
	Unsterilized <sub>60</sub> × Inoculated <sub>60</sub>	2.337
Grassland	Unsterilized <sub>60</sub> × Sterilized <sub>60</sub>	0.44
	Inoculated <sub>60</sub> × Sterilized <sub>60</sub>	0.306
	Unsterilized <sub>60</sub> × Inoculated <sub>60</sub>	0.132

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Unsterilized	Forest interior <sub>60</sub> × Forest edge <sub>60</sub>	-1.172
	Forest interior <sub>60</sub> × Shrubland <sub>60</sub>	0.156
	Forest interior <sub>60</sub> × Grassland <sub>60</sub>	0.595
	Forest edge <sub>60</sub> × Shrubland <sub>60</sub>	1.324
	Forest edge <sub>60</sub> × Grassland <sub>60</sub>	1.741
	Shrubland <sub>60</sub> × Grassland <sub>60</sub>	0.44
Inoculated	Forest interior <sub>60</sub> × Forest edge <sub>60</sub>	-1.616
	Forest interior <sub>60</sub> × Shrubland <sub>60</sub>	1.016
	Forest interior <sub>60</sub> × Grassland <sub>60</sub>	1.443
	Forest edge <sub>60</sub> × Shrubland <sub>60</sub>	2.533
	Forest edge <sub>60</sub> × Grassland <sub>60</sub>	2.899
	Shrubland <sub>60</sub> × Grassland <sub>60</sub>	0.444
Sterilized	Forest interior <sub>60</sub> × Forest edge <sub>60</sub>	-1.182
	Forest interior <sub>60</sub> × Shrubland <sub>60</sub>	-1.397
	Forest interior <sub>60</sub> × Grassland <sub>60</sub>	1.324
	Forest edge <sub>60</sub> × Shrubland <sub>60</sub>	-0.226
	Forest edge <sub>60</sub> × Grassland <sub>60</sub>	2.426
	Shrubland <sub>60</sub> × Grassland <sub>60</sub>	2.614
Forest interior	Sterilized <sub>30</sub> × Sterilized <sub>60</sub>	-5.908***
	Unsterilized <sub>30</sub> × Unsterilized <sub>60</sub>	-8.695***
	Inoculated <sub>30</sub> × Inoculated <sub>60</sub>	-7.35***
Forest edge	Sterilized <sub>30</sub> × Sterilized <sub>60</sub>	-7.264***
	Unsterilized <sub>30</sub> × Unsterilized <sub>60</sub>	-7.999***
	Inoculated <sub>30</sub> × Inoculated <sub>60</sub>	-7.299***
Shrubland	Sterilized <sub>30</sub> × Sterilized <sub>60</sub>	-6.755***
	Unsterilized <sub>30</sub> × Unsterilized <sub>60</sub>	-7.862***

	Inoculated <sub>30</sub> × Inoculated <sub>60</sub>	-8.6***
Grassland	Sterilized <sub>30</sub> × Sterilized <sub>60</sub>	-7.387***
	Unsterilized <sub>30</sub> × Unsterilized <sub>60</sub>	-6.336***
	Inoculated <sub>30</sub> × Inoculated <sub>60</sub>	-7.599***

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## 7. Considerações Finais

A presente tese teve por objetivo geral avaliar os efeitos das práticas tradicionais de manejo na paisagem e na biodiversidade e verificar os efeitos da microbiota do solo em uma espécie-chave, a qual está diretamente envolvida na expansão da floresta com araucária sobre os campos de altitude. O primeiro capítulo desta tese objetivou avaliar o tipo de manejo utilizado por proprietários rurais (denominados “gestores locais”) e verificar os efeitos desse manejo nos campos subtropicais de altitude. Este estudo foi realizado em uma escala espacial e temporal mais ampla que os demais, e nossa hipótese foi de que a dinâmica da vegetação é determinada pelo tipo de manejo utilizado (com manejo tradicional e sem manejo tradicional). Especificamente, locais sem manejo tradicional possivelmente são afetados pelo adensamento de arbustos ao longo do tempo. A taxa de adensamento de arbustos é ainda pouco conhecida para os campos subtropicais de altitude no Brasil. Além disso, a dinâmica da vegetação nunca foi examinada comparativamente entre áreas com e sem manejo tradicional. Para testar estas hipóteses, entrevistamos gestores locais atuais, e antigos para compreender suas práticas de manejo e o destino dos campos. Em seguida, foram analisadas fotografias aéreas do ano de 1978 e imagens de satélite de 2018 para quantificar a mudança de vegetação. Essas duas fontes de informação foram utilizadas para verificar se as mudanças na vegetação dependiam do tipo de manejo. Esperávamos que o manejo tradicional feito por gestores locais manteria os campos subtropicais de altitude ao impedir o adensamento de arbustos, ao passo que as áreas protegidas, onde os distúrbios são evitados, permitiriam um adensamento de arbustos em um ritmo mais acelerado. Nossos resultados demonstraram que todos os entrevistados utilizam ou utilizavam o gado em suas áreas como fonte primária de renda. A criação de gado é feita de forma extensiva, isto é, há uma baixa densidade de animais circulando livremente pelos campos. A densidade média foi de 41 animais ( $\pm 13$ ) a cada 100 hectares. O fogo foi ou é usado por gestores locais em intervalos de 2 anos, ao final do inverno, para incentivar o rebrote dos campos. Na opinião da maioria dos entrevistados (55,6%), se o

gado e o fogo são suprimidos, os campos são substituídos por arbustais. Com a análise de dados de sensoriamento remoto, demonstramos que alguns anos após a remoção do fogo e do gado, as taxas de adensamento de arbustos nos campos aumentaram exponencialmente. Os campos foram substituídos por densos arbustais, de forma geral dominados pela espécie *Baccharis uncinella*, uma espécie nativa e comum nas regiões altas do planalto. A taxa de transformação do campo em arbustais foi tão expressiva que a extrapolação sugere que bastam 30 anos sem manejo tradicional para que 99% dos campos tornem-se arbustais. Enquanto isso, os campos manejados por de gestores locais, através de práticas tradicionais de manejo (*i.e.*, com utilização do gado e do fogo), permanecem praticamente inalterados nos últimos 40 anos. Tais componentes, além de manterem os campos subtropicais de altitude, também previnem incêndios florestais em larga escala, que podem ser catastróficos para a biodiversidade. Assim, é fundamental o reconhecimento do fogo como um elemento integrado a esses sistemas, uma vez que o ritmo acelerado de adensamento de arbustos nos campos subtropicais de altitude ameaça não apenas sua biodiversidade e os seus serviços ecossistêmicos, mas também o bem-estar humano e o patrimônio cultural associado.

Além do manejo, outros processos podem estruturar a comunidade biológica nestes ecossistemas, como a facilitação, que tem importante papel na dinâmica da expansão da floresta sobre os campos. Assim, objetivamos com o segundo capítulo investigar como o manejo, as interações bióticas e fatores abióticos afetam a riqueza, a abundância e a composição de espécies florestais em áreas de campo. Neste capítulo, realizamos um estudo empírico em um mosaico de campo subtropical de altitude e floresta com araucária. Buscamos entender como que os campos são mantidos pelas práticas de manejo, se as araucárias contribuem para a expansão florestal e o papel das interações bióticas e fatores abióticos (por exemplo, rochas) envolvidos nesse processo. Nossas hipóteses foram (1) o manejo reduz a riqueza e a abundância e muda a composição de espécies florestais, (2) araucárias aumentam a riqueza e abundância mudam a composição de espécies florestais, e (3) o efeito interativo



entre o manejo e a araucária é responsável por moldar padrões de riqueza, abundância e composição das espécies florestais. Esperávamos que o manejo tradicional afetaria negativamente espécies florestais, porque a supressão da vegetação pelo uso do fogo e o pastoreio podem matar ou danificar plântulas e indivíduos juvenis. Por outro lado, esperávamos que as araucárias favoreceriam a colonização de espécies florestais devido ao efeito poleiro e facilitação. Por fim, esperávamos também que o efeito do manejo tradicional suplantasse o efeito facilitador das araucárias. Os dados de riqueza, abundância e composição de espécies florestais juvenis foram coletados em condições sem manejo tradicional e com manejo tradicional, sob as copas de araucária e em áreas adjacentes. Nossos resultados indicaram que a abundância e a composição de espécies são afetadas pela forma de manejo e influência da copa das araucárias. Os maiores valores de abundância foram encontrados sob as copas das araucárias em áreas sem o manejo tradicional. A composição das espécies mudou em todas as combinações avaliadas de manejo e influência da copa. Nosso estudo demonstrou os papéis principais do manejo e da facilitação na estruturação de comunidades, além dos efeitos da cobertura de rochas e gramíneas. Além disso, nossos resultados esclareceram padrões e processos que emergem nos campos de altitude, como a conversão de campos em florestas e a perda de paisagens culturais quando as principais ações de manejo tradicional são excluídas. Figura 9.

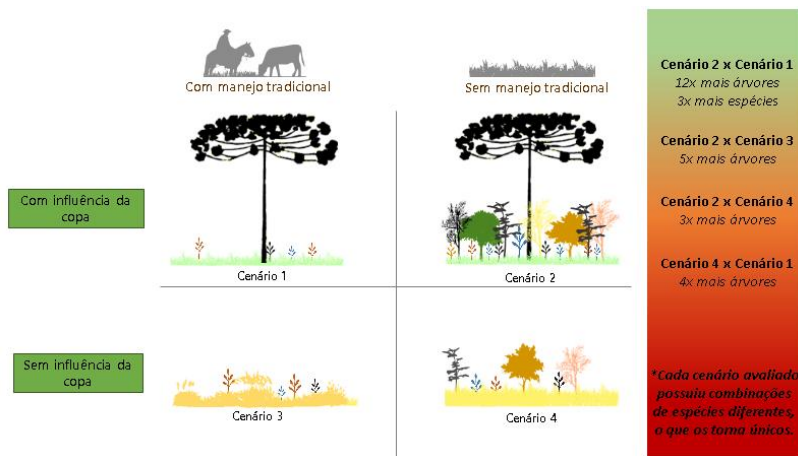


Figura 9. Representação esquemática dos resultados obtidos no capítulo 2.

Sabendo do importante papel das interações bióticas no processo de expansão da floresta sobre os campos, demonstrado no capítulo 1 e 2, no terceiro capítulo avaliamos experimentalmente os efeitos do tipo de solo e de comunidades microbianas do solo nos estágios iniciais de desenvolvimento da araucária, uma espécie-chave na expansão da floresta sobre os campos de altitude no sul do Brasil. O efeito da comunidade microbiana do solo na germinação e no crescimento inicial de plantas permanece pouco conhecido. Sendo a araucária uma espécie-chave na dinâmica sucessional e na expansão da floresta sobre o campo, nossa hipótese foi de que as comunidades microbianas do solo e tipos de solos comumente associados a essa espécie são benéficas para sua germinação e crescimento inicial, ao passo que solos e comunidades microbianas onde campos e arbustais ocorrem seriam prejudiciais. Nesse experimento, avaliamos o desempenho da araucária em solos onde a vegetação refletia diferentes unidades no gradiente floresta-campo (interior de floresta, borda de floresta, arbustais e campos) e com comunidades microbianas não-esterilizadas, esterilizadas ou inoculadas. Contrariando nossas expectativas, a germinação da araucária não foi afetada pelo tipo de solo nem pelos tratamentos,

mudando apenas em virtude do tempo. Por outro lado, as comunidades microbianas do solo afetaram negativamente o crescimento inicial da araucária, enquanto que as diferenças de solo, por não terem afetado as plântulas, podem permitir um ritmo mais rápido de expansão da floresta sobre os campos.

### 7.1 Consequências para a dinâmica floresta-campo

Sabendo-se que a expansão da floresta com araucária sobre os campos subtropicais de altitude é mediada e impulsionada pela araucária, os aspectos relacionados aos seus mecanismos de facilitação, estabelecimento e sucesso de colonização nos campos são de relevante interesse. O conjunto de mecanismos subjacentes a expansão da floresta com araucária sobre os campos de altitude ainda não foram completamente elucidados e muitas questões requerem respostas (FRANCO et al., 2005; SILVA; ANAND, 2011). No que se refere ao estabelecimento da araucária, por exemplo, a baixa quantidade de plântulas desenvolvendo-se fora de ambientes florestais, especialmente nos campos de altitude (FRANCO et al., 2005; GUGLIELME; GANADE, 2006) é resultado provável de uma série de fatores. Por exemplo, a dispersão de sementes tem papel importante no sucesso de estabelecimento da araucária. A araucária é dispersa por vários animais, especialmente aves (principalmente psitacídeos e gralhas), pequenos roedores, cotias (BRUM; DUARTE; HARTZ, 2010; IOB; VIEIRA, 2008; REITZ; KLEIN, 1966) e o próprio humano (BITENCOURT; KRAUSPENHAR, 2006; LAUTERJUNG et al., 2018; REITZ; KLEIN, 1966; SEDREZ DOS REIS; LADIO; PERONI, 2014). A limitação da dispersão não parece ser um problema para a araucária, uma vez que a maioria destes agentes estão presentes na região (BOGONI, 2018). Com base nos resultados do segundo capítulo da presente tese (SÜHS; GIEHL; PERONI, 2018), demonstramos que o manejo tradicional é um dos grandes mecanismos atuando na restrição do desenvolvimento de plântulas de araucária em áreas de campo. Nossos resultados demonstraram que em áreas onde o manejo tradicional é empregado, a ocorrência de plântulas de araucária (e de outras espécies florestais) é reduzida, tanto sob a copa de araucárias adultas (que facilitam o estabelecimento de

espécies florestais) quanto em áreas adjacentes. O pastoreio pelo gado e o uso frequente do fogo compõem práticas comuns de manejo nas regiões altas do planalto sul-brasileiro (NABINGER; MORAES; MARASCHIN, 2000). Essas práticas tradicionais são importantes para a manutenção dos campos de altitude uma vez que podem reduzir a velocidade de expansão da floresta sobre os campos (DUARTE et al., 2006; OVERBECK et al., 2007; PILLAR, 2003; SÜHS; GIEHL; PERONI, 2018).

Além do manejo tradicional, podemos inferir que os principais fatores impedindo o estabelecimento da araucária nos campos de altitude sejam (1) competição com espécies de gramíneas, (2) altas taxas de predação e herbivoria, (3) componentes do solo e (4) condições ambientais extremas. Quanto à competição com gramíneas, estas podem impedir o estabelecimento de espécies lenhosas através da competição por luz e recursos (SCHOLLES; ARCHER, 1997). Na interação com araucária, espécies de gramíneas podem ter sucesso sobre plântulas e juvenis por conta da barreira física imposta pelo rápido crescimento da biomassa da parte aérea (ZANDAVALLI; DILLENBURG, 2015). Além da competição, predação e herbivoria também têm um importante papel nestes sistemas. Por exemplo, GUGLIELME; GANADE (2006) demonstraram que 100% das sementes da araucária foram predadas (especialmente por pequenos roedores) em menos de 10 dias e a principal causa de mortalidade de plântulas foi ocasionada por formigas cortadeiras. Estas altas taxas de predação estão provavelmente relacionadas ao grande tamanho e alto valor nutricional das sementes de araucária comparado a espécies nativas (GUGLIELME; GANADE, 2006). Os componentes do solo, por sua vez, também podem afetar o desenvolvimento de plântulas. Demonstramos o efeito negativo de solos com microbiota viva em plântulas de araucária, revelando que a microbiota do solo pode dificultar o desenvolvimento inicial das plântulas. Por fim, sementes e plântulas da araucária precisam resistir a condições ambientais, tanto para eventos estocásticos quanto determinísticos. Por exemplo, as sementes da araucária têm um tempo curto de conservação em condições naturais devido à suas características recalcitrantes, sendo, portanto, suscetível à dessecação (ARALDI;

COELHO, 2015). Além disso, estresse hídrico e compactação do solo podem reduzir tanto o crescimento radicular como da parte aérea das plântulas (MÓSENA; DILLENBURG, 2004), embora a araucária tenha boa performance ecofisiológica sob condições estressantes (FRANCO et al., 2005). O próprio fogo, que pode ocorrer também de forma natural na região dos campos, pode representar uma ameaça para as sementes e plântulas da araucária. Além disso, áreas naturalmente úmidas (banhados), afloramentos rochosos superficiais e subsuperficiais, onde solos tendem a ser muito rasos, também previnem o estabelecimento de araucárias. Desta forma, o sucesso na germinação das sementes desta espécie está condicionado a uma série de filtros ambientais (abióticos e bióticos) até seus indivíduos alcançarem a fase reprodutiva, para então facilitar o estabelecimento de outras espécies florestais, contribuindo no processo de expansão da floresta sobre os campos.

## 7.2 Implicações para a conservação da floresta com araucária e dos campos de altitude

A floresta com araucária e os campos de altitude são ecossistemas frágeis que atualmente se encontram em elevado grau de fragmentação e alto risco de extinção. Hoje, a floresta com araucária ocupa apenas 12% de seu território original (RIBEIRO et al., 2009) e a própria araucária está criticamente ameaçada de extinção (IUCN, 2017). Ao mesmo tempo, os campos estão ameaçados por uma série de fatores, como invasão por espécies exóticas (CORDERO et al., 2016; ZALBA; VILLAMIL, 2002), práticas de gestão inapropriadas (OVERBECK et al., 2007) e mudanças climáticas (ASSIS; DE MATTOS, 2016). Tanto os campos como a floresta com araucária e ecossistemas associados são negligenciados por políticas públicas de conservação (CARLUCCI et al., 2016). Nas regiões acima de 900 m do nível do mar (ambientes montanos e alto-montanos), a floresta com araucária é frequentemente encontrada associada aos campos de altitude, formando mosaicos (KLEIN, 1984). Esses ambientes permanecem pouco estudados (DA SILVA et al., 2017; HIGUCHI et al., 2013; MARTINS-RAMOS et al., 2011) e detém peculiaridades importantes para a conservação, como um elevado número de

espécies e alta diversidade genética (BARROS et al., 2015), além de abrigarem espécies ameaçadas e endêmicas (e.g., SÜHS et al., 2019) e possivelmente servirem como refúgio para espécies migrantes de elevações mais baixas por consequência das mudanças climáticas (FEELEY et al., 2011).

Uma mudança no clima foi um dos eventos responsáveis pela expansão da floresta com araucária sobre os campos há cerca de 4000 anos A.P. Por isso, é indispensável discutir o papel das mudanças climáticas globais atuais no contexto da floresta com araucária e dos campos de altitude e nos processos influenciadores deste padrão (e.g., facilitação, distúrbio). As mudanças climáticas globais (MCGs) estão e continuarão afetando ecossistemas e comunidades vegetais. Os múltiplos efeitos das MCGs afetam diretamente as comunidades, através do aumento de temperatura, mudanças na disponibilidade de água e maior incidência de eventos extremos (IPCC, 2018), e indiretamente, através de modificações nas interações entre espécies (ANTHELME; CAVIERES; DANGLES, 2014). O aquecimento global age sobre ecossistemas aquáticos e terrestres, favorecendo espécies adaptadas a condições mais quentes e/ou reduzindo a abundância de espécies adaptadas ao frio, um fenômeno chamado de termofilização (“*thermophilization*”) (DE FRENNE et al., 2013; DUQUE; STEVENSON; FEELEY, 2015). Com relação às espécies arbóreas, além de registros de aumento de mortalidade, há evidências de que muitas espécies tropicais estão migrando para regiões mais altas (FEELEY et al., 2011, 2013). Esta migração muda a composição de florestas montanas tropicais levando a uma maior abundância de espécies cujas distribuições eram centradas em elevações mais baixas e mais quentes (DUQUE; STEVENSON; FEELEY, 2015) (DUQUE; STEVENSON; FEELEY, 2015).

Por outro lado, muitas espécies arbóreas montanas têm uma baixa capacidade de persistir sob aumento rápido de temperatura, o que sugere um alto risco de perda de espécies nestas condições de altitude (DUQUE; STEVENSON; FEELEY, 2015). Neste sentido, a facilitação pode ter um papel importante nesses sistemas, uma vez que a ausência de uma espécie facilitadora eficiente poderá tornar

essa migração mais lenta e assim resultar em extinções de espécies (ANTHELME; CAVIERES; DANGLES, 2014; CAVIERES et al., 2014). Diante disso, caso a floresta alto-montana não se estabeleça nos campos e assim não se expanda, poderá ser substituída por espécies migratórias de regiões mais baixas (FEELEY et al., 2011, 2013). Por outro lado, caso a floresta consiga se estabelecer nos campos, essa expansão florestal se tornará uma ameaça para os campos (OVERBECK et al., 2007), que não terão para onde migrar. Dessa maneira, é provável que tanto a floresta com araucária, como os campos de altitude, se tornem ainda mais ameaçados. Isto comprometerá não apenas as espécies que ocorrem nesses ecossistemas, mas suas histórias evolutivas, heranças culturais e todos os benefícios de seus serviços.

Neste sentido, tanto a ecologia histórica, como programa de pesquisa, quanto as interações bióticas, como processos, desempenham papéis cruciais na conservação dos campos subtropicais de altitude e da floresta com araucária. Interações bióticas que ocorrem por intermédio da facilitação atuam na organização de comunidades ao permitir que espécies consigam se estabelecer em locais antes inapropriados. Este é o caso da araucária, uma espécie-chave nos altiplanos sul-brasileiros. Interações negativas como a predação, competição e inibição, são importantes processos atuando no processo de expansão e na manutenção dos campos. Já a ecologia histórica é um programa de pesquisa que traz perspectivas mais amplas sobre as formas de uso e manejo das paisagens, e torna-se uma ferramenta essencial para a compreensão da dinâmica da expansão da floresta sobre os campos. As práticas de manejo tradicional secularmente utilizadas por gestores locais, principalmente através da utilização do fogo e do gado, podem contribuir para a manutenção dos campos ao conter o adensamento de arbustos e a expansão da floresta. Ao mesmo tempo, a utilização da araucária como fonte de alimento e renda pelos gestores locais e a ampliação de áreas protegidas poderão contribuir para a manutenção das populações de araucária na paisagem. Dessa forma, áreas protegidas e áreas tradicionalmente manejadas poderão juntas manter uma maior diversidade de paisagens e espécies,

resguardando paisagens culturais e processos ecológicos fundamentais que atuam na expansão da floresta sobre o campo. No entanto, integrar essas duas perspectivas torna-se um grande desafio no contexto da legislação atual. É preciso reconhecer a importância dos campos como paisagens culturais e do fogo como elemento natural nesses ecossistemas. A valorização das práticas seculares de manejo tradicional nos campos se faz necessária para estabelecer políticas públicas efetivas de conservação e manejo. Somente assim será possível evitar um colapso desses ecossistemas e de suas paisagens culturais.



## 8. Conclusões

- O fogo e a pecuária extensiva fazem parte do manejo tradicional secular utilizado por gestores locais das regiões de campos subtropicais de altitude para a manutenção dos campos;
- Sem o manejo tradicional, os campos subtropicais de altitude são rapidamente substituídos por densos arbustais, que podem representar uma ameaça à biodiversidade e às paisagens culturais;
- A araucária (*Araucaria angustifolia*) é uma espécie-chave nessas regiões de elevado estresse abiótico, promovendo o estabelecimento de outras espécies arbóreas e acelerando o processo de expansão da floresta sobre o campo; afloramentos rochosos também contribuem para esse processo, em determinadas situações;
- Os campos, em termos de volume de biomassa, oferecem uma resistência ao estabelecimento de espécies florestais, ao passo que o manejo tradicional, embora reduzindo essa biomassa, consegue se sobrepor ao efeito facilitador das araucárias, dificultando o estabelecimento de espécies arbóreas;
- A germinação da araucária não é afetada pelo tipo ou microbiota do solo do gradiente floresta-campo;
- A microbiota do solo do gradiente floresta-campo afeta o desenvolvimento da araucária, sendo um dos fatores atuantes como obstáculos no processo de expansão da araucária sobre os campos.

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