# Estrategias ecológicas de *Cynodon dactylon*: de la competencia por nutrientes a la degradación de mutualismos micorrícicos

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## Resumen

*Cynodon dactylon* es la principal gramínea invasora de Uruguay. Su expansión amenaza la biodiversidad de los pastizales naturales, especialmente frente a procesos de intensificación del manejo, como los mejoramientos de campo (fertilización fosforada y siembra de leguminosas). Los mecanismos que explican su éxito invasor aún no están claros. Dada su habilidad competitiva, su potencial alelopático y su baja dependencia de micorrizas arbusculares —mutualismo extendido en los pastizales uruguayos—, las interacciones bióticas desempeñan un papel central en su establecimiento. El objetivo general de este trabajo fue explorar el rol de la competencia interespecífica y del mutualismo micorrícico arbuscular en la invasión de *C. dactylon*. Específicamente, se evaluaron interacciones entre *C. dactylon* y dos gramíneas nativas (*Paspalum notatum* y *Mnesithea selloana*) bajo distintos niveles de disponibilidad de nutrientes, analizando el efecto relativo de la competencia y la alelopatía, y el papel de la asignación de biomasa en dichas interacciones. Además, se examinó si el aumento del nivel de invasión se asociaba con una reducción en la colonización micorrícica, el crecimiento y el contenido de nutrientes de *P. notatum*. Se realizaron tres experimentos distintos en condiciones controladas. Por un lado, dos experimentos de interacciones interespecíficas en donde las especies se cultivaron con o sin interacción, aplicando tres niveles de fertilización (sin, simple y doble dosis de nitrógeno y fósforo) y dos niveles de carbón activado (con y sin) para aislar los efectos de la alelopatía. La biomasa se midió a los 120 días y se calculó el índice de interacción relativo. Por otro lado, se realizaron muestreos en pastizales naturales y mejorados con diferentes coberturas de *C. dactylon* (0-10 %, 30-50 % y 70-90 %), midiendo biomasa, fósforo y colonización micorrícica en *P. notatum*. Los resultados mostraron que *C. dactylon* posee una alta habilidad competitiva, explicada por su tolerancia al estrés biótico y su asignación de biomasa a estructuras aéreas (estolones). La micorrización en *P. notatum* disminuyó con el incremento de la invasión, especialmente en pastizales intensivamente manejados, lo que sugiere que la degradación del mutualismo planta-hongo contribuiría al éxito invasor de *C. dactylon*. En conjunto, este trabajo aporta

evidencia ecológica clave para comprender los mecanismos bióticos de la invasión de *C. dactylon* y para orientar el manejo y la conservación de los pastizales naturales del Uruguay frente a la intensificación productiva.

**Palabras clave**: invasiones biológicas, pastizales, habilidad competitiva, micorrizas arbusculares, alelopatía

## Ecological strategies of *Cynodon dactylon*: from nutrient competition to the degradation of mycorrhizal mutualisms


### Summary

*Cynodon dactylon* is the main invasive grass species in Uruguay. Its expansion threatens the biodiversity of natural grasslands, particularly under management intensification such as fertilization with phosphorus and overseeding with legumes. The mechanisms underlying its invasive success are still unclear. Given its competitive ability, allelopathic potential, and low dependence on arbuscular mycorrhizas—a widespread mutualism in Uruguayan grasslands—biotic interactions play a central role in its establishment. The general objective of this study was to explore the role of interspecific competition and arbuscular mycorrhizal mutualism in the invasion of *C. dactylon*. Specifically, interactions between *C. dactylon* and two native grasses (*Paspalum notatum* and *Mnesithea selloana*) were evaluated under different nutrient availability levels, analyzing the relative effects of competition and allelopathy, and the role of biomass allocation in these interactions. In addition, the study examined whether increasing invasion levels were associated with a reduction in mycorrhizal colonization, growth, and nutrient content of *P. notatum*. Three different experiments were conducted under controlled conditions. Two interspecific interaction experiments were carried out in which the species were grown either together or separately, applying three fertilization levels (none, single, and double doses of nitrogen and phosphorus) and two levels of activated carbon (with and without) to isolate allelopathic effects. Biomass was measured after 120 days, and the Relative Interaction Index was calculated. Additionally, field samples were collected from natural and intensively managed grasslands with different *C. dactylon* cover levels (0–10%, 30–50%, and 70–90%), measuring biomass, phosphorus content, and mycorrhizal colonization in *P. notatum*. Results showed that *C. dactylon* exhibits high competitive ability, explained by its tolerance to biotic stress and its biomass allocation to aboveground structures (stolons). Mycorrhizal colonization in *P. notatum* decreased with increasing invasion, especially in intensively managed grasslands, suggesting that the degradation of the plant–fungus mutualism may contribute to the invasive success

of *C. dactylon*. Overall, this work provides key ecological evidence to understand the biotic mechanisms underlying the invasion of *C. dactylon* and to guide the management and conservation of Uruguay's natural grasslands under increasing production intensification.

**Keywords**: biological invasions, grasslands, competitive ability, arbuscular mycorrhizae, allelopathy

# 1. Introducción

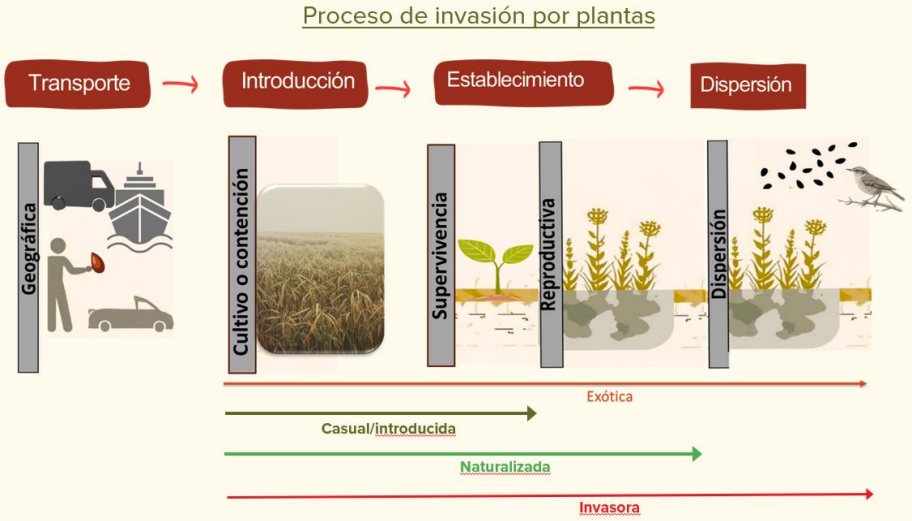
## 1.1. Invasiones biológicas

Las invasiones biológicas constituyen una de las principales amenazas para la biodiversidad en el mundo, con impactos que se extienden desde la modificación de la estructura y función de los ecosistemas hasta la pérdida de especies nativas (IPBES, 2023; Mack et al., 2000; Vilà e Ibañez, 2011).

El proceso de invasión en plantas constituye el llamado *continuum introducción-naturalización-invasión* (figura 1; Blackburn et al., 2011; Richardson y Pysek, 2006). El proceso comienza con el transporte intencional o accidental y la introducción humana de una especie introducida, también denominada *exótica*, desde su área de distribución original (rango nativo) hacia otras áreas fuera de esta (rango exótico). Esto permite que dicha especie supere determinadas barreras geográficas y se distribuya a distancias mayores a las que alcanzaría naturalmente (figura 1; Blackburn et al., 2011; Richardson et al., 2000; Richardson y Pysek, 2006). Una vez introducida, la especie puede alcanzar la etapa de establecimiento formando poblaciones autónomas. En algunos casos, las especies introducidas no logran reproducirse, denominándose *casuales*. Otras especies, en cambio, superan barreras ambientales y reproductivas, por lo que logran la supervivencia y reproducción, lo que asegura su establecimiento y naturalización en la comunidad receptora (Blackburn et al., 2011). Durante el establecimiento operan filtros de tipo bióticos, como por ejemplo la competencia por recursos, que pueden limitar el tamaño poblacional de la especie invasora (Theoharides y Dukes, 2007). Finalmente, algunas de estas especies naturalizadas logran superar los filtros bióticos y alcanzan una rápida expansión poblacional, con un aumento en el número de individuos y una mayor capacidad de dispersión en el nuevo ambiente, tal como caracteriza a una especie invasora (Blackburn et al., 2011; Fristoe et al., 2021).

**Figura 1**

Proceso de invasión destacando el continuo de naturalización-invasión, resaltando las diversas barreras que una planta debe superar para convertirse en una especie exótica, casual, naturalizada o invasora en un nuevo ambiente.




Nota. Elaboración propia basada en Richardson y Pysek (2006).

Los mecanismos que operan en los procesos de invasión son variables, actuando en las sucesivas etapas, a diferentes escalas espaciales. De forma general, Lonsdale (1999) postula que el éxito de invasión es el resultado de tres factores principales: la presión de propágulos (esfuerzo de introducción), la capacidad de una especie de invadir (invasividad) y la susceptibilidad del ambiente a ser invadido (invasibilidad). A escala regional, la invasividad de una especie está determinada por atributos como su amplitud de nicho climático (Bustamante et al., 2020; Higgins y Richardson, 2014) o edáfico (Skálová et al., 2019) o su capacidad de dispersión regional (Irl et al., 2021; Zhang et al., 2023; Zhou et al., 2021). Por otro lado, la invasibilidad de un ambiente está explicada por condiciones climáticas (Hellmann et al., 2007), la configuración del paisaje (With, 2002) y el régimen de perturbaciones (*e. g.*, Burke y Grime, 1996).

A escalas espaciales menores, los mecanismos que actúan en los procesos de invasión se vinculan principalmente a las interacciones bióticas entre las especies invasoras y nativas (Blackburn et al., 2011). Aunque se cumplan sus requerimientos ecológicos, una especie no nativa puede enfrentar resistencia biótica, manifestada a través de la competencia por recursos con las especies nativas de la comunidad

receptora (Elton, 1958), la presión de nuevos enemigos naturales (Parker et al., 2007) o la ausencia de mutualistas (Mitchel et al., 2006) que sí estaban presentes en su rango nativo.

## 1.2. Rol de las interacciones bióticas en el proceso de invasión de comunidades vegetales

### 1.2.1. Habilidad competitiva e invasión

Una vez introducidas, el éxito de la invasión por plantas ha sido explicado, en gran medida, a partir de teorías que destacan las interacciones antagónicas entre las especies invasoras y la comunidad nativa. En la etapa de establecimiento, la invasividad en plantas puede estar explicada por una superioridad en la habilidad competitiva de la especie no nativa, que le permitirá utilizar más eficientemente los recursos disponibles, o mostrar una mayor tasa de adquisición de estos en comparación con las especies de la comunidad nativa (Gioria y Osborne, 2014; Vilà y Weiner, 2004). La habilidad competitiva está definida por dos componentes: el efecto competitivo, es decir, la capacidad de un individuo para adquirir recursos a tasas lo suficientemente altas como para reducir su disponibilidad para las plantas vecinas, y la respuesta competitiva, que es su capacidad para tolerar la competencia y desempeñarse con bajos niveles de recursos (Goldberg, 1990).

Algunas de las hipótesis vinculadas a las interacciones bióticas se enfocan en los atributos de las plantas invasoras los cuales favorecen su invasividad en determinadas condiciones (Rejmanek y Richardson 1996; Baker, 1965). Los mecanismos que se han propuesto para explicar la mayor habilidad competitiva de las plantas invasoras dependen de las condiciones ambientales y de la disponibilidad de recursos. En ambientes pobres en recursos, especies invasoras cuentan con estrategias tanto de conservación de recursos —por ejemplo, una alta eficiencia en el uso de dichos recursos— como de adquisición rápida de recursos —por ejemplo, hojas ricas en nutrientes, gran área foliar específica y una vida media foliar corta (Funk, 2013), que le confieren una alta tolerancia a la competencia con especeies vecinas Por otro lado, en ambientes con alta disponibilidad de recursos predominan atributos ecofisiológicos

de adquisición rápida de recursos (Burke y Grime, 1996; Heunneke et al., 1990; Liu y van Kleunen, 2017).

Otro atributo que explica el éxito es la capacidad de propagarse rápidamente de forma clonal, lo que le permite acaparar una mayor cantidad de recursos en comparación con plantas no clonales (Pyšek, 1997; Wang et al., 2024). También se ha reportado que las plantas invasoras asignan mayor energía a órganos aéreos en comparación con las especies nativas. Una mayor asignación a biomasa aérea permite a las invasoras competir eficazmente por la luz, ocupar rápido espacios, reproducirse abundantemente, alterar el entorno a su favor y desplazar a las especies nativas (van Kleunen et al., 2010).

Otro rasgo que puede aumentar la habilidad competitiva de las especies invasoras es la plasticidad fenotípica, es decir, la capacidad de modificar la morfología, fisiología o fenología, según las condiciones del ambiente, para lograr ampliar su nicho ecológico (Richards et al., 2006). Esta plasticidad, que le permite ajustar atributos claves frente a vecinos competidores (Davidson et al., 2011) o a fluctuaciones de recursos (Li et al., 2023), puede mejorar su desempeño en ambientes nuevos y variables, lo que favorece la colonización y el establecimiento.

Otro de los mecanismos que puede aumentar la habilidad competitiva de las plantas invasoras es la alelopatía, mecanismo de competencia por interferencia que reduce la competencia por recursos. De acuerdo con la hipótesis de las armas novedosas (*novel weapon hypothesis*; Callaway y Ridenour, 2004; Ridenour y Callaway, 2001), algunas especies invasoras tendrían ventaja en los ecosistemas que colonizan porque producen sustancias químicas que resultan nuevas para las comunidades receptoras, frente a las cuales las especies nativas no están evolutivamente adaptadas. A través de la liberación de compuestos alelopáticos, una planta puede inhibir la germinación, el crecimiento o la actividad fisiológica de las plantas nativas (Callaway y Ridenour, 2004; Ridenour y Callaway, 2001). La producción de metabolitos secundarios es evolutivamente nueva en el rango de introducción y estos pueden ser inhibitorios para otras plantas o para microorganismos benéficos de las especies residentes no adaptadas a dichos compuestos (Callaway y Ridenour, 2004; Ridenour y Callaway, 2001).

La invasividad de una especie está ligada a la invasibilidad de la comunidad receptora: el éxito de una especie invasora no depende únicamente de sus características biológicas, sino también de las propiedades del ambiente receptor (Lonsdale, 1999). Una planta con alta invasividad solo logrará invadir si encuentra un entorno suficientemente susceptible, así como también un ambiente vulnerable solo será colonizado si llegan especies con las características adecuadas.

Un determinante clave del éxito de una invasión biológica es la correspondencia entre la historia ecoevolutiva de las especies invasoras y la de las comunidades receptoras. En este marco, existen hipótesis que plantean que la similitud funcional y filogenética entre especies invasoras y nativas pueden influenciar el establecimiento de plantas exóticas. La hipótesis de similitud filogenética (o *naturalización de Darwin*) propone que las plantas exóticas presentan mayor probabilidad de establecimiento y éxito invasor en comunidades compuestas por especies filogenéticamente distantes, debido a una menor competencia y a la ausencia de enemigos naturales compartidos (Daehler, 2001; Darwin, 1859). Por su parte, la hipótesis de la similitud límite sostiene que el éxito de una especie invasora aumenta cuando sus atributos funcionales difieren de los de las especies nativas, reduciendo así la superposición de nichos y la competencia directa (Macarthur y Levis, 1967). En contraposicón la hipótesis de adaptación sugiere que especeis exóticas cercanas filogenéticamente y/funcionalmente, tienen mayores éxitos para invadir, por haber compartido historia evolutiva con especies nativas (Duncan y Williams, 2002). De acuerdo a la evidencia, que se cumplan o no dchas hipótesis depende de las escalas espaciales del estudio: a escalas pequeñas operan vinculados mecanismos que reafirman la hipótesis de similitud filogenética y similitud límite, mientras que a mayores escalas mayores la hipótesis de adaptación tiene mayor fuerza (Daly et al. 2023).

Otro de los filtros bióticos que una planta invasora debe superar es la resistencia biótica de la comunidad receptora (Elton 1958). De acuerdo a esta hipótesis, a escalas espaciales locales, ambientes con alta diversidad de especies nativas presentan menor invasibilidad (Liu et al., 2025; Traveset y Richardson, 2020), ya que existe mayor complementariedad en el uso de recursos y menos recursos disponibles (Lambers et al., 2010; Olde Venterink, 2011). Además, las especies nativas tienen ventajas

adaptativas en dichos ambientes en comparación con las exóticas (Alpert et al., 2000; Daehler, 2003).

La invasibilidad de un ambiente, o de una comunidad, está determinada por varias propiedades de los ecosistemas como el régimen de disturbios, la disponibilidad y fluctuación de recursos, la heterogeneidad ambiental, y la presencia de enemigos naturales y de mutualistas (Callaway y Ridenour, 2004; Davis et al., 2000; Huenneke et al., 1990; Traveset y Richardson, 2014, 2020).

Los disturbios promueven la invasión, ya que mayoritariamente aumentan la disponibilidad de recursos y crean espacios libres en la vegetación, situaciones que son aprovechadas por las plantas invasoras (Davis et al., 2000; Frioste et al., 2021). Ambientes con alta disponibilidad de recursos son más susceptibles a ser invadidos y presentan mayor riqueza de especies exóticas que ambientes pobres en recursos (Gross et al., 2005; Huenneke et al., 1990; Stohlgren et al., 2008). En este sentido, la hipótesis de los recursos fluctuantes propone que, en una comunidad de plantas, la invasibilidad aumenta inmediatamente después de un incremento en la disponibilidad de recursos, debido a una reducción en el uso de estos por la comunidad residente o por el aumento en su suministro (Davis et al., 2000). En conjunto, la interacción entre disturbios, disponibilidad de recursos y características de la comunidad residente emerge como un determinante clave de la invasibilidad de los ecosistemas.

### 1.2.2. Interacciones bióticas positivas e invasión

Aunque la mayoría de las hipótesis que buscan explicar los patrones de invasión se refieren a interacciones antagónicas, existe cada vez mayor evidencia de la importancia de las interacciones positivas (facilitadoras o mutualistas) en el proceso de invasión por plantas (Pyšek et al., 2019; Traveset y Richardson, 2014, 2020).

Por un lado, la diversidad de mutualistas presentes en la comunidad nativa —como polinizadores, dispersores y microrganismos— influye en la invasibilidad (Richardson et al., 2000; Wolfe y Klironomos, 2005). Por otro lado, muchas veces las especies no nativas requieren establecer asociaciones mutualistas para poder asentarse con éxito. En algunos casos la planta no nativa puede verse limitada por falta de mutualistas,tal como establece la hipótesis del mutualismo perdido (Mitchell et al., 2006). En otros

casos, las especeis exóticas pueden formar nuevas interacciones bióticas con miembros de la comunidad receptora y establecer mutualismos que favorezcan su establecimiento, como proponen la hipótesis del mutualismo mejorado (Reinhart y Callaway, 2006). Además, también puede suceder que las especies invasoras supriman los mutualismos en el área invadida de manera más acentuada que en su rango nativo, como lo plantea la hipótesis del mutualismo degradado (Hale y Kalisz, 2012; Roche et al., 2023; Traveset y Richardson, 2014; Vogelsang et al., 2004; Vogelsang y Bever, 2009). Esta supresión confiere una ventaja competitiva a la especie invasora sobre aquellas nativas que dependen en mayor medida de dichos mutualismos (Callaway et al., 2008; Callaway y Ridenour, 2004; Vogelsang y Bever, 2009).

Uno de los mutualismos más extendidos en los sistemas terrestres son las micorrizas arbusculares (MA), relación que se establece entre hongos pertenecientes al *phylum* Glomeromycota y las raíces de la mayoría de las plantas vasculares (Smith y Read, 2008). Esta simbiosis otorga beneficios a las plantas, entre los que se destaca el aumento en la absorción de agua (Marulanda et al., 2003) y nutrientes, particularmente fósforo (Smith y Smith, 2011). La planta, por su parte, destina entre el 10 % y el 60 % de los carbohidratos que produce por fotosíntesis para mantener esta interacción (Stribley et al., 1980), por lo que se ha postulado un continuo mutualismo-parasitismo (Klironomos, 2003). La interacción micorrícica arbuscular se caracteriza por tener muy baja especificidad entre los simbiontes (Smith y Read, 2008). Sin embargo, la respuesta de las plantas a los hongos dependerá del genotipo de ambos simbiontes y de las condiciones ambientales (Bever et al., 1996). Esto significa que el resultado de la interacción entre una planta introducida y un hongo nativo podrá tener consecuencias positivas o negativas para la planta invasora; como resultado, se promoverá o limitará el proceso de invasión (Klironomos, 2003).

Es evidente la importancia de las micorrizas arbusculares en la estructuración de comunidades vegetales, dado que estas pueden mediar interacciones bióticas entre las plantas (van der Heijden et al., 1998). Si bien el rol de estas interacciones en los procesos de invasión ha ganado reconocimiento en los últimos años, persiste un debate acerca de los mecanismos subyacentes, ya que múltiples factores y atributos ecológicos pueden estar operando de forma concurrente. Entre estos atributos se

cuentan, el satus, la dependencia, la respuesta y la flexibilidad micorrícica de la planta invasora y de las nativas. El status micorrícico se define como la capacidad o propensión de una especie vegetal a formar micorrizas, por lo que una planta puede ser micorrícica obligada (necesita de las micorrícicas para cumplir su ciclo de vidao crecer), facultativa (es capaz de prescindir de las micorrizas bajo ciertas condiciones ambientales), o no micorrícica (nunca forma micorrizas) (Smith y Read, 2008; Menzel et al. 2017). La dependencia micorrícica, en tanto, refiere al grado en que una planta depende de las micorrizas para sobrevivir o crecer en ambientes con bajos niveles de fertilidad del suelo (Janos, 2007). La respuesta micorrícica es la diferencia en crecimiento de una planta creciendo con y sin micorrizas (Janos, 2007). La flexibilidad micorrícica en este trabajo la definimos como la capacidad de una planta de asociarse a diferentes taxas de hongos micorrícicos.

Algunos estudios no encontraron relación entre las interacciones micorrícicas arbusculares y el desempeño de las plantas invasoras en el nuevo ambiente (Bunn et al. 2015). Otros trabajos que utilizaron datos de flora globales y regionales encontraron que el número de especies de plantas introducidas con MA era menor que las nativas con dichas interacciones (Delavaux et al., 2019; Pringle et al., 2009) y, además, hallaron que las especies introducidas que sí se micorrizaban mostraban menores respuestas a las micorrizas en su crecimiento en comparación con las especies nativas (Pringle et al., 2009). Sin embargo, existen estudios que sugieren que estos mutualismos, sí serían relevantes en mantener la performance de las especies exóticas y podría conferirle ventajas para invadir. Dichos trabajos revelaron, que las especies vegetales exóticas que mostraban mayor capacidad de naturalización y dispersión fueron las que presentaron MA (Pysek et al., 2019; Menzel et al., 2017; Menzel et al., 2018; Pysek et al., 2019). Esto es explicado particularmente porque la fase de establecimiento de las plántulas es promovida por la micorrización (van der Heijden et al., 2003). En cuanto al status micorrícico las plantas invasoras que mostraron interacciones del tipo facultativas también presentaron una mayor probabilidad de naturalización (Pysek et al., 2019) y mayor rango de ocupación que las de interacción de tipo obligada (Menzel et al., 2017; Menzel et al., 2018; Pysek et al., 2019). Esto se debe a que, en las interacciones de tipo obligada y alta dependencia micorrícica, la

falta de un simbionte fúngico en el nuevo ambiente constituye una limitante para el establecimiento y dispersión de la planta invasora, tal como propone la hipótesis del mutualismo perdido (Pringle et al., 2009). La flexibilidad de la interacción, es decir la capacidad de una especie introducida de establecer MA con varios simbiontes de la comunidad nativa, es otro aspecto importante en el éxito de la invasión, principalmente durante la etapa de dispersión (Pringle et al., 2009; Pysek et al., 2019).

De acuerdo a la literatura, los mecanismos mediante los cuales las interacciones micorrícicas median el proceso de invasión son diversos. Entre estos, por ejemplo el mecanismo que subyace a la hipótesis del mutalismo mejorado, propone que plantas invasoras con MA tendrían acceso a la red de micelio del suelo, lo que les proporcionaría nutrientes y agua a un bajo costo energético y les otorgaría ventajas competitivas con respecto a las especies introducidas no micorrícicas (Menzel et al., 2017). Otro mecanismo identificado en la literatura es el que ocurre a través de la degradación de las interacciones micorrícicas de la comunidad nativa (Vogelsang y Bever, 2009). Esto puede ocurrir en casos en que la planta introducida sea no micorrícica o poco dependiente de las micorrizas, lo que, en el mediano-largo plazo, podría reducir el banco de esporas del suelo, hecho que perjudicaría a las plantas nativas que sí se favorecen de las micorrizas (Callaway et al., 2008; Shah et al., 2009; Vogelsang y Bever, 2009; Wilson et al., 2012). En otros casos, las plantas invasoras son capaces de alterar la composición de la comunidad fúngica del suelo, seleccionando las especies de hongos más beneficiosas para su propio desempeño (Day et al., 2015; Zhang et al., 2017).

### 1.3. Invasiones biológicas en Uruguay

En Uruguay, las especies exóticas invasoras representan una de las principales amenazas para la biodiversidad y el funcionamiento de los ecosistemas terrestres y acuáticos. En el marco de los compromisos internacionales y ante el creciente impacto de las invasiones biológicas, Uruguay creó en 2008 el Comité Nacional de Especies Exóticas Invasoras (CEEI), que desde entonces ha trabajado en la identificación, priorización y gestión de estas especies. Sus acciones incluyen la elaboración de listas y diagnósticos, la definición de especies prioritarias de actuación, el desarrollo de

planes piloto de control y la aplicación de metodologías de análisis de riesgo, avanzando en la construcción de bases de datos y protocolos de respuesta. Si bien aún persisten vacíos legales e institucionales, el CEEI ha impulsado un enfoque integral basado en la prevención, la detección temprana, el control y la restauración, subrayando la importancia de la participación social, la investigación y la educación para enfrentar este problema. En este contexto, resulta relevante destacar las principales especies exóticas invasoras registradas en el país

Aunque se han registrado más de 350 especies exóticas, unas 42 son consideradas invasoras por sus impactos ambientales, sociales y productivos. Entre estas especies exóticas invasoras hay 12 que se consideran de alto riesgo (Brazeiro et al. 2021), incluyendo cuatro plantas vasculares, cuatro invertebrados y cuatro vertebrados. Entre las especies de plantas vasculares se destacan *Eragrostis plana* (Capin annoni), *Ulex europaeus* (tojo), *Ligustrum lucidum* (ligustro) y *Gleditsia triacanthos* (corona de Cristo). Entre los vertebrados de alto riesgo se encuentran *Sus scrofa* (jabalí), *Axis axis* (ciervo axis), *Lithobates catesbeianus* (rana toro) y *Cyprinus carpio* (carpa). En cuanto a los invertebrados se cuentan *Limnoperna fortunei* (mejillón dorado), *Rapana venosa* (caracol rapana)*, Corbicula* sp. (almeja asiática) y *Aedes aegypti* (mosquito del dengue). Sus efectos incluyen la alteración de comunidades nativas, pérdida de servicios ecosistémicos y perjuicios a sectores productivos como la ganadería, la forestación y la pesca. Si bien en la última década se avanzó en la identificación y gestión de estas especies, persisten vacíos de conocimiento y la necesidad de políticas más proactivas para la prevención, control y mitigación, en línea con los compromisos internacionales asumidos por el país.

La invasión por plantas es una de las principales amenazas de los pastizales naturales (PN), bioma en el que se encuentra contenido Uruguay. Estos pastizales, junto con la *Pampa* (este de Argentina) y los *Campos* (sur de Brasil), integran el sistema de pastizales del Río de la Plata. Su alta relevancia se explica, por un lado, porque constituyen uno de los pastizales más conservados del mundo (Gallego et al., 2024; Baeza et al., 2022; Andrade et al., 2018) y, por otro, porque se ubican en una zona de transición climática (templada–subtropical), que les confieren una notable diversidad vegetal con 4836 especies de plantas y la presencia de 403 especies

endémicas (Andrade et al., 2018). Se trata de pastizales mixtos, en los que convergen gramíneas estivales C4 —mayoritarias en los pastizales del norte— y gramíneas invernales C3, predominantes en el sur de Uruguay (Gibson, 2009). En Uruguay los pastizales naturales, ocupan el 55 % de su territorio (Baeza et al., 2022; MapBiomas Uruguay Initiative, 2023), están conformados principalmente por gramíneas C4 (que predominan en el norte del país y C3 (en el sur del territorio; Pallarés et al 2005), aunque también hay presencia de Asteraceae, Fabaceae y Cyperaceae (Andrade et al., 2018). Albergan una gran diversidad vegetal, con 2756 species de plantas, de las cuales 128 son exclusivas de Uruguay (Andrade et al. 2018) y animal ya que más de la mitad de las especies de aves, mamíferos y reptiles que caracterizan la fauna uruguaya utilizan pastizales como hábitat (Brazeiro et al. 2020). Además de la biodiversidad, los pastizales naturales proveen de otra cantidad de servicios ecosistémicos intermedios como protección del suelo contr la erosión y la captura de carbono (Gallego et al., 2020; Modernel et al., 2016; Paruelo et al., 2024) y servicios finales como la producción de forraje que sostiene la ganadería extensiva, una de las principales actividades económicas del país. La productividad primaria producción primaria media oscila entre de 2900 a 6300 kg de MS ha−1 año−1 (Berretta et al., 2000; Bendersky et al., 2017 en: Jaurena et al 2021) y una producción secundaria media de 60 a 70 kg de peso vivo ha−1 año−1 (Carvalho et al., 2006). Tanto la producción como el valor nutricional de forraje tienen una marcada estacionalidad con mayores valores en primavera-verano (Berretta et al., 2000 en: Jaurena et al 2021). En este contexto, la conservación de los pastizales resulta fundamental tanto para la sustentabilidad ecológica como para la viabilidad socioeconómica de Uruguay.

El escenario de cambios en el uso del suelo ocurrido en las últimas décadas, caracterizado por el aumento del área destinada a la agricultura y la forestación, constituye una importante perturbación para los pastizales naturales y genera oportunidades para la colonización y el establecimiento de plantas invasoras (Bresciano et al., 2014; Pañella et al., 2022). Los pastizales muestran limitación principalmente por N (Cardozo et al., 2024; Rodríguez Palma et al., 2024), con relaciones N:P en los tejidos de entre 11 y 15, aunque también hay evidencia experimental de co-limitación N–P (Fedrigo et al., 2021). Estas limitaciones

nutricionales redundan en bajas productividad secundaria. En el caso de la ganadería, con el fin de aumentar la producción y el valor nutritivo del forraje, se llevan a cabo prácticas agronómicas de intensificación productiva, tales como los mejoramientos de campo o mejoramientos extensivos del pastizal natural (PN + LP). Dichas prácticas de manejo consisten en la fertilización fosforada anual y la siembra en cobertura de leguminosas para incrementar los niveles de fósforo y nitrógeno disponibles, respectivamente (Jaurena et al., 2021). Aunque estas prácticas de manejo provocan un incremento en la productividad primaria, en el largo plazo pueden causar reducción de la diversidad de la vegetación nativa y un incremento de especies exóticas invasoras (Cáceres, 2019; Jaurena et al., 2015; Pañella et al., 2022). Se ha propuesto que las situaciones de PN y PN + LP corresponden a dos condiciones del sistema pastizal, donde el PN permitiría sostener una mayor diversidad vegetal, tanto en el espacio como en el tiempo, lo que estaría explicado por una heterogeneidad en los recursos por los cuales las especies compiten (Pañella et al., 2022). Sin embargo, la perturbación y el aumento de la disponibilidad de recursos que representan los PN + LP ofrecen oportunidades para el establecimiento de especies exóticas, entre las que se destaca la gramínea *Cynodon dactylon* (Jaurena et al., 2015; Pañella et al., 2022).

### 1.4. Invasiones de pastizales de Uruguay por *Cynodon dactylon*

*Cynodon dactylon*, de procedencia africana y europea, es considerada una de las cinco especies invasoras más importantes en todo el mundo (Harlan y De Wet, 1969; Holm et al., 1977). Fue introducida intencionalmente en América del Sur para controlar la erosión en las ferrovías. Se trata de una gramínea estival de metabolismo fotosintético $C_4$. Si bien tiene bajo éxito de propagación reproductivo y un largo período de implantación y emergencia, presenta una exitosa propagación clonal a través de rizomas y estolones (Dong y De Kron, 1994; Fernández, 2003), así como también órganos de reserva que aumentan su supervivencia (Horowitz, 1996; Moreira, 1975; Taliaferro et al., 2004). Se ha demostrado que presenta una gran habilidad competitiva por nutrientes (Horowitz, 1996) y que las restricciones por luz no afectan su capacidad de dispersarse vegetativamente (Guglielmini y Satorre, 2004). Se trata

de una especie capaz de formar interacciones micorrícicas arbusculares, aunque con menor presencia fúngica en sus raíces que las especies nativas y con una presencia muy baja de estructuras de intercambio (Endresz et al., 2013; Michelini et al., 2024; Wu et al., 2010). Por otro lado, se ha documentado que produce compuestos alelopáticos capaces de inhibir el crecimiento de otras plantas (Golparvar et al., 2015; Guido et al., 2020; Petrova et al., 2015). Estos atributos le confieren sin duda una enorme habilidad para competir y establecerse en nuevos ambientes, lo que le otorga un gran potencial como especie invasora.

*Cynodon dactylon* es la especie invasora con mayor área de ocupación de Uruguay (Bresciano et al., 2014; Guido et al., 2024; Ríos, 1999). Se observa en pastizales con signos de degradación de las propiedades físicas del suelo (Millot et al., 1987; Panario, 1994) y sobrepastoreados (Altesor et al., 1998; Millot et al., 1987), dado que presenta pobre calidad forrajera y es poco apetecida por el ganado (Ríos, 1999; Rosengurtt, 1979). Recientemente se reportó que en pastizales naturales esta especie invasora está positivamente asociada a posiciones topográficas altas del paisaje, posiblemente vinculadas a los descansos del ganado (dormideros), donde existe mayor grado de perturbación (Guido et al., 2024).

Aunque *C. dactylon* es indiscutiblemente una especie invasora de gran relevancia y su presencia resulta muy conspicua en nuestros pastizales, los mecanismos que explican su invasión en Uruguay —y en particular en los PN + LP— aún no están claramente comprendidos. A campo, *Cynodon dactylon* invade principalmente en sistemas enriquecidos, pero allí co-ocurren múltiples factores —fertilización, disturbio, pastoreo selectivo— que dificultan identificar mecanismos. Dado que se ha reportado una alta habilidad compettiva por nutrientes en *C. dactylon*, estudiar el desempeño de las plantas invasora y nativas en un escenario de limitación por nutrientes, permite aislar la competencia por nutrientes y evaluar si la limitación natural del pastizal constituye una barrera competitiva para *C. datylon*. Es decir, el diseño apunta a entender la resistencia del sistema, independientemente de las condiciones ya perturbadas. Por un lado, su éxito podría vincularse con atributos propios de la especie, tal como su habilidad competitiva por nutrientes (Horowitz, 1996), la producción de aleloquímicos Bresciano et al., 2022; Golparvar et al., 2015;

Guido et al., 2020; Petrova et al., 2015) o su capacidad de alterar mutualismos micorrícicos arbusculares de nuestros pastizales. Por otro lado, su invasión podría estar asociada a características vinculadas a la invasibilidad de los PN + LP, como el aumento de disponibilidad de recursos que ocurre de forma anual durante la fertilización, lo que causa una disminución de la resistencia biótica de las comunidades nativas.

A pesar de la marcada presencia de *C. dactylon* en los pastizales del Uruguay, aún persisten vacíos en el conocimiento sobre los mecanismos que favorecen su establecimiento y expansión, así como sobre las condiciones de mayor vulnerabilidad del sistema. En esta tesis se exploraron diferentes estrategias de la invasora para establecerse en los pastizales de Uruguay y busca aportar evidencia clave para comprender la dinámica de su invasión. De este modo, se generan insumos valiosos tanto para el manejo sustentable del pastizal natural como para el diseño de estrategias de prevención y mitigación que contribuyan a conservar la biodiversidad y funcionalidad de estos ecosistemas.

### 1.5. Objetivos

El objetivo general de esta tesis fue estudiar el papel de las interacciones bióticas, en particular la competencia y el mutualismo micorrícico arbuscular, entre la especie invasora *Cynodon dactylon* y gramíneas nativas de Uruguay.

Los objetivos específicos fueron los siguientes:

1. Evaluar la habilidad competitiva de *Cynodon dactylon*, en situaciones de limitación por nutrientes del suelo, ante dos gramíneas nativas muy frecuentes de los pastizales de Uruguay: *Paspalum notatum* y *Mnesithea selloana*.

2. Estudiar la habilidad competitiva de *Cynodon dactylon* y una gramínea nativa dominante del pastizal natural, *P. notatum,* en altos y bajos niveles de nutrientes.

3. Explorar la importancia relativa de la alelopatía en las interacciones bióticas entre la especie invasora *C. dactylon* y especies nativas bajo diferentes niveles de disponibilidad de nutrientes.

4. Explorar el rol de los patrones de asignación de biomasa en la habilidad competitiva de *Cynodon dactylon*.

5. Analizar si existe una asociación negativa entre la invasión por *C. dactylon* y la presencia de micorrizas arbusculares en raíces de la gramínea nativa *P. notatum* como posibles mecanismos de invasión.

A modo de hipótesis, planteamos que la invasión de *Cynodon dactylon* en pastizales de Uruguay resultaría de la interacción entre disturbios ligados a la intensificación productiva, que reducen la resistencia biótica y liberan recursos, y mecanismos bióticos como la alta captación de recursos, la interferencia química y la degradación de mutualismos micorrícicos.

### 1.6. Estrategia de investigación

La tesis se estructuró en tres etapas que permitieron abordar, desde distintos enfoques experimentales, los mecanismos que explican el éxito de invasión de *Cynodon dactylon* en pastizales de Uruguay. En una primera etapa, teniendo en cuenta las condiciones de limitación por nutrientes de los pastizales naturales se evaluó la competencia por nutrientes entre *C. dactylon* y dos especies de gramíneas nativas frecuentes en los pastizales uruguayos (*P. notatum* y *M. selloana*). Para ello, se diseñó un experimento en macetas con el objetivo de cuantificar la habilidad competitiva entre las especies en condiciones de baja disponibilidad de nutrientes. En la segunda etapa, se exploraron mecanismos específicos que podrían contribuir a la habilidad competitiva de *C. dactylon*, particularmente aquellos relacionados con la adquisición rápida de nutrientes y la alelopatía. Se llevó a cabo un experimento de interacción planta-planta en macetas en el que se seleccionó como especie nativa a *P. notatum*, dada su alta frecuencia en los pastizales y su mayor tolerancia a la competencia en comparación a *M. selloana*, observada en la etapa anterior (capítulo 2). Este experimento incluyó tratamientos con diferentes niveles de disponibilidad de nutrientes y una segunda aplicación cuando las plantas ya estaban establecidas, con el fin de simular condiciones de incrementos de nutrientes característicos en los pastizales sujetos a fertilización. Además, se incorporaron tratamientos con neutralización de compuestos alelopáticos para evaluar la importancia relativa de la

alelopatía en las interacciones interespecíficas. Finalmente, en una tercera etapa, se investigó si el nivel de invasión por *C. dactylon* se asociaba a una menor colonización micorrícica arbuscular, tal como propone la hipótesis del mutualismo degradado. Esta evaluación se realizó mediante el muestreo de monolitos de vegetación en pastizales naturales y en pastizales sujetos a un manejo más intensivo.

### 1.7. Estructura general de la tesis

Esta tesis se presenta en el formato de compendio de artículos científicos y se organiza en cinco capítulos que consisten en una introducción general, tres capítulos en formato de artículos y una discusión general.

Capítulo 1. Introducción general. Este capítulo contextualiza el problema de investigación, planteando la situación de invasión de los pastizales de Uruguay por *Cynodon dactylon* y exponiendo los antecedentes sobre los mecanismos que explican el éxito de su invasión. Se plantean los objetivos generales y específicos de la tesis y el marco conceptual que guía la investigación.

Capítulo 2. Artículo publicado. García, S., Guido, A., Pezzani, F. y Lattanzi, F. A. (2023). Invasion strategies of *Cynodon dactylon*: Competitive ability under low-nutrient conditions. *Austral Ecology*, *48*(6), 1107-1120. https://doi.org/10.1111/aec.13341

Capítulo 3. Este capítulo presenta un artículo adaptado al formato de la revista *Plant and Soil*, en proceso para su envío. García, S., Guido, A., Pezzani, F. y Lattanzi, F. A. (en preparación). Disentangling the invasion strategies of *Cynodon dactylon*: The role of resource competition and allelopathy under contrasting nutrient scenarios.

Capítulo 4. Artículo publicado. García, S., Pezzani, F., Guido, A. y Lattanzi, F. A. (2025). Degradation of arbuscular mycorrhizal symbiosis: A mechanism underlying *Cynodon dactylon* invasion in grasslands? *Journal of Vegetation Science*, *36*(1), e70010. https://doi.org/10.1111/jvs.70010

Capítulo 5. Discusión general. En este capítulo se integran los principales hallazgos de los estudios presentados y se discute su contribución al conocimiento de los mecanismos de invasión en pastizales subtropicales y templados. Se reflexiona

sobre las implicancias ecológicas y de manejo derivadas de los resultados y se plantean líneas futuras de investigació

## 2. Invasion strategies of *Cynodon dactylon*: Competitive ability under low-nutrient conditions[1]

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### 2.1. Resumen

*Cynodon dactylon* es una de las cinco especies exóticas invasoras más importantes a nivel mundial. Es la especie invasora con mayor rango de distribución en Uruguay, y su expansión se asocia frecuentemente a disturbios. Dado que los pastizales naturales enfrentan procesos de intensificación productiva, *C. dactylon* representa una amenaza, ya que podría desplazar a especies nativas. Sin embargo, los mecanismos que explican su éxito invasor aún no están claros. El objetivo de este estudio fue analizar las interacciones interespecíficas bajo condiciones de baja disponibilidad de nutrientes entre *C. dactylon* y dos especies nativas de los pastizales del Campo uruguayo. Específicamente, se evaluaron diferencias en los componentes de la habilidad competitiva, tanto en sus efectos como en sus respuestas (o tolerancia), como posibles mecanismos involucrados en la invasividad de *C. dactylon*. Se realizó un experimento en invernáculo, en macetas con sustrato de bajo contenido nutricional, evaluando interacciones por pares entre *C. dactylon*, *Mnesithea selloana* y *Paspalum notatum*, además de macetas control con individuos aislados de cada especie. La

especie invasora mostró una mayor habilidad competitiva que ambas gramíneas nativas, ya que redujo su biomasa aérea y subterránea. Por el contrario, el tamaño de las plantas de *C. dactylon* que interactuaban con especies nativas fue similar al de plantas creciendo solas (controles). Esto revela que la mayor habilidad competitiva de la especie invasora se debió a una mayor tolerancia a crecer en presencia de plantas vecinas. La causa subyacente de esta tolerancia fue un marcado aumento en la asignación de biomasa hacia estolones y hojas, en detrimento de las raíces. En contraste, las especies nativas apenas modificaron su patrón de asignación aérea-radicular al interactuar con vecinas. Además, *C. dactylon* indujo desarrollo reproductivo únicamente cuando interactuó con plantas vecinas. Junto con el hecho de que la tasa potencial de crecimiento de la especie invasora y las nativas fue bastante similar, estos resultados sugieren que respuestas de evitación de sombra, sensibles y rápidamente activadas, podrían ser uno de los mecanismos involucrados en el éxito invasor de *C. dactylon*.

Palabras clave: invasiones biológicas, capacidad competitiva, *Paspalum notatum*, *Mnesithea selloana*, pastizales de los Campos uruguayos.

## 2.2. Abstract

*Cynodon dactylon* is one of the five most important invasive alien species worldwide. It is the invasive alien species with the broadest distribution range in Uruguay, and its expansion is frequently associated with disturbances. Since natural grasslands are facing processes of productive intensification, *C. dactylon* represents a threat as it could displace native species. However, the mechanisms that explain its invasion success remain unclear. The objective of this study was to analyse interspecific interactions under low nutrient conditions between *C. dactylon* and two species that are native to the Campo grasslands in Uruguay. Specifically, we assessed differences in the components of competitive ability effects and responses (or tolerance) as possible mechanisms involved in *C. dactylon* invasiveness. We performed a greenhouse experiment in pots with low- nutrient substrate assessing pair-wise interactions between *C. dactylon*, *Mnesithea selloana* and *Paspalum notatum* plus control pots consisting of single individual of each species. The invasive species

showed greater competitive ability than both native grasses, as it reduced their below and above- biomass. Conversely, the size of *C. dactylon* plants interacting with native species was similar to that of single *C. dactylon* plants growing alone (controls). This reveals that the greater competitive ability of the invasive species was due to a greater tolerance to grow with neighboring plants. The reason underlying this tolerance was a marked increase in biomass allocation towards stolons and leaves, at the expense of roots. Conversely, native species barely changed their shoot- root allocation pattern when interacting with neighbors. Furthermore, *C. dactylon* induced reproductive development solely when interacting with neighbors. Along with the fact that the potential growth rate of the invasive and native species was quite similar, these results suggest that sensitive and rapidly triggered shade avoidance responses could be one mechanism involved in the invasion success of *C. dactylon*.

Keywords: biological invasions, competitive ability, *Mnesithea selloana*, *Paspalum notatum*, Uruguayan campo grasslands

## 2.3. Introduction

Invasive plant species impact terrestrial ecosystems by reducing their biodiversity, changing nutrient pools and altering disturbance regimes (Reichmann et al., 2016), with potentially significant changes in the functioning of, and the services provided by, natural ecosystems (Mack et al., 2000; Pysek et al., 2012; Vilà et al., 2011). *Cynodon dactylon* is one of the five most important invasive alien species worldwide, colonizing different types of ecosystems around the world (Holm et al., 1977). It is a perennial, prostrate, fine- leaved and warm season grass, which is native to Africa and Europe. It extends throughout tropical and subtropical areas, well-established into temperate climates and coastal zones (Harlan and de Wet, 1969; Holm et al., 1977). In Uruguay, as in many places worldwide (Holm et al., 1977), *C. dactylon* is the most frequent and extended invasive alien plant species (Bresciano et al., 2014). In agroecosystems dominated by crop - pasture sequences, *C. dactylon* expands during the pasture phase but it is effectively controlled with herbicides during the phase with glyphosate- resistant crops. Currently, *C. dactylon* is also becoming a frequent problem in agroecosystems dominated by native grasslands, particularly when overgrazed

(Altesor et al., 1998, 2005) or overseeded with legumes and fertilized with phosphorus (P; Jaurena et al., 2015; Pañella et al., 2022).

Campo grasslands, part of the Río de la Plata grasslands and occupying more than 60% of Uruguay (Baeza and Paruelo, 2020; MGAP, 2011), are highly biodiverse, with more than 3000 species of temperate and subtropical plants (Andrade et al., 2018; Bilenca and Minarro, 2004). In Uruguay, five physiognomic types of Campo grassland communities are distinguished based on the density and height of the vegetation, but in all cases, it is a heterogeneous mosaic composed of warm season shortgrasses and to a lesser extent of tall grasses warm season shortgrasses (Lezama et al., 2019). *C. dactylon* invasions easily trigger almost irreversible processes of degradation that end up in monospecific stands (e.g. Altesor et al., 2019; Jaurena et al., 2015; Pañella et al., 2022). Undisturbed Campo grasslands still show low *C. dactylon* incidence (Bresciano et al., 2014; Lezama et al., 2019; Pañella et al., 2022), but its presence is increasing under the current context of intensification of livestock production (Pañella et al., 2022), threatening its productivity and resilience which are both based on the high biodiversity of this agroecosystem (Jaurena et al., 2021). Furthermore, since it does not exist as a selective herbicide against *C. dactylon*, its chemical control in native grassland would also affect non- target native vegetation.

What underlies the success of invasive alien species is not always clear and often depends on the specific invasive species and invaded com- munity considered (Vilà et al., 2011; Vilà and Ibañez, 2011), but their ability to out- compete native species from the recipient community is relevant, particularly during early invasion stages (Gioria and Osborne, 2014; Vilà and Weiner, 2004). Two components define competitive ability: a 'competitive effect'— that is the capacity of an individual to acquire resources at rates high enough to reduce its availability to neighboring plants— and a 'competitive response'— that is its capacity to perform with low resources (Goldberg, 1990; Goldberg et al., 1999). Successful invasive alien species have been shown to exhibit traits that allow higher rates of resource acquisition than native species (Callaway and Aschehoug, 2000; Pysek and Richardson, 2007; van Kleunen, Dawson, et al., 2010; van Kleunen, Weber, et al., 2010), as well as the ability to tolerate better the lower availability of resources due to competition (e.g. Craine et al., 2005; Funk,

2013; Weiner, 1993). However, the specific relevance of each component in a given situation depends on the availability of resources (Gioria and Osborne, 2014). For instance, invasive alien plants have been found to be better at acquiring nutrients than natives in nutrient- enriched environments (Burke and Grime, 1996; Harrison, 1999; Heunneke et al., 1990; Liu and van Kleunen, 2017) and to tolerate better low- nutrient availability in ecosystems characterized by their scarcity (Funk, 2013; Groves et al., 2003).

Plant invasions are explained by many factors that operate at different scales in the successive stages of the invasions process: transport, colonization, establishment and landscape spread (Theoharides and Dukes, 2007). In regards to *C. dactylon* in grasslands, the mechanisms that explain its great success at the different stages of the invasion process remain unclear and little studied. Ultimately, the competitive ability of a species results from traits that determine how plants acquire and use resources, and how it interacts with neighbors. *C. dactylon* can spread rapidly by stolons and rhizomes (Moreira, 1975; Taliaferro et al., 2004), which could confer a high ability to compete for nutrients and light in open ecosystems, as stolons enable it to forage for light while rhizomes serve as organs for storage of resources and as a bud bank (Dong and de Kroon, 1994). Little is known about the role of biomass allocation to leaves, stolons, rhizomes and roots play in *C. dactylon* in competitive interactions with native species, during the invasion process. In grasses, Reichmann et al. (2016) showed that greater allocation to roots in native species can constrain their relative growth rate (RGR), perhaps due to the trade- off between allocation to roots and specific leaf are (SLA; Reich, 2014). Indeed, alien invasive species are often thought to have inherently higher growth rates than co- occurring native species (Arredondo et al., 1998; James and Drenovsky, 2007).

Traits providing competitive ability may be modulated by convergence or divergence in plant physiology and morphology between invasive and native species. Alternative hypotheses have been put forward about mechanisms of invasion and community resistance that differ in how functional traits are expected to vary in native vs. invasive species (Drenovsky et al., 2012, van Kleunen, Dawson, et al., 2010, van Kleunen, Weber, and Fischer, 2010). On one hand, ‘habitat filtering and neutral

processes hypotheses' propose that invasive and dominant native species share similar functional traits, which would confer a pre- adaptation in being introduced to new environments (Duncan and Williams, 2002). On the other hand, 'limit similarity' hypotheses (Catford et al., 2009) predict that invasive and native plants should differ in morphological and physiological traits to coexist (Funk, 2008).

The objective of this study was to analyse the interspecific interactions between *C. dactylon* and two native species under low-nutrient conditions, as a possible mechanism for its invasion success in grasslands. For this, we evaluated the competitive ability of *C. dactylon*, *Paspalum notatum* and *Mnesithea selloana* against each other when grown in a low- nutrient substrate. *Paspalum notatum* and *M. selloana* are perennial $C_4$ grasses of high forage value that are native and frequent and/or dominant in Campo grasslands of Uruguay (Pizarro, 2000; Rosengurtt, 1979). Specifically, we aim to answer whether, compared to these two natives, *C. dactylon* has (i) a greater competitive effect, (ii) greater tolerance to plant interaction and (iii) what role does above- vs. below- ground allocation of biomass play in regard to the competitive ability of the invasive alien species.

## 2.4. Methods

### 2.4.1. Experimetal design

We performed an experiment using pair- wise interactions between *C. dactylon* (Invasive), *M. selloana* (Native 1) and *P. notatum* (Native 2) growing in a greenhouse from the Instituto Nacional de Investigación Agropecuaria (INIA, Treinta y Tres) over one growing season (180 days). Six treatments resulted from the combination of one individual per species: (i, ii and iii) were the controls (each species alone without

neighbor), (iv) *M. selloana* + *P. notatum* (Native 1 + Native 2), (v) *M. selloana* + *C. dactylon* (Native 1 + Invasive) and (vi) *P. notatum* + *C. dactylon* (Native 2 + Invasive). Four replicates (i.e. pots) per treatment were arranged in a completely randomized design. As a result, the experiment involved a total of 24 pots.

2.4.2. Experiment management

Pots (15 cm diameter, 15 cm height) were filled with a nutrient- low substrate obtained by mixing sand with white peat (2:1 volume ratio), that had 2.9% organic carbon, 0.09% total N. The C:N ratio was greater than 30, 1.7 ppm of plant available P (Bray) and less than 20 ppm of plant available K. These nutrient levels are lower than that of natural grasslands soils in Uruguay, which are typically greater than 0.20% total N, C:N ratios of 10– 11, 4 ppm P Bray, and 150 ppm plant available K (Altamirano et al., 1976). In November 2018, at the beginning of the growing season of $C_4$ species (mid- spring), four seeds of each species were sown in each pot. We used commercial seeds of *P. notatum* cv INIA Sepé, a cultivar developed from a local ecotype, and commercial seeds of *C. dactylon*. For M. selloana, we collected seeds from Campo grasslands in Treinta y Tres department located in the east of Uruguay, in September and October 2018. One month later, pots were thinned to either one seedling per pot in controls (treatments i– iii), or to one seedling of each species in interaction (treatments iv– vi). Irrigation was automated, and pots were randomly rotated weekly inside the greenhouse so that light, water and nutrient availability were the same for all pots during the experiment.

2.4.3. Measurements and calculations

After 6 months, in May 2019, at the end of the growing season of $C_4$ species (late autumn), we recorded the number of inflorescences in each plant and then we harvested all individuals. Each plant was separated into above (shoot, stolons, leaves and inflorescences) and below- ground organs (roots and rhizomes). Samples were dried at 60°C until constant weight (48 to 72 h with forced air) and then weighed. Shoot N concentration was measured by Kjeldhal (Sommers and Nelson, 1972) but due to small sample sizes, all replicates of each treatment had to be pooled. The relative interaction intensity index (RII) was used to evaluate the result of each interaction treatment for each target species (Armas et al., 2004). As it quantifies the proportional change in plant biomass due to interaction, it allows the comparison of the interaction effect between interacting species. Thus, for each target species, RII was estimated from total biomass at the end of the experiment as

$$RII = (Bc - Bo)/(Bc + B0)$$

where Bc is the total biomass of the target plant when competing with a neighbour (for treatments iv– vi), and Bo is the biomass of the target plant without competition (the average biomass of the corresponding species in treatment i). Thus, RII ranges from −1 to 1 and is symmetrical around zero; and more negative values indicate higher competition effect (Armas et al., 2004).

2.4.4. Statistical analyses

We compared plant biomass (above and belowground) and the number of inflorescences of each target species (i.e. *M. selloana*, *P. notatum* and *C. dactylon*) between neighboring interaction treatments using ANOVAs. Similarly, the RII values were compared between neighboring interaction treatments within and between each target species using ANOVAs, including neighbor species identity and target species identity as the factors in the model.

Biomass allocation to above- vs. below- ground organs of each target species was assessed through ANOVA of shoot: root ratios and via allometric relationships of the following general form:

$$log\ (MY) = log\ (\beta) + \alpha log\ (MX)$$

where MY and MX represent the biomass of organs Y and X, respectively, α reflects the RGR ratio between organs X and Y (Ledig and Perry, 1966; Poorter et al., 2012) and b is a scaling parameter. We compared these relationships between species by combining data from all treatments using model II linear regression (Falster et al., 2006).

## 2.5. Results

2.5.1. Nutrient deficiency

N concentration in shoots was very low in all species, with values between 0.46% and 0.80%, reflecting the limited nutrient availability of the substrate. An inverse relationship with shoot mass was apparent across species, which indicated more N concentration in small plants (Figure 1).

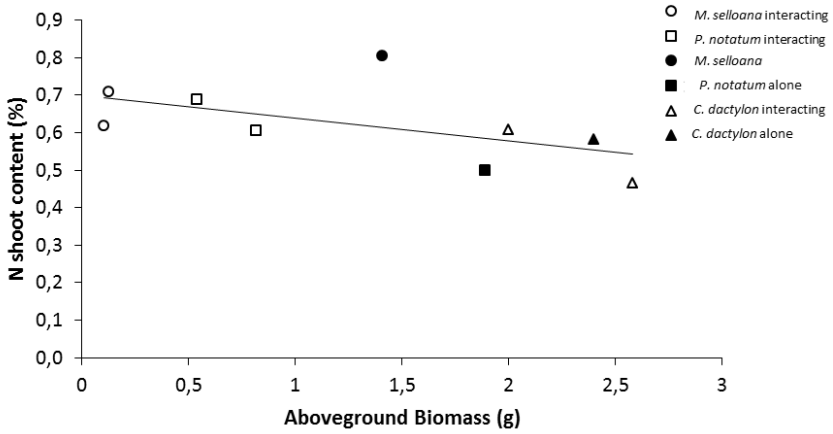


Figure 1 Relationship between N concentration in shoots and aboveground biomass of native grasses *Mnesithea selloana* (circles) and *Paspalum notatum* (squares) and the invasive species *Cynodon dactylon* (triangles), when grown alone (closed symbols) or interacting with other species (open symbols). Each data point is a composed sample obtained from the mean of four replications from each treatment.

2.5.2. Competitive ability: Competitive effects and competitive responses

The three species differed in their competitive ability. The relative interaction intensity index (RII) based on whole- plant biomass was always lower than zero for both native grasses (Figure 2), indicating *M. selloana* and *P. notatum* were negatively affected by neighboring species (neighbor effect). However, there were no differences in the identity of the neighbor, as we observed similar effects when the neighbor was the invasive *C. dactylon* or one of the natives (Figure 2). *M. selloana* (Native 1) was negatively and equally affected when interacting with either *C. dactylon* (Invasive) or *P. notatum* (Native 2), as indicated by the similar RII values (−0.86). *Paspalum notatum* (Native2) was less affected than Native 1 when interacting with neighbors, and again these effects were similar when the neighbor was the *Invasive* or *Native 1* (Figure 2). Conversely, RII was not statistically different from zero for *C. dactylon* (Invasive) when interacting with either of both native species, which indicated no neighbor effect (Figure 2).

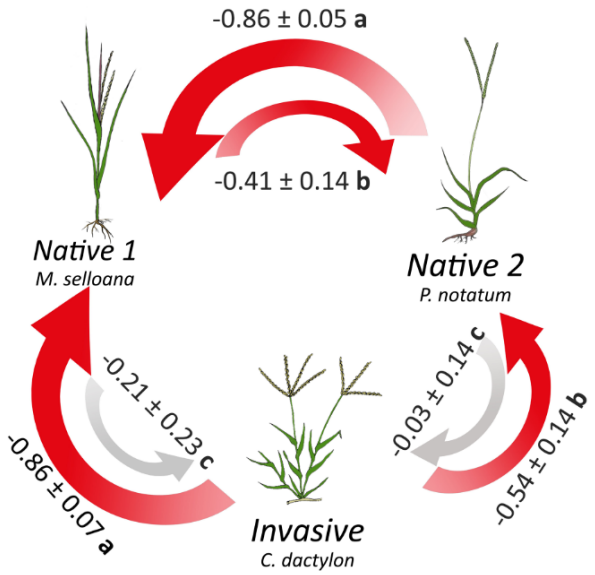


Figure 2 Effect of neighboring interaction treatments on the relative interaction intensity index (RII) for the three species: *Cynodon dactylon* (Invasive), *Mnesithea selloana* (Native 1) and *Paspalum notatum* (Native 2). The RII values (mean ± SD; n = 4) are based on the total plant biomass of individual plants at the end of the 6- month experimental period (Armas et al., 2004). Arrows (scaled to RII value) indicate RII that differs (red) or not from zero (grey). Different letters indicate statistically significant differences ($p < 0.05$) between different species, and for any given species, in the different treatments. Images: invasive (*C. dactylon*) and Native 2 (*P. notatum*), adapted from free vectors from Depositphotos and Shutterstock, respectively. Native 1 (*M. selloana*), own.

The negative impact of the invasive *C. dactylon* on native's biomass was due to effects on both above- and below- ground biomass. Likewise, neg- ative effects between the native species were also due to effects on both shoot and roots (Figure 3), since both native plant biomass fractions were reduced by plant interaction. For *C. dactylon*, none of the biomass fractions were affected by neighbor interaction (Figure 3). The biomass of *M. selloana* (Native 1) decreased by 90% when interacting with either *C. dactylon* (Invasive) or *P. notatum* (Native 2; Figure 3), whereas *P. notatum* showed a 70% biomass reduction when interacting with either Invasive or Native 1 (Figure 3). Notably, when grown alone (control) total biomass production was lowest for the Invasive (3.4 g plant−1; Figure 3a), intermediate for

M. selloana (4.0 g plant−1; Figure 3c) and had the highest values for P. notatum (6.0 g plant−1; Figure 3b). When comparing Invasive and both native grasses when grown alone (control), there were major differences in root biomass with greater root biomass in native plants; shoot biomass of control plants was similar for the three species (Figure 3).

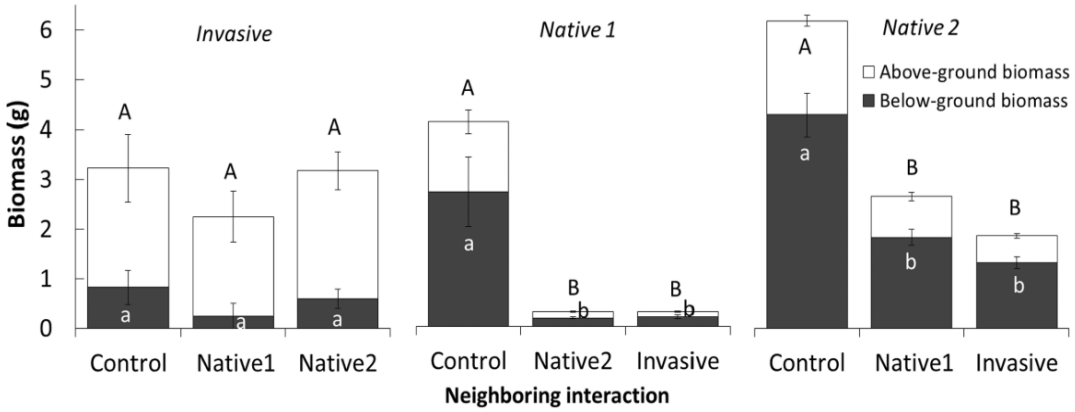


Figure 3. Above (light bars)— and below- ground (dark bars) biomass of Native 1 (*Mnesithea selloana*), Native 2 (*Paspalum notatum*) and invasive (*Cynodon dactylon*) species when grown alone (Control) or in neighboring interaction treatments. Different capital and lowercase letters indicate statistically significant differences ($p < 0.05$) in above and below- ground biomass, respectively, between neighbor treatments for each species. Bars indicate SE (n = 4).

2.5.3. Above- and belowground biomass allocation patterns

The invasive species had a higher allocation of biomass to aboveground organs compared to both natives (+0.48 vs. −0.34 shoot per unit root; $p = 0.006$). Furthermore, this preferential allocation to shoot was far less affected by neighboring interactions compared with native species (0.38 vs. 0.96 change in shoot per unit change in root; $p = 0.006$). Native species showed a similar pattern of biomass allocation (Figure 4; $p = 0.830$). Both native species allocated biomass to rhizomes at a constant proportion versus roots and this pattern did not change due to neighboring interaction treatments; meanwhile, *C. dactylon* did not produce rhizomes (Figure 5).

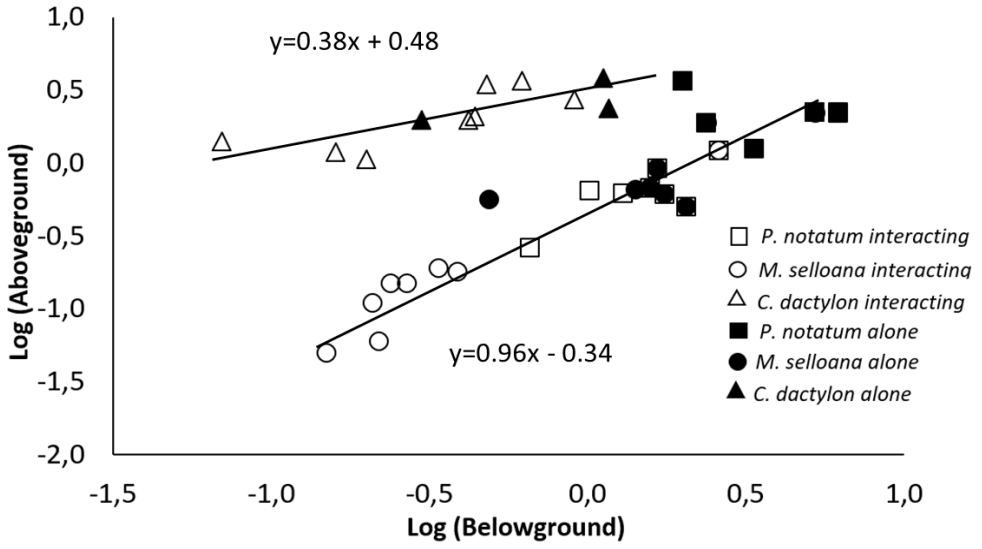


Figure 4. Allometric relationship between above-and belowground biomass of native species *Mnesithea selloana* (circles) and *Paspalum notatum* (squares) and the invasive species *Cynodon dactylon* (triangles), when grown alone (closed symbols, n = 4) or in neighboring interaction treatments (open symbols, n = 8). Each data point is a replicate**.**

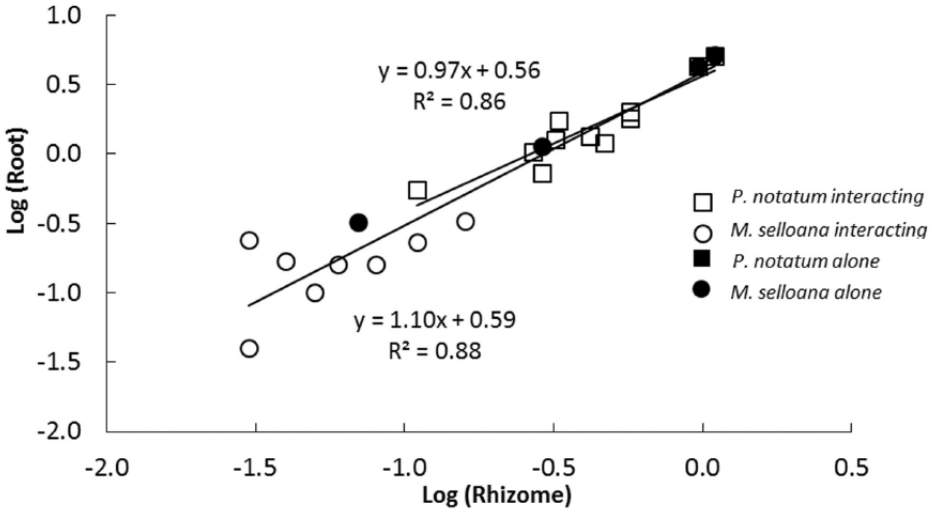


Figure 5. Allometric relationship between roots and rhizomes of native species *Mnesithea selloana* (circles) and *Paspalum notatum* (squares) when grown alone

(closed symbols, n = 4) or in neighboring interaction treatments (open symbols, n = 8). Each data point is a replicate.

2.5.4. Reproductive effort

After 6 months, *P. notatum* (Native 2) did not allocate energy to sexual reproduction (Table 1). For *M. selloana* (Native 1), the number of inflorescences was significantly reduced when interacting with *P. notatum* (Native 2) or the invasive *C. dactylon*. For *C. dactylon* the opposite response was observed, as the inflorescences developed only when interacting with either native species (Table 1).

Table 1. Mean number of inflorescences for each target species when grown alone (control) or when interacting with each neighboring species.

| **Target species** | **Neighboring Interaction** | | | |
|---|---|---|---|---|
| | Control | Invasive | Native 1 | Native 2 |
| Invasive | 0 a | - | 5.5 ± 1.84 b | 1.5 ± 0.86 ab |
| Native 1 | 4 ± 1.29 a | 0.25 ± 0.28 b | - | 0.5 ± 0.25 b |
| Native 2 | 0 | 0 | 0 | - |

*Note*: Invasive: *Cynodon dactylon*; Native 1: *Mnesithea selloana* and Native 2: *Paspalum notatum*. Different letters indicate statistically significant differences ($p < 0.05$) between neighboring interactions within target species ($n = 4 \pm SE$).

## 2.6. Discussion

The invasive alien species *C. dactylon* showed greater competitive ability than both native grasses. Nutrient availability was scarce in the present study, and this was reflected in plant N concentration (0.46%–0.8%), which was lower than sufficiency values for $C_4$ grasses (e.g. 2%–5% Greenwood et al., 1990; 2%–4% Mathews et al., 2016). The competitive ability of a species is explained by the combination of its effects on neighbor species and by its response to the conditions of lower resource available due to neighbors (Goldberg et al., 1999). In the present study, the invasive species negatively affected native plant biomass; however, this effect was similar to that observed in native-native interactions.

Rather, the greater competitive ability of *C. dactylon* emerged as a consequence of its capacity to tolerate the conditions created by the presence of natives, as it was not affected by any of the neighboring plants. The invasive alien plant preferentially

allocated biomass to aboveground organs (which included stolons), and when interacting with natives its allocation to roots decreased. Conversely, both natives had a greater allocation to belowground organs, which included storage organs (rhizomes), and did not modify this pattern when interacting with the invasive species.

The invasive grass showed negative effects on native species by decreasing their below-and above-biomass, as was evidenced by negative values of RII for both native grasses. However, competitive effects of the invasive species were not a relevant component explaining its greater competitive ability, because the inclusion of native-native treatment in our experimental design allowed us to show that the invasive effect was not greater than a co-occurring native. If an invasive alien species is competitively superior to co-occurring natives, we would expect a greater suppression of target species (greater effect of the invader) and more ability to tolerate competition with neighboring plants (weaker response of the invader; Guido et al., 2019; Vilà and Weiner, 2004). Effects in plant competition are explained by the capacity of an individual to acquire resources at rates high enough to reduce their availability to neighboring plants (Goldberg et al., 1999; Leishman et al., 2007). Traits associated with competitive effects such as the growth rate (GR) are strongly habitat-dependent and are most important in high-resource environments (Gioria and Osborne, 2014). In fact, we found that *C. dactylon* did not show intrinsically higher GR than the natives under low –nutrient availability, since total plant biomass growing alone (a proxy for GR in this experiment) was lower than that in native grasses.

Interaction with native species did not affect the plant biomass of *C. dactylon* (RII not statistically different from zero). This indicated a high tolerance to lower availability of resources resulting from competition with neighbors (Goldberg et al., 1999). Such an ability has been found to be relevant in explaining invasive plant success (Craine et al., 2005; Funk, 2013). Under poor resource environments, invasive alien species can display traits indicative of both resource acquisition and resource conservation strategies to tolerate neighboring interaction (Funk, 2013). This author examined the physiological and morphological traits of native and invasive species in low-resource environments and found that the main trait explaining the success of invasive plants was their efficiency of use of nutrients (EUN). Indeed, 12 out of 19

studies reported greater EUN in invasive than native species (Drenovsky et al., 2008; Firn et al., 2012; Funk and Vitousek, 2007; Godoy et al., 2011; Matzek, 2011). A greater EUN can be reached due to physiological mechanisms like the ability to resorb nutrients from senescing tissues or constructing cheaper tissues (Drenovsky et al., 2012). Although we did not analyse variables related to EUN, we suggest that traits explaining *C. dactylon* invasiveness may be related to resource conservation strategies such as EUN.

Another mechanism that could explain tolerance in plant competition is biomass allocation to different organs, as a strategy to cope with limited resources. In our experiment, *C. dactylon* allocated more biomass to aerial organs (including stolons) than both native species. Furthermore, preferential biomass allocation to shoots was actually exacerbated by interaction with native grasses. Conversely, above-and belowground biomass of native grasses were equally affected by the presence of neighbors. Our results about biomass allocation patterns suggested that invasive and native species showed divergence in this growth strategy. Previous studies show that there may exist convergence (Drenovsky et al., 2021) or divergence (Leishman et al., 2007) in traits between natives and invasive species. This is in line with hypothesis based on limit similarity, which predicts invasive and native plants should differ in their morphological and physiological traits to coexist, as biotic resistance mechanism in recipient community (Catford et al., 2009).

Considering that this experiment was carried out in low nutrient conditions, and that native grasses allocated more to belowground organs than *C. dactylon*, it would be expected that native species would cope better with nutrient competition. Yet, the invasive plant showed a superior competitive ability than native plants. This response suggests that other mechanisms than nutrient competition may be underlying plant-plant interaction. Instead, *C. dactylon* responses are consistent with shade avoidance syndrome. Previous studies have shown that this species is indeed highly sensitive to shading (Guglielmini and Satorre, 2002; Adamipour et al., 2016). It is well-known that when neighbor proximity changes light quality, plants activate responses leading to elongation growth and also to acceleration of flowering (Smith, 1995; Ballaré and Pierik, 2017). Taking into account that invasive grass did not show greater GR than

native, we suggest that in a stoloniferous species like *C. dactylon,* shade avoidance could be one of the mechanisms explaining the higher allocation to aboveground biomass when interacting with natives. Indeed, shade avoidance syndrome has already been mentioned as a putative reason underlying the success of invasive herbaceous plants (van Kleunnen et al*.,* 2011; Du et al., 2017) when invading native systems; however, a specific study should be performed to confirm that. Another factor explaining invasive success in our research may be allelopathy, since it has been reported that this invasive grass produces allelopathic compounds that would displace recipient species (Golparvar et al*.*, 2015; Favaretto et al*.,* 2018; Guido et al*.*, 2020; Bresciano et al*.*, 2022). For *M. selloana*, the number of inflorescences was significantly reduced due to plant interaction, while, *P. notatum* did not produce inflorescences, which could be due to the short term of the experiment. On the other hand, invasive grass flowered only when interacting with both native species, which could be another response related to shade avoidance syndrome in *C. dactylon* (Guglielmini and Satorre, 2002; Adamipour et al., 2016).

### 2.7. Conclusions

*C. dactylon*, an invasive alien plant worldwide, had a superior competitive ability than two co-occurring native grasses that are frequent in Uruguayan natural Campo grasslands. This was mostly explained by *C. dactylon* tolerance to the presence of neighbors under low nutrient conditions. The invasive grass allocated more biomass to above-ground organs than both native species, especially when interacting with them. Such a response, consistent with classic shade-avoidance syndrome, could be one of the mechanisms explaining greater tolerance in *C. dacylon* when interacting with natives. Furthermore, it did not produce belowground storage organs (rhizomes), as both native species did, and solely induced sexual reproduction when interacting with natives. These results suggest that the main driver underlying plant-plant interaction in this experiment may not be the ability to acquire nutrients, although higher nutrient use efficiency in the generation of aboveground tissue could have played a role as well.

Biomass allocation pattern observed in *C. dactylon* should be considered in management plans for its control. Since this invasive grass presented greater biomass allocation to above-ground parts, and lower to belowground organs, decreasing its aerial biomass would imply a limitation for its recovery. In this sense, management practices focused on removing *C. dactylon* above biomass, could be effective to control it.

Since our results suggest that *C. dactylon* success would not be explained by higher competitive effects mediated by nutrient acquisition, future research would need to focus on other mechanisms behind, such as allelopathy and traits related to nutrient use efficiency.

The present research focuses on plant-plant interactions, therefore scaling up inferences must be done with caution. In future studies it would be necessary to consider population or community variables, as well as to take into account direct and indirect interactions involving native and invasive species. On the other hand, the outcomes of biotic interactions are context dependent, so it would be crucial to expand research to different environmental conditions to assess the driven factors of this invasion process. In this sense, due to *C. dactylon* is invading disturbed Uruguayan grasslands, it is necessary to explore its competitive ability at high nutrient levels.

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## 3. Disentangling the invasion strategies of *Cynodon dactylon*: the role of resource competition and allelopathy under contrasting nutrient scenarios[2]

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### 3.1. Resumen

*Antecedentes*

*Cynodon dactylon* es una gramínea invasora de distribución mundial que amenaza los pastizales nativos de Uruguay. Aunque la eficiencia en el uso de los recursos y la alelopatía se han propuesto como mecanismos que explican las invasiones, su papel aún no está claro. Nuestro objetivo fue evaluar las interacciones competitivas entre *C. dactylon* y la gramínea nativa *Paspalum notatum*, bajo distintas condiciones de disponibilidad de nutrientes y carbón activado, para aislar los efectos de la competencia por nutrientes de los efectos alelopáticos. Además, se evaluó el papel de la asignación de biomasa para explicar los resultados competitivos entre especies.

*Métodos*

Se realizó un experimento en invernadero utilizando macetas, donde plantas individuales de *C. dactylon* y *P. notatum* se cultivaron solas o en mezclas durante una estación de crecimiento. Se aplicaron tres tratamientos de fertilización (sin



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fertilización, dosis simple y dosis doble de nitrógeno y fósforo) y dos condiciones de carbón activado (con y sin). La biomasa de las plantas se midió a los 120 días y el efecto de la competencia se evaluó mediante el índice de interacción relativo (RII). La asignación de biomasa se analizó mediante relaciones alométricas y también se registró el esfuerzo reproductivo.

*Resultados*

*P. notatum* fue negativamente afectada por *C. dactylon* en todas las situaciones evaluadas. En cambio, *C. dactylon* toleró la competencia. El carbón activado no modificó estos resultados. Esto sugiere que la alelopatía no sería el mecanismo más relevante en explicar la mayor habilidad competitiva de la invasora, aunque tampoco es posble descartar la presencia de alelopatía por ser nuestra metodología, una forma indirecta de evaluarla. La especie invasora asignó más biomasa a los órganos aéreos (estolones), mientras que la nativa invirtió más en estructuras subterráneas (rizomas). La floración de *C. dactylon* solo se observó bajo competencia y en presencia de carbón activado.

Conclusión

La capacidad competitiva de *C. dactylon* estaría basada en una estrategia de asignación de biomasa hacia estructuras de ocupación del espacio. Las herramientas de manejo que eviten la creación de espacios vacíos o que apunten a los estolones de *C. dactylon* podrían ser claves para impedir su colonización y minimizar su propagación, contribuyendo así a prevenir o reducir las invasiones en los pastizales.

*Palabras clave*: habilidad competitiva, carbón activado, *Paspalum notatum*, pastizales naturales, asignación de biomasa.

## 3.2. Abstract

*Background and Aims*

*Cynodon dactylon* is a globally invasive grass that threatens native grasslands in the grasslands of Southeastern South America. While resource use efficiency and allelopathy have been proposed as mechanisms underlying invasions, their actual roles remain unclear. This study aimed to evaluate the competitive interactions between *C.*



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*dactylon* and the native grass *Paspalum notatum* under varying nutrient conditions and in the presence or absence of activated carbon to isolate nutrient competition effects from allelopathic effects. Furthermore, it assessed the role of differences in growth rate vs. differences in biomass allocation in explaining species' competitive outcomes.

*Methods*

We conducted a greenhouse pot experiment, growing individual *C. dactylon* and *P. notatum* plants either alone or in mixtures, for a whole growth season. We employed three fertilization treatments (none, single, and double rate of nitrogen and phosphorous addition) and two activated carbon conditions (with/without). Plant mass was measured after 120 days, and competitive outcomes were evaluated using the Relative Interaction Index (RII). Biomass allocation was assessed through allometric analyses. Reproductive effort was recorded as well.

*Results*

*P. notatum* was negatively affected by *C. dactylon* (*i.e.* it had less biomass when grown in mixtures than alone), regardless of nutrient levels.In contrast, *Cynodon dactylon* exhibited greater tolerance to competition than the native grass, particularly under low nutrient conditions. Activated carbon did not alter these outcomes, suggesting that allelopathy would not be the primary factor driving the observed patterns. The invader invested more biomass in aboveground organs (stolons); the native, more in belowground rhizomes. Flowering in *C. dactylon* occurred only when in competition and with activated carbon.

*Conclusion*

The superior competitive ability of *C. dactylon* appears driven by its tolerance to biotic stress based on a strategy of biomass allocation towards space-occupation structures, rather than by allelopathy or an inherently higher growth rate. Management tools preventing the creation of empty spaces or targeting *C. dactylon* stolons may be key for thwart colonization and minimize propagation, respectively, and thus avert or reduce invasions in grasslands.

*Keywords*: plant invasion, competitive ability, activated carbon, *Paspalum notatum*, natural grasslands, biomass allocation



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## 3.3. Introduction

Plant invasions are a major threat to the structure and functioning of natural ecosystems (Mack et al. 2000; Parra-Tabla and Arceo-Gómez 2021). To identify mechanisms underlying plant invasions, studies have mainly focused on competition, suggesting that competitive superiority of invaders is a key factor (Levine et al. 2004). Competitive ability is explained by the capacity of a species to affect its neighbor. This may be due to the ability to acquire resources at rates high enough to reduce their availability for neighboring plants, and/or to tolerate the presence of a neighbor, explained mainly by its capacity to perform with less resources (Gioria and Osborne 2014; Goldberg 1990; Goldberg et al. 1999; Vilà and Weiner 2004). Invasive plants appear to use scarce resources more efficiently than native residents, while also being able to capitalize on increases in resource availability (Davis et al. 2000; Liu and van Kleunen 2017).

As proposed by the fluctuating resource hypothesis, the susceptibility of a plant community to invasion increases immediately after an increase in resource availability, due either to a decrease in the use of these resources by the resident community or an increase in their supply (Davis et al. 2000). In plant communities, soil resources play an important role mediating biotic interactions, particularly nitrogen, which is the most commonly limiting nutrient in many ecosystems (Chapin et al. 1990). In fact, several studies report that environments with high soil resource availability are more susceptible to invasion and have a greater richness of exotic species (Flores-Moreno et al. 2016; Huenneke et al. 1990; Parepa et al. 2013; Zhang et al. 2022). An ability to exploit fluctuating environmental conditions might be conferred by plastic changes in biomass allocation in plants (Hutchings and de Kroon 1994), which has been widely reported in invasive plants (Davidson et al. 2011; Funk 2008; Zhang et al. 2021).

In addition to physiological efficiency in resource utilization, allelopathy could also be a mechanism behind the success of plant invasions (Callaway and Ridenour 2004; Ridenour and Callaway 2001). It involves the production and release of secondary metabolites, such as phenolic compounds, terpenoids and alkaloids, that can be inhibitory to other plants and thus increase competitive ability (Cipollini et al. 2008;



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Zhang et al. 2021). According to the novel weapon hypothesis, compounds that are relatively ineffective against their adapted neighbors in the native range may be highly inhibitory to newly encountered plants in the invasive range (Callaway and Ridenour 2004). The study of allelopathy is challenging because different approaches present inherent limitations. Laboratory assays allow the detection of phytotoxic effects under controlled conditions; however, their low similarity to natural environments limits the extrapolation of results to field conditions. Greenhouse or common garden experiments represent a step toward more realistic systems, although they often fail to fully isolate allelopathy from confounding processes such as resource competition or changes in nutrient availability. Field studies, in turn, are essential for assessing the ecological relevance of these effects, but they also present limitations in unequivocally disentangling allelopathy from other biotic and abiotic factors that structure plant communities (da Silva et al. 2017). A strategy for determining the relative contribution of allelopathy to the outcome of biotic interactions is the use of activated carbon, which has the ability to adsorb many large organic compounds and thus neutralize allelopathic effects (Callaway and Ashehoug 2000; Callaway and Ridenour 2004). However, this approach presents important methodological limitations when confirming the presence of allelopathy. On the one hand, there is no certainty that all allelopathic compounds are effectively retained by activated carbon, nor that such retention occurs at ecologically relevant concentrations (Inderjit and Callaway 2003 in da Silva et al. 2017). In addition, activated carbon can alter soil physicochemical properties, including pH, water retention, and nutrient availability, potentially stimulating plant growth independently of allelochemical neutralization (Lau et al 2008). Therefore, results obtained using this approach should be interpreted with caution when inferring the presence of allelopathy.

*Cynodon dactylon* is among the most common invaders worldwide, colonizing different types of ecosystems (Holm et al. 1977). It is a perennial, prostrate, fine-leaved, warm season grass, native from Africa and Europe. It extends throughout tropical and subtropical areas and well into temperate climates and coastal zones (Harlan and de Wet 1969; Holm et al. 1977). *C. dactylon* exhibits successful clonal



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propagation through stolons, forgaging organs, and rhizomes, reserve organs and to a lesser extent, exploration, that increase its survival (Moreira 1975; Taliaferro et al. 2004). It is highly competitive for nutrients (García et al. 2023; Horowitz 1996), which could be due in part to its plastic morphology and biomass allocation (Dong and de Kroon 1994). Moreover, this species produces allelopathic compounds that can inhibit the growth and germination of other plant species (Golparvar et al. 2015), although evidence for the relevance of this mechanism under field conditions remains limited. (Bresciano et al. 2022; Guido et al. 2020).

In Uruguay, *C. dactylon* is the most frequent and extended invasive alien species in natural grasslands (Bresciano et al. 2014; Guido et al. 2024). It was positively associated with higher topographic positions at the landscape scale, areas where trampling and cattle deposition are concentrated and soil disturbance is consequently higher (Guido et al. 2024). The ability of *C. dactylon* to invade natural grasslands may be due to its greater competitive ability for nutrients than native grasses. In a previous study (García et al. 2023), we found that *C. dactylon* exhibited greater competitive ability than two native grasses when grown under low-nutrient conditions. This advantage was primarily explained by its capacity to tolerate the conditions created by the presence of the two native species and by its nutrient-use efficiency, rather than by its effects on neighboring plants. Its tolerance could be probably because this alien plant preferentially allocated biomass to aboveground organs. It is unclear whether the same pattern would emerge when nutrient availability is higher.

For instance, a scenario in which *C. dactylon* invasion has been particularly favoured is that of natural grasslands (NG) oversown with legumes and fertilized with P (NG+LP; Cáceres 2019; Jaurena et al. 2015; Pañella et al. 2022). This agronomic practice, carried out to increase forage productivity and nutritive value, represents a complex disturbance that involves chronically increased overall N and P availability, and seasonally contrasting grazing intensities. The mechanisms driving the successful invasion of *C. dactylon* on these more intensively managed grasslands are still not clear, but its competitive ability by nutrients as its capacity to take advantage of increased nutrients availability could be involved. In addition, allelopathy could



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further modify these resource-mediated biotic interactions between native and invasive plants.

### 3.4. Objectives

In the present study, we proposed to investigate the response to the competitive capacity of *Cynodon dactylon* and the native grass *Paspalum notatum* at varying nutrient levels, and the role of the allelopathic capacity of *C. dactylon* in altering such competition, using activated carbon. Additionally, we wanted to assess the response of the competitive capacity of this invasive plant to a further sudden increase in nutrient availability when plants are already established.

Specifically, we aim to answer whether:

i) Does *C. dactylon* show greater competitive ability than a native grass under different levels of nutrient availability?

ii) Which is the effect of a second dose of nutrients on the competitive ability of the native and the invader species when plants are already established?

iii) What is the relative importance of allelopathy in plant interaction outcomes under low nutrient conditions and under fertilization at sowing??

iv) Could patterns of biomass allocation explain differences in competitive ability between plant species?

A modo de hipótesis se plantea que la mayor habilidad competitiva de *Cynodon dactylon* frente a *Paspalum notatum* estaría modulada por la disponibilidad de nutrientes y por mecanismos de interferencia química. En particular, se espera que el aumento en los nutrientes favorezca a la habilidad competitiva de la especie invasora y a que su potencial alelopático contribuya a alterar la competencia interespecífica.

### 3.5. Methods

#### 3.5.1. Experimental design

To study biotic interactions between *Cynodon dactylon* (invasive) and *Paspalum notatum* (native), we performed an experiment in pots growing in a greenhouse, at the Instituto Nacional de Investigación Agropecuaria (INIA, Treinta y Tres,



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33°15'17.74"S, 54°25'38.67"O), over one growing season. An incomplete factorial experiment with 15 treatments was set up. The factor biotic interaction had two levels: species growing either alone or in interaction; nutrient availability had three levels: without fertilization (F-), with fertilization at sowing (F+), and with fertilization at sowing plus an additional fertilization when plants were already established (F++). Finally, to investigate the role of allelopathy in plant interactions, we added activated carbon (AC+) to retain allelochemicals to half of the pots with no fertilization (F-) and with fertilization at sowing (F+), and to all pots fertilized twice (F++). The 15 treatments were arranged in a completely randomized design with 4 replicates (Fig. 1).

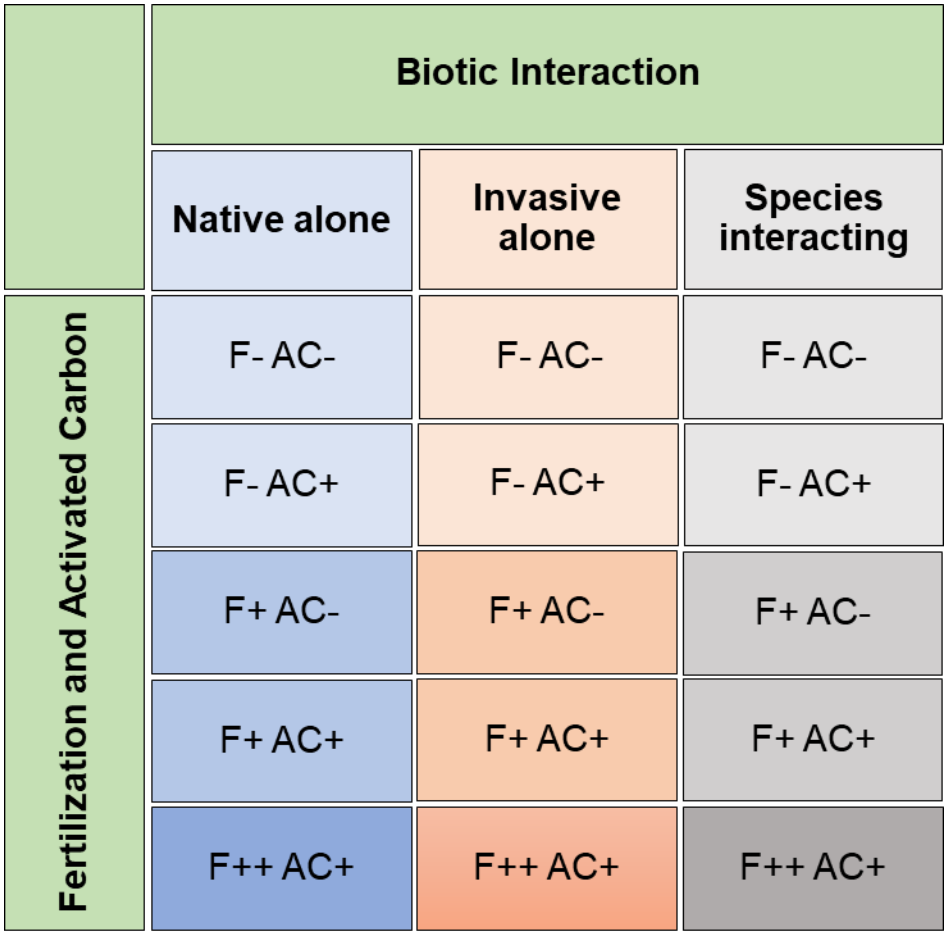


**Figure 1**. Experimental design. Native plant: *Paspalum notatum*, invasive plant: *Cynodon dactylon*, F-: no fertilization treatment, F+: fertilization at sowing, F++: double fertilization: at both sowing and at established phases (3 months later), AC- pots with no activated carbon, AC+: pots with activated carbon.



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3.5.2. Experiment management

The invasive *C. dactylon* was sown from commercial seed, while native *P. notatum* plants were obtained from tillers of similar size from a natural grassland community, as this species had a low germination rate. The initial weight of the tillers of *P. notatum* was recorded.

Pots (10 cm diameter, 14 cm height) were filled with a mix of commercial soil and sand (3:1 volume ratio). Activated carbon was added to pots corresponding to AC+ (2% w/w). Table 1 shows the nutrient content of the substrate employed.

**Table 1**. Nutrient content of the substrate employed with (AC+) and without (AC-) activated carbon.

| | Available N-NO3 (ppm) | Available P* (ppm) | pH | E.C. (µS) |
|---|---|---|---|---|
| AC+ | 19.55 | 115 | 7.18 | 426 |
| AC- | 54.26 | 69 | 7.56 | 392 |

*P Bray

In November 2019, at the beginning of the growing season of $C_4$ species in the mid-spring of the southern hemisphere, four tillers of *P. notatum* and four commercial seeds of *C. dactylon* were sown in each pot. One month later, pots were thinned to either one (species growing alone) or two seedlings per pot (plants interacting). Irrigation was automated, and pots were randomly rotated weekly inside the greenhouse to homogenize the conditions (e.g. light) between the pots. In fertilized treatments, pots were irrigated with nitrogen as urea (0.15 g/pot, equivalent to 60 kg N/ha) and phosphorous (0.13 g/pot, equivalent to 20 ppm of plant-available P). Three months later, in February 2020, we applied a second dose of nutrients to the F++ treatment, when the plants were already established.

3.5.3 Measurements and calculations

In March 2020, after 120 days of growth, the number of inflorescences, tillers and leaves in each plant was recorded. Then each plant was harvested and separated



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into above- (tillers, stolons, live and senescent leaves, and inflorescences) and below-ground organs (roots and rhizomes). Samples were dried at 60°C with forced air until constant weight (48 to 72 h) and then weighted. To estimate the growth rate, we subtracted the weight of the initial tiller from the total biomass of *P. notatum*.

To quantify the competitive ability of each species, the relative interaction intensity index (RII) was estimated using total biomass, and above- and belowground biomass (Armas et al. 2004) as

$$RII = (Bc - Bo) / (Bc + Bo)$$

where Bc is the total, above or belowground biomass of the target plant when competing with a neighbor, and Bo is the average of the total, above or belowground biomass of the target plant in the treatments without interaction. Thus, RII ranges from −1 to 1, and is symmetrical around zero. The RII quantifies the proportional change in plant biomass due to interaction, and thus it allows the comparison of the interaction effect between species. More negative values indicate higher competition effect (Armas et al. 2004). We estimated whether RII values differed from zero by estimating the 95% confidence intervals. If the limits did not include zero, we concluded the RII mean was significantly different from zero.

3.5.4. Statistical analyses

Because the experimental design was an incomplete factorial, statistical analyses were conducted in two separate steps. First, the effect of the nutrient gradient was evaluated using only pots with activated carbon (AC+). Second, the effects of fertilization and activated carbon were assessed using a subset of treatments that included pots without nutrient addition and pots receiving a single fertilizer dose. The relative interaction intensity index (RII), considering total biomass, aboveground biomass and belowground biomass of each species, was analyzed with ANOVA. In the first analysis, nutrient level and target species was included as the fixed effects, whereas in the second analysis the fixed factors were target species, nutrient level, the presence of activated carbon, and the interaction between these factors.

The number of inflorescences for each target species (P. notatum and C. dactylon) was analyzed with Generalized linear models (GLM), using a Poisson error



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distribution, as no overdispersion was detected. In the first analysis, nutrient level was included as the fixed effects, whereas in the second analysis the fixed factors were nutrient level, the presence of activated carbon, and the interaction between these factors.

Whenever significant differences were found, Least Significance Difference (LSD) was used as *a posteriori* test.

The allocation of biomass between aboveground and belowground structures, and between different organs, was assessed using allometric relationships fitted *via* the standardized major axis (SMA) regression, using the following general form:

$$\log(MY) = \log(\beta) + \alpha \log(MX)$$

where MY and MX represent the biomass of Y and X structures or organs, α reflects the RGR ratio between organs X and Y (Ledig and Perry, 1966; Poorter et al. 2012), and β is a scaling parameter.

We compared these relationships between species, fertilization, activated carbon and its interactions using Model II linear regression (Falster et al. 2006). The confidence intervals (95%) of the slope (α) and y-intercept (log β), calculation of common slopes, and test for homogeneity of slopes were determined by the software Standardised Major Axis Tests and Routines Version 2.0.



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## 3.6. Results

### 3.6.1. Competitive ability in native and invasive grasses

Overall, the invasive species *Cynodon dactylon* and the native *Paspalum notatum* differed in their competitive ability. All RII values estimated (considering total, aboveground or belowground biomass) differed between plant species, with more negative values in the native than in the invasive species (Fig 2). Therefore, the native grass was negatively affected when interacting with the invasive species in all evaluated situations. Conversely, the invasive grass showed greater tolerance to plant interaction in all treatments.

According to the first analysis (analysis I), regardless of the biomass fraction considered, RII values in the native grass were lesser than zero in all situations evaluated, ranging from -0.67 to -0.75, and similar between fertilization treatments (Figs. 2a, c and e; p=0.329, p=0.098, p=0.411). For RII values based on aboveground biomass, the effect of fertilization depended on the species considered (interaction target species*fertilization; p=0.007). For the invasive grass, RII was slightly lesser than zero under fertilized conditions, reaching values of about -0.2 (Fig. 2d). On the other hand, with no fertilization, this species was unaffected by biotic interactions and had a RII similar to zero.

The second analysis (analysis II) showed that activated carbon had no significant effects (p = 0.329, p = 0.329, p = 0.098, p = 0.411) on any of the estimated RII values. Likewise, fertilization treatments did not alter the RII of the native grass (Fig.2 b, d and f).



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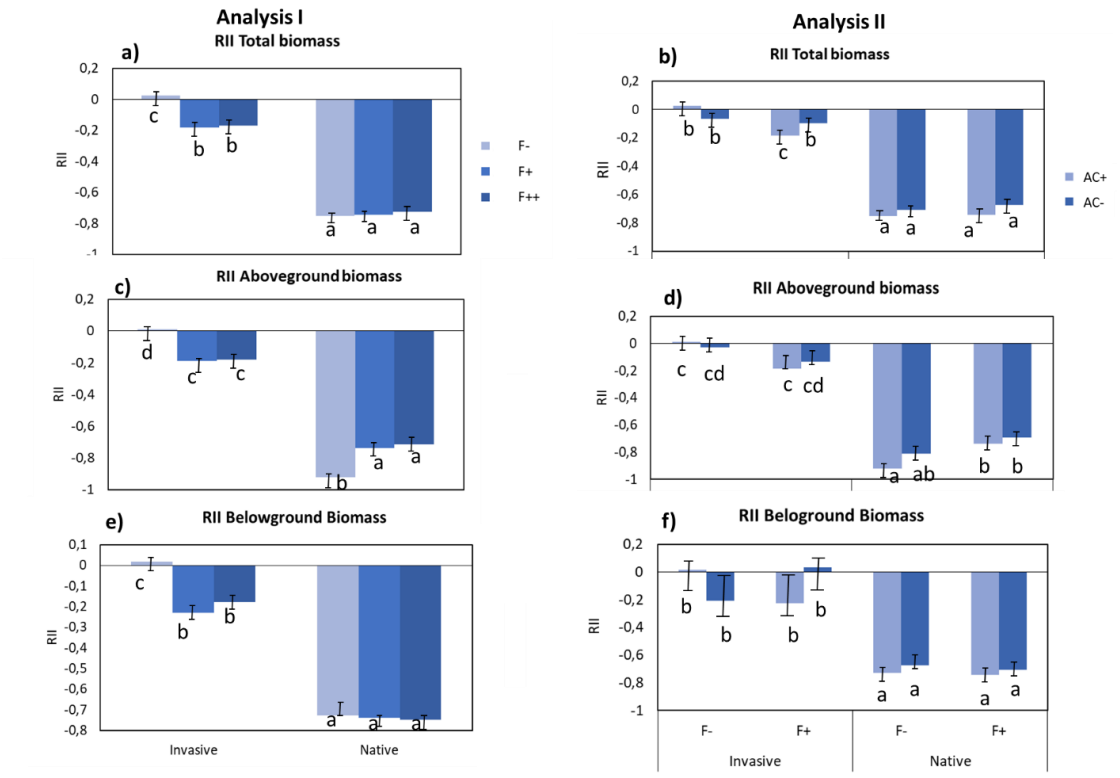


Figure 2. Relative Interaction Intensity index (RII) considering total biomass (a-b), aboveground biomass (c-d) and belowground biomass (e-f) in native (P. notatum) and invasive (C. dactylon) grasses under different levels of fertilization (F-, F+ and F++; analysis I) and fertilization and activated carbon (F- or F+ and AC+ or AC-; analysis II). The RII values (mean ± SD; n = 4) are based on plant biomass of individual plants at the end of the 120-days experimental period. Different letters indicate statistically significant differences according to LSD test ($p < 0.05$) between different treatments within each species.3.6.2. Biomass allocation patterns in native and invasive grasses

Relationship between above- and belowground biomass was affected by species, the interaction between Species and Fertilization (S*F) and the interaction between Species and Biotic Interaction (S*BI) (Fig. 3: Table S1, S2). The invasive species allocated more biomass to above-ground organs compared to the native grass. Overall, the slope of the relationship between above- and belowground biomass was similar in both species (1.088 and 0.969 for native and invasive species, respectively; p=0.469),



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and not different from 1 (p=0.417 and p=0.369 for native and invasive species, respectively). This indicates that, in both species, above and below ground biomass changed isometrically as plant size changed. However, invasive plants had substantially more above-ground biomass per unit of belowground biomass across all plant sizes (shift in elevation p<0.001, Table S1, 2).

Furthermore, as indicated by S*B, the invasive species plants were of similar size when growing in interaction or alone, whereas in the native grass plants were smaller when growing in interaction (shift along common slope and shift in elevation, p<0.001, fig 3). Thus, while the interaction with the invasive species reduced plant size in the native grass, it did not modify how plants allocated biomass between above- and belowground organs. Fertilization did not change allocation patterns in native grass but appeared to affect biomass allocation in the invasive species: in unfertilized pots, C. dactylon exhibited a steeper slope than in fertilized pots (p=0.009; Table S1, S2; Figure S1). In addition, fertilization increased the allocation to aboveground biomass per unit of belowground biomass in the invasive species (Figure S1).

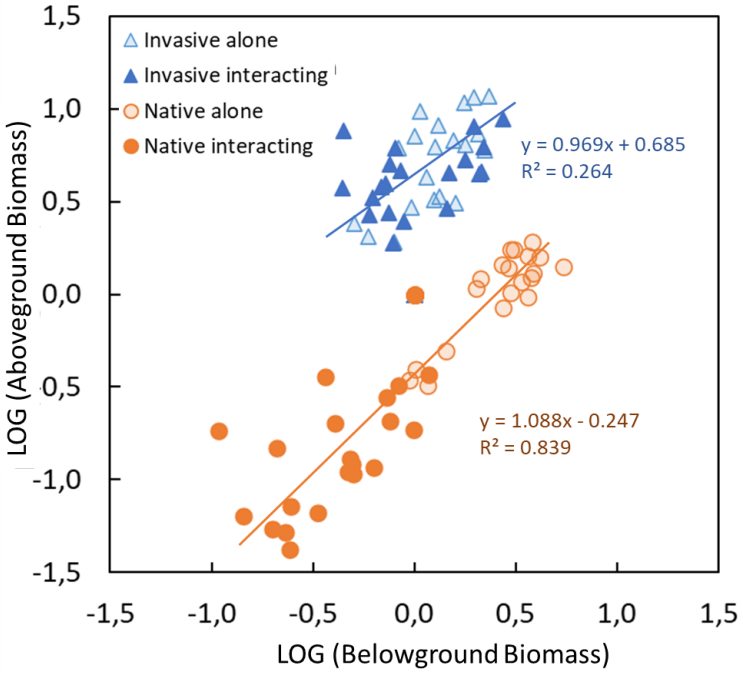




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**Figure 3**. Allometric relationship between above- and below- ground biomass of native species (circles) and the invasive species (triangles) grown either alone (open symbols, n=20) or in interaction (closed symbols, n=20).

The contrasting pattern of above and belowground biomass allocation between species (Fig. 3), in part, reflected changes in allocation between leaves and roots. Invasive plants invested more biomass in leaves per unit of biomass allocated to roots than natives (1.939 vs. 1.305, p=0.015. Fig. 4). The invasive species had similar root and leaf mass when growing in interaction or alone, whereas the native grass had less root and leaf mass when growing in interaction (shift along common slope, p<0.001). Thus, while the interaction with the invasive plant reduced the size of both these organs, it did not modify how native plants allocated biomass between them. Again, fertilization had a slight effect on the invasive grass: in unfertilized pots, C. dactylon exhibited a steeper slope than in fertilized pots (p = 0.033; Table S1, S2; Figure S2).

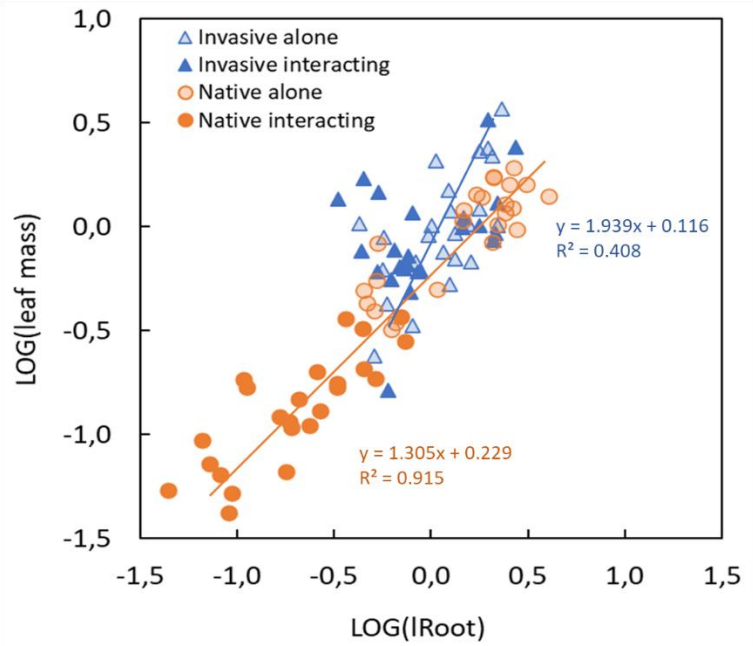


**Fig. 4**. Allometric relationship between root mass and leaf mass (live leaves plus dead leaves) of native species *Paspalum notatum* (circles) and the invasive species *Cynodon dactylon* (triangles), when grown alone (open symbols, n=20) or in neighboring interaction treatments (closed symbols, n=20).



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The contrasting pattern of above and belowground biomass allocation between species (Fig. 3) was mainly caused by changes in allocation of biomass to stolons and rhizomes. Per unit leaf mass, the invasive grass allocated more biomass to stolons (above-ground organ) than the native to rhizomes (belowground organ; shift in elevation $p<0.001$, Fig. 5a; Table S1, S2).

Changes in biomass allocation to stolons and rhizomes were associated to differences in the number of leaves produced. Invasive plants had almost one order of magnitude more leaves than the native and the native species in interaction had substantially less leaves than when grown alone (shift along common slope, $p=0.028$, Fig 5b). Furthermore, per produced leaf, invasive species allocated more mass to stolons than native grass to rhizomes, as shown by different slopes (1.223 vs. 0.853, $p=0.028$, Fig. 5b). Indeed, while the invasive plant deposited proportionally more stolon mass as the number of leaves increased (slope greater than 1), the native species deposited proportionally less to rhizomes (slope lower than 1).



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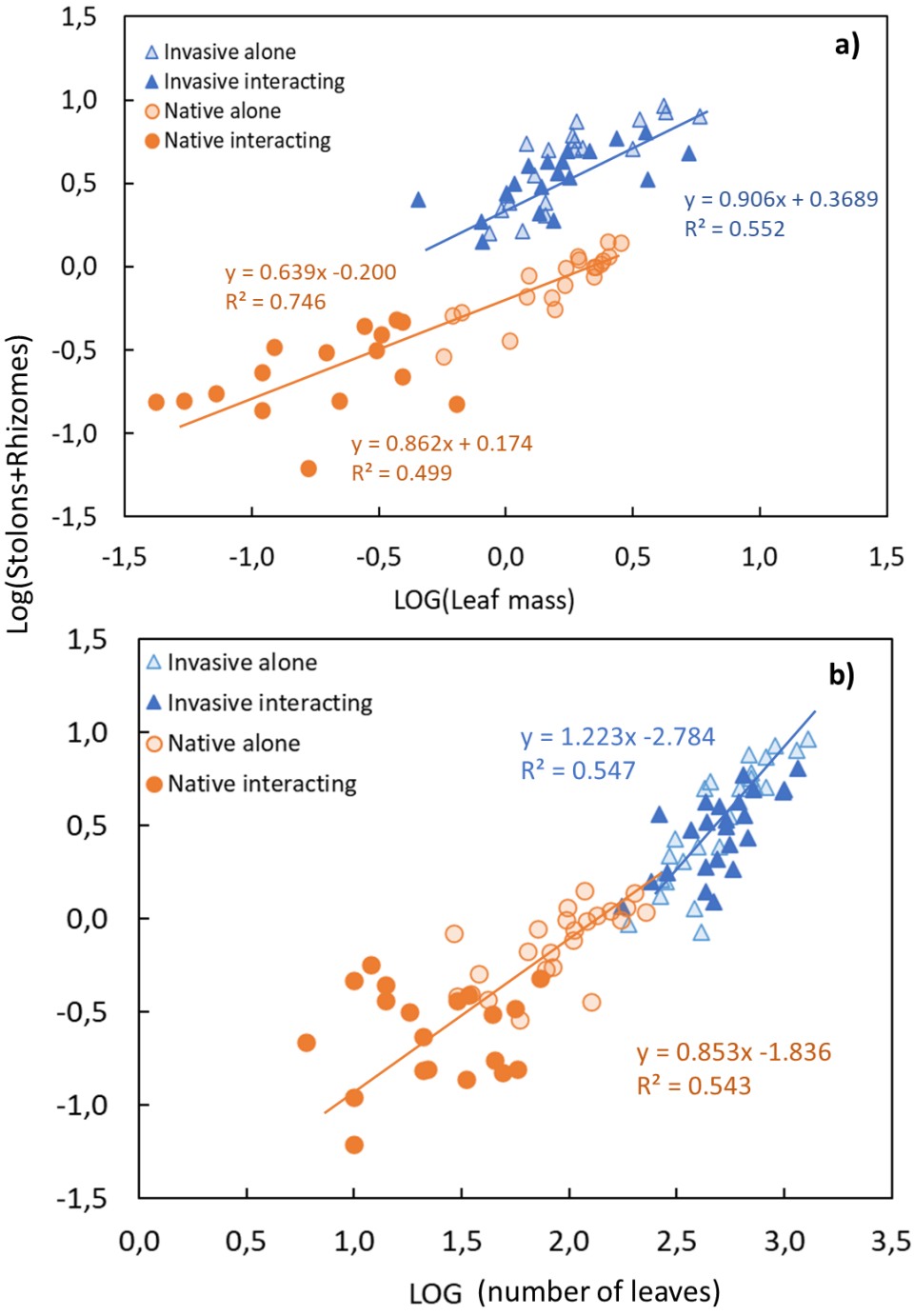


**Figure 5.** Allometric relationship between stolon+rhizomes mass vs. leaf mass (a) and number of leaves (b) of native species *Paspalum notatum* (circles) and the invasive species *Cynodon dactylon* (triangles), when grown alone (open symbols, n=20) or in neighboring interaction treatments (closed symbols, n=20).



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The much larger number of leaves produced by the invasive than native species was in part due to more active branching (tillering). Indeed, in invasive plants, each increase in the number of leaves produced led to a greater number of tillers than in the native species (slopes 1.239 vs. 0.951 in invasive vs. native, respectively; p=0.018, Fig. 6). Moreover, we found effect of the S*B in the shift along common slope, p<0.001; shift in elevation p=0.027): the invasive species plants had similar leaf and tiller numbers when growing in interaction or alone, whereas in the native grass plants had less leaves and tillers when growing in interaction. Thus, while the interaction with the invasive grass reduced the number of leaves and tillers in native species, it did not modify the branching pattern.

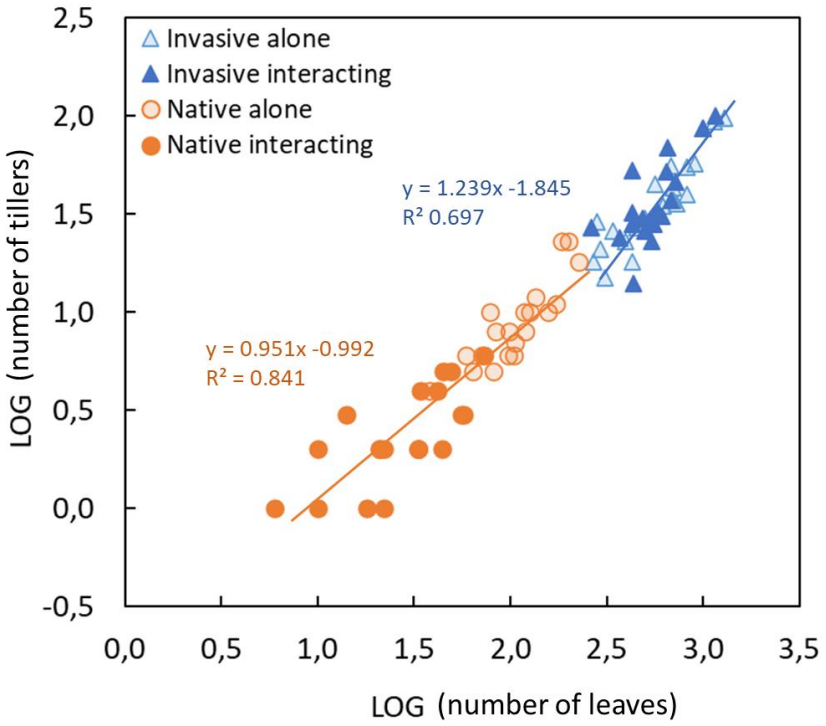


**Fig. 6**. Allometric relationship between number of tillers and number of leaves of native species *Paspalum notatum* (circles) and the invasive species *Cynodon dactylon* (triangles), when grown alone (open symbols, n=20) or in neighboring interaction treatments (closed symbols, n=20).



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3.6.3. Reproductive biomass allocation

Native grass *P. notatum* allocated energy to sexual reproduction in all treatments and it was not affected by fertilization nor activated carbon, while the invasive *C. dactylon* produced flowers only when interacting with *P. notatum* in activated carbon treatments (Table 2).

**Table 2.** Mean number of inflorescences (n=4±SE) in native grass *Paspalum notatum* and the invasive species *Cynodon dactylon*, when grown alone or in interacting treatments, under different levels of fertilization (F+ or F-) and activated carbon (AC+ or AC-).

| | **Treatments** | | | | |
|---|---|---|---|---|---|
| **Target species in treatments** | F+AC+ | F+AC- | F-AC+ | F-AC- | F++AC+ |
| Native alone | 0.5±0.5 | 0.75±0.43 | 0.5±0.5 | 0.5±0.86 | 0.25±0.5 |
| Native interacting | 0.25±0.43 | 0.5±0.47 | 0.5±0.5 | 0.75±0.83 | 0.5±0.5 |
| Invasive alone | 0 | 0 | 0 | 0 | 0 |
| Invasive interacting | 0.25±0.43 | 0 | 0.25±0.43 | 0 | 0.25±0.43 |



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## 3.7. Discussion

The invasive species C. dactylon showed greater competitive ability than the native grass, regarding both above and below ground biomass, at different nutrient levels, and these results were not affected by the presence of activated carbon. On the other hand, the invasive grass showed high tolerance to the presence of native neighbors, as its biomass was generally unaffected in the evaluated situations.

In native grass, RII values constructed with different biomass fractions were always lower than zero and similar between treatments, reflecting that this species was always negatively affected by the presence of the invasive. In fact, RII ranged from -0.67 to -0.75 for total biomass, showing that the effect size of plant interaction on P. notatum is quite important when coexisting with the invasive grass. Similar results had been reported for the native P. notatum when competing with invasive species C. dactylon or E. plana in similar experimental designs (García et al. 2023; Guido et al. 2019). Here we further show that increasing nutrient availability did not modify the outcome of biotic interaction for native species. For the invasive grass, on the other hand, biomass production was not affected by the interaction under low nutrient conditions, which is consistent with our previous findings (García et al. 2023). As the invasive species C. dactylon proliferates in fertilized and oversown natural grasslands (Cáceres 2019; Jaurena et al. 2015; Pañella et al. 2022), we would have expected this species to exhibit greater competitive ability under the fertilized treatments. However, its aboveground biomass was slightly reduced in the fertilized treatment, and its RII decreased in these situations.



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Since the invasion by C. dactylon is reported mainly in fields fertilized annually, when the plants are already established, we would have expected that a second dose of nutrients would result in a greater competitive ability for the invader. However, adding a second dose of nutrients when the species are already established had similar effects: when we compared single vs. double doses of nutrient, no differences were found on the RII values. Although it is widely reported in the literature that increased nutrient availability promotes the richness and cover of invasive plants (Flores-Moreno et al. 2016¸Huenneke et al. 1990; Parepa et al. 2013; Zhang et al. 2022), our results suggest that factors other than nutrient competition may be explaining the success of C. dactylon.



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Adding activated carbon to minimize the chances of allelopathic compounds to play a role in the interaction did not modify these response patterns. These results suggest that allelopathy would not significantly affect the outcome of these biotic interactions. As is propose by Novel Weapons Hypothesis, allelopathy has been widely proposed as a mechanism facilitating plant invasions (Callaway and Ridenour 2004; Ridenour and Callaway 2001). However, demonstrating allelopathy is challenging, and it is rarely confirmed conclusively because most methodological approaches have inherent limitations that restrict their extrapolation to field conditions. In particular, the methodology used in our study allows only an indirect estimation of allelopathic effects and therefore does not completely rule out their occurrence. One of the main limitations is that we cannot confirm the presence of allelopathic compounds in the treatment without activated carbon. Previous studies conducted at the national level have also found limited evidence of allelopathy in C. dactylon. For instance, Bresciano et al. (2022) reported no significant effects of allelochemical production on the germination of native species. Similarly, Guido et al. (2020) observed some inhibitory effects, but these were not stronger than those produced by a native species from the resident community. Importantly, our study did not directly test the effect of allelopathy on plant performance. Given the methodological limitations described above, future experiments could directly test the effects of adding allelopathic compounds on already established native plants, such as those used in this study, under conditions that more closely resemble those of invaded grasslands.

Biomass allocation patterns differed between the invader and the native species: the invader allocated more mass to aerial organs, regardless of the situation evaluated. Conversely, the native grass always allocated more biomass to belowground organs. A previous experiment yielded similar results, with a greater allocation of biomass towards aboveground organs in C. dactylon than in P. notatum (García et al. 2023). Different biomass patterns between species were explained only in part by differences in root/leaf partitioning, which suggest little difference between species in the partitioning of biomass between C assimilation vs. nutrient and water uptake organs. In the invasive grass, steeper slopes were observed under unfertilized conditions than



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under fertilized conditions, which would reflect an increase of aboveground and leaves biomass allocation under competition. This result is counterintuitive, as one would expect plants growing under nutrient limitation to allocate more biomass to belowground structures in order to enhance nutrient acquisition. This pattern may reflect the high variability in the data rather than a biologically meaningful effect (Figure S1 and Figure S2). Moreover, fertilization increased biomass allocation to aboveground tissues per unit of belowground biomass, which was reflected in a higher intercept in fertilized pots (Figure S1). On the other hand, a quite contrasting allocation of biomass to stolons vs. rhizomes was detected between invasive and native, respectively. This was evidenced by a higher production of stolons in relation to roots and leaves in the invasive C. dactylon, than of rhizomes in native P. notatum. Production of stolons requires initiation of axillary bud's growth, which are created at the same rate as leaves are produced (Hutchings and Mogie 1990). The invasive grass showed a far greater capability of producing leaves than the native, which appeared to result from both a higher intrinsic leaf production as well as more active branching (tillering), thus exponentially increasing the initial difference. This high capacity to produce leaves faster in the invasive species than its competitor would explain its high stolon production.

Notably, native plants in interaction reacted primarily be reducing its size, but did almost no adjustment in their allocation pattern. Moreover, allocation results indicate that invasive and native species develop starkly contrasting strategies regarding organs no directly involved water, nutrient or C capture. While the invasive plant prioritizes stolon development (and thus horizontal space colonization), native grass prioritizes the construction of belowground organs as rhizomes that would serve as a bud bank and carbohydrates storage (Eggers et al. 2004; White 1973).

Sexual reproduction was different between species: C. dactylon flowered only in interaction with P. notatum, a response already reported in our previous studies (García et al. 2023), and this flower production only occurred in pots with activated carbon. Invasive plants often exhibit phenotypic plasticity in reproductive traits, as they encounter considerable environmental heterogeneity during the invasion process



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(Silva et al. 2020; Zhang et al. 2021). The deployment of sexual or clonal reproduction depends on resource availability and the stage of invasion. In general, sexual reproduction is associated with low stress and high resource availability, so competition among plants tends to reduce this propagation strategy (Zhang et al. 2024). However, there are also studies reporting that increased competition intensity promotes sexual reproduction as a means to ensure offspring under biotic stress (Aarssen 2008). Experimental studies under controlled conditions have shown that C. dactylon responds to shading by producing vertical (orthotropic) tillers that typically develop inflorescences (Dong and De Kroon 1994; Dong and Pierdominici 1995). In field conditions, this response may provide adaptive advantages by allowing the species to adjust its reproductive investment according to competition intensity, thereby facilitating its spread in heterogeneous environments. In more conserved grasslands—characterized by low grazing pressure and greater vegetation height and density—C. dactylon may increase investment in sexual reproduction as a strategy to disperse over longer distances and avoid patches dominated by competing species. Understanding the reproductive strategy of this invasive species is therefore essential for designing grassland restoration and conservation actions. In highly degraded or overgrazed systems, management should focus on limiting vegetative propagation, whereas in well-conserved grasslands efforts should prioritize preventing sexual dispersal and maintaining native diversity.

According to laboratory studies, Paspalum notatum has been reported to exhibit allelopathic potential (Favaretto et al. 2018), which could have a negative impact on the reproduction of the invader species. However, activated carbon could eliminate these signals, enabling the invader to flower. Flowering could therefore serve as a reproductive escape strategy when competing with the invader in an environment free from inhibitory chemical signals, facilitated by the activated carbon. In P. notatum flowers were registered in all situations studied and they were not affected by any treatment. In our experiment, the native species was planted from adult individuals, whereas the invasive species was sown from seed, which could be an energetic advantage for the native plant.



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Overall, the invasive species C. dactylon showed greater competitive ability than the native grass, mostly due to its tolerance to growth with neighboring plants across the whole range of nutrient situations, and the same results were found when allelopathic effects were controlled. The superior competitive ability of the invasive species appears to emerge from a basic difference between species in biomass allocation: invasive plant prioritizes stolons, organs whose function is vegetative reproduction and allows plants to expand faster. In turn, such a large production of stolons is based on a high rate of leaf emergence, which provides potential sites for the production of tillers. Stolons are highly efficient and economical structures for colonizing empty spaces (Holm eta. 1977; Horomitz 1996) and this strategy is particularly advantageous in disturbed environments such as the fertilized and oversewn grasslands. In such conditions, the lack of persistence of the sownanual legumes (Jaurena et al. 2015; Pañella et al. 2022) creates open gaps in the sward, providing opportunities for the establishment of Cynodon dactylon. Conversely, native species prioritizes the construction of roots and short rhizomes that would serve as a bud bank and also carbohydrates storage (Eggers et al. 2004; White 1973), from which it can recover in the event of disturbances.

Future research should be directed towards understanding other factors related to the invasiveness of this species, such as water availability and shading. Moreover, further research on biotic interactions between C. dactylon and native species—particularly those functionally similar to C. dactylon, such as Axonopus affinis—is essential for developing more effective management strategies. However, important questions remain regarding the competitive ability and field performance of native stoloniferous grasses. This is because stolons represent a considerable proportion of aboveground biomass and are exposed to removal events such as grazing, a very frequent disturbance in the grasslands of our region.

Because resource release favors the spread of C. dactylon, management practices that minimize the creation of open space (e.g., seasonal adjustment of stocking rates) are crucial to reduce disturbances and thereby limit the expansion of this invasive species.



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Overall, these findings highlight the importance of understanding the allocation patterns and biotic interactions that contribute to the success of invasions. This knowledge is crucial for predicting future spread and developing effective, sustainable management strategies.

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**Authors contribution**

All authors contributed to the study conception and design**.** The installation, maintenance, and harvest of the experiment were carried out by Anaclara Guido, Silvina García, and Fabiana Pezzani. Data analysis was conducted by Fernando Lattanzi and Silvina García. The first draft of the manuscript was written by Silvina García, and all authors contributed with comments on previous versions. All authors have read and approved the final manuscript.

**Competing interest:** the authors declare that there are no known competing interests.

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## 4. Degradation of arbuscular mycorrhizal symbiosis: A mechanism underlying *Cynodon dactylon* invasion in grasslands?[3]

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### 4.1. Resumen

Preguntas. La degradación de las interacciones mutualistas de las especies nativas puede desempeñar un papel importante en el establecimiento y expansión de plantas invasoras en las comunidades. Evaluamos la relación entre el nivel de invasión de *Cynodon dactylon* y la colonización micorrícica arbuscular de la especie nativa *Paspalum notatum* en pastizales naturales (PN) o pastizales sembrados con leguminosas exóticas y fertilizados con fósforo (PN+LP). Específicamente, investigamos si los niveles crecientes de invasión se asociaban con reducciones en la colonización micorrícica, el crecimiento y el contenido de nutrientes en *P. notatum.*

Localización. Pastizales uruguayos de la región de los pastizales del Río de la Plata.

Métodos. Se seleccionaron dos potreros bajo manejo PN+LP, contiguos a dos potreros con manejo PN. En cada potrero se recolectaron nueve monolitos (0,2 m de diámetro x 0,3 m de profundidad) que contenían individuos de *P. notatum* y diferentes porcentajes de cobertura de *C. dactylon*, (0–10%), (30–50%) y (70–90%). Después de

[3] García, S., Pezzani, F., Guido, A., and Lattanzi, F. A. (2025). Degradation of arbuscular mycorrhizal symbiosis: A mechanism underlying *Cynodon dactylon* invasion in grasslands? *Journal of Vegetation Science* 36(1). https://doi.org/10.1111/jvs.70010. Doctorado en Ciencias Agrarias, Facultad de Agronomía, UdelaR

diez meses de crecimiento sin interrupciones, se evaluaron en las plantas de *P. notatum* la biomasa aérea, la concentración de fósforo y nitrógeno en el brote, y la colonización micorrícica arbuscular.

Resultados. La micorrización disminuyó con el aumento de la invasión de *C. dactylon*, siendo esta disminución más pronunciada en pastizales manejados intensivamente (PN+LP). Sin embargo, la menor colonización micorrícica arbuscular no se asoció con una menor biomasa aérea ni con una menor concentración de fósforo o nitrógeno en los brotes. Además, en niveles bajos de invasión, la colonización micorrícica fue similar entre PN y PN+LP, a pesar de sus contrastantes disponibilidades de fósforo en el suelo.

Conclusiones. La presencia de micorrizas arbusculares en la gramínea nativa objetivo *P. notatum* se asoció negativamente con el nivel de invasión de *C. dactylon*. Por lo tanto, la degradación del mutualismo podría ser un mecanismo subyacente al éxito invasivo de *C. dactylon* en pastizales, particularmente en aquellos manejados intensivamente, aunque probablemente no a través de efectos nutricionales relacionados con el fósforo.

**Palabras clave:** micorrizas arbusculares, invasiones biológicas, disponibilidad de nutrientes, *Paspalum notatum,* pastizales del Río de la Plata.

## 4.2. Abstract

**Questions**. Degradation of facilitative interactions of native species can play an important role in the establishment and expansion of invasive plants in communities. We evaluated the relationship between the level of invasion of *Cynodon dactylon* and the arbuscular mycorrhizal colonization of the native *Paspalum notatum* in Uruguayan grasslands, which were either extensively managed (NG) or oversown with exotic legumes and fertilized with phosphorus (NG+LP). Specifically, we investigated whether increasing invasion levels were associated with reductions in *P. notatum* mycorrhizal colonization, growth and nutrient content.

**Location**. Uruguayan grasslands of Río de la Plata grasslands region.

**Methods.** Two paddocks with 19 and 27 years under NG+LP management were selected contiguous to two paddocks with NG management. In each paddock, we

collected nine monoliths (0.2 m diameter x 0.3 m depth) that had *P. notatum* and increasing percentages of *C. dactylon* cover, classified as low (0-10%), medium (30-50%) or high (70-90%) invasion levels. After ten months of uninterrupted growth, shoot mass, phosphorus and nitrogen shoot concentration and arbuscular mycorrhizal colonization were assessed in *P. notatum* plants.

**Results**. Mycorrhization decreased with increasing *C. dactylon* invasion. This was greater in more intensively managed grasslands (NG+LP). However, lower arbuscular mycorrhizal colonization was not associated with either lower aboveground growth nor phosphorus or nitrogen shoot concentration. Furthermore, at low invasion levels, mycorrhizal colonization was similar between NG and NG+LP, despite their contrasting soil P availability.

**Conclusions**. The presence of arbuscular mycorrhizae in the target native grass *P. notatum* was negatively associated with the invasion level of *C. dactylon.* Therefore, mutualism degradation might be a mechanism underlying the success of *C. dactylon* invading grasslands, particularly those intensively managed, albeit probably not *via* phosphorus nutritional effects.

**Keywords:** arbuscular mycorrhizae, biological invasions, nutrient availability, *Paspalum notatum*, Río de la Plata grasslands.

### 4.3. Introduction

Biological invasions are a leading cause of global biodiversity loss, impacting the structure and functioning of ecosystems (Mack et al. 2000; Vilà et al. 2011; IPBES, 2023). At the local scale, the mechanisms involved in invasion processes are primarily linked to biotic interactions between the invasive species and native species from the recipient community (Richardson et al. 2000a; Mitchell et al. 2006; Blackburn et al. 2011; Traveset and Richardson, 2020).

Most studies have focused on the role of negative interactions between native and invasive species during the invasion process, such as competition and predation, as mechanisms underlying invasion success (Elton, 1959; Mitchell et al. 2006; Traveset and Richardson, 2020). However, increasing evidence suggests that facilitative interactions can also play an important role in the establishment and spread

of invasive species (Traveset and Richardson, 2014, 2020; Pysek et al. 2019). In some cases, non-native plants may form mutualistic relationships with native soil microbes, enhancing their ability to outcompete with natives species (Shah et al. 2009). Whereas in other cases, invasive species negatively affect mutualistic interactions essential for some native species of the recipient community, which is referred to as the "degraded mutualism" or "mutualism disruption" hypothesis (Vogelsang et al. 2004; Vogelsang and Bever, 2009; Hale and Kalisz, 2012; Traveset and Richardson, 2014; Roche et al. 2023).

Among mutualisms, arbuscular mycorrhizae stand out because they play a critical role in determining patterns of abundance and invasiveness (Pringle et al. 2009; Traveset and Richardson, 2020). Arbuscular mycorrhizal mutualisms derive from the association between fungi belonging to the phylum *Glomeromycota* and the roots of most terrestrial plants (Smith and Read, 2008). Chief among the benefits that plants get from mycorrhizae is the enhanced absorption of slowly mobile nutrients, such as P (Grimoldi et al. 2005; Smith and Read, 2008). Improved water uptake (Kakouridis et al. 2022; Romero-Munar et al. 2024) and protection against pests and diseases have also been documented (Newsham et al. 1995; Schausberger et al. 2012; Weng et al. 2022). Furthermore, arbuscular mycorrhizae may also interact with other symbionts, such as rhizobia and epichloe endophytes (García Parisi et al. 2017). Not surprisingly, this symbiosis is very conspicuous in natural ecosystems (Smith and Read, 2008; Brundrett, 2009).

The precise mechanisms underlying mutualism degradation during invasions are yet unclear (Grove et al. 2017) but can be indirectly via the host plant or due to a direct effect on the mycorrhizal interaction (Groves et al. 2017). Indirect effects occur for example when invaders can affect mycorrhizae by competitively reducing or excluding suitable plant hosts, and this mechanism is particularly important when the invader is not mycorrhizal (Vogelsang and Bever, 2009; Zubek et al. 2016). In these cases, low dependence on mycorrhizae cause a reduced bank of arbuscular mycorrhizal spores in invaded communities (Vogelsang and Bever, 2009; Wilson et al. 2012; Traveset and Richardson, 2014; Zubek et al. 2016; Roche et al. 2023). On the other hand, an invasive plant can directly affect the mycorrhizal community in the soil, for example by

increasing soil N content (Castro-Díez et al. 2014), which can affect the composition of the fungal community (Jumpponen et al. 2005) or reduce mycorrhizal structures associated with nutrient uptake (i.e., arbuscules; Johnson et al. 2010). Another direct effect is due to allelopathy, as there is evidence that secondary metabolites inhibit spore germination and the establishment of the symbiosis (Robert and Anderson, 2001; Hale and Kalisz, 2012; Pinzone et al. 2018; Roche et al. 2021, 2023). Moreover, since arbuscular mycorrhizae benefits are more important in nutrient-poor environments, increasing nutrient availability may act as a selection pressure favoring less efficient fungal species (Johnson et al. 1997; Grove et al. 2017). Some situations of increased soil promote mycorrhizal disruption via a phytocentric control because plants take up nutrients directly rather than via mycorrhizae (Paries and Gutjahr, 2023). In other cases, mutualisms can turn into parasitism (Johnson et al. 1997). Finally, invasive plants are capable of selecting more efficient arbuscular mycorrhizal symbionts, which can generate positive feedback that enhances their performance (Bever, 2002; Bever et al. 2009; Zhang et al. 2010; Wilson et al. 2012).

Arbuscular mycorrhizal colonization is widespread among native species in the highly biodiverse Río de la Plata grasslands, a temperate-subhumid region in southern South America (Parodi and Pezzani, 2011; Garcia et al. 2016, 2019; del Pino et al. 2021), particularly in thicker-rooted species (Michelini et al. 2024). In Uruguay, grasslands persist either as natural vegetation (NG) or subject to overseeding with legumes coupled with phosphorus (P) fertilization, to increase forage productivity and nutritive value (NG+LP; Jaurena et al. 2021). However, the invasion of *Cynodon dactylon* was frequently reported as a result of agronomic practices such as legume and P addition on NG (Jaurena et al. 2015; Pañella et al. 2022). *Cynodon dactylon* is a perennial warm season grass native to eastern Africa and southern Europe that has become one of the five most important invasive alien species worldwide (Holm et al. 1977).

The mechanisms underlying the success of *C. dactylon* invasion of NG+LP are unclear. It exhibits successful clonal propagation through rhizomes and stolons, as well as reserve organs that enhance its survival (Horowitz, 1996; Taliaferro et al. 2004). Moreover, it produces allelopathic compounds capable of inhibiting the growth of

other plants (Golparvar et al. 2015; Mahmoodzadeh et al. 2014; Guido et al. 2020;). The competitive ability of *C. dactylon* is greater than that of dominant native species of the Río de la Plata grasslands, which seems to be related to a greater plasticity associated with root:shoot allocation (Garcia et al. 2023). Among the native grasses, *Paspalum notatum* stands out for its abundance and forage value (Pizarro, 2000) and, together with *Axonopus affinis,* represent the most frequent species in grassland communities of Uruguay (Lezama et al. 2019). It is a perennial species with a prostrate habit, summer cycle (Rosengurtt, 1979), and $C_4$ photosynthetic metabolism that coexists with the invasive *C. dactylon. Paspalum notatum* showed high levels of arbuscular mycorrhizal colonization, which is expected in plants with thick roots (Hartnett and Wilson, 1999; Pezzani et al. 2006; García et al. 2019; Michelini et al. 2024). In this species, mycorrhizae play a crucial role in water and nutrient acquisition due to the lack of fine roots. Another strategy explaining the success of *C. dactylon* in NG+LP could be the degradation of arbuscular mycorrhizal fungi mutualism with due to greater *C. dactylon* coverage than in NG (Garcia et al. 2016). Although arbuscular mycorrhizae would not be as needed in high nutrient availability, they may develop another function as protection against noxious organisms in NG+LP. *Cynodon dactylon* is capable of forming arbuscular mycorrhizal interactions, but it develops a very low presence of nutrient exchange structures (arbuscles and coils) between symbionts (Pezzani et al. 2011; Endresz et al. 2013; Lugo et al. 2018), so it has been considered non-mycorrhizal or facultative mycorrhizal (Muthukumar and Udaiyan, 2000; Muthukumar et al. 2006). This could suggest that this invasive grass would be allocating less energy to mycorrhizal fungi than native plants. However, experimental studies that examine the role of arbuscular mycorrhizae on *C. dactylon* invasion are lacking.

The aim of this study was to explore the relationship between the level of invasion of *C. dactylon* and the mycorrhizal colonization of a conspicuous native species from the recipient community. Specifically, we addressed the following questions: i) what is the association between the levels of *C. dactylon* invasion and the arbuscular mycorrhizal colonization of a dominant native grass (*P. notatum*)?; ii) is this association modified by the addition of legumes and P on natural grasslands

(NG+LP)?; and (iii) does a reduction of mycorrhizal colonization in *P. notatum* involve a decline in its growth and nutrient content?. We expected that higher levels of invasion would be associated with a reduction in mycorrhizal colonization of *P. notatum*, that this effect would be more marked in NG+LP, and that mutualism degradation would be negatively associated with growth and nutritional status of *P. notatum*.

## 4.4. Methods

### 4.4.1. Collection and maintenance of monoliths

In June 2019, at the "*Centro de Investigación y Experimentación Dr. Alejandro Gallinal*" of the *Secretariado Uruguayo de la Lana*, located in south center region of Uruguay (CIEDAG-SUL 33.86932 °S, 55.57273 °W), we selected two paddocks with 19 (site A) and 27 (site B) years under NG+LP management contiguous to two paddocks with NG communities (Appendix S1). The study area is framed within Community IV "Densely vegetated grasslands of the Eastern Hills, Northeastern Sedimentary Basin and South Central Region", in accordance with the classification of NG communities of Uruguay (Lezama et. al. 2019).

NG+LP agronomic management consisted in broadcasting 5 kg/ha of *Lotus subbiflorus* cv El Rincón upon native grasslands, plus annual fertilization with 20 kg P/ha as either superphosphate (until the year 2000) or hyperphosphate (after 2000) in sites A and B, respectively. In contrast, native grasslands paddocks under NG management received no fertilizer nor legume addition.

All sampled paddocks had similar soil type and topographical position, and were continuously stocked with cattle and sheep, although at seasonally varying stocking rates. Soil analysis showed that NG and NG+LP paddocks had similar pH and organic carbon concentration, but plant available P was 80% higher in NG+LP (Appendix S2).

In each paddock, we selected a small area (radius less than 200 m) where *P. notatum* had a similar plant cover but coexisting with different levels of invasion by *C. dactylon*: low (0-10%), medium (30-50%) or high (70-90%) cover. This ensured homogeneous edaphic conditions, topographical position, and grazing regime, as well as minimized the possibility of initial differences between invasion levels (Figure 1).

At each invasion level, we collected three monoliths (depth 0.20 m and diameter 0.20 m), at least 5 m apart from each other. Monoliths were placed in plastic boxes, and immediately transported to a controlled garden for maintenance.

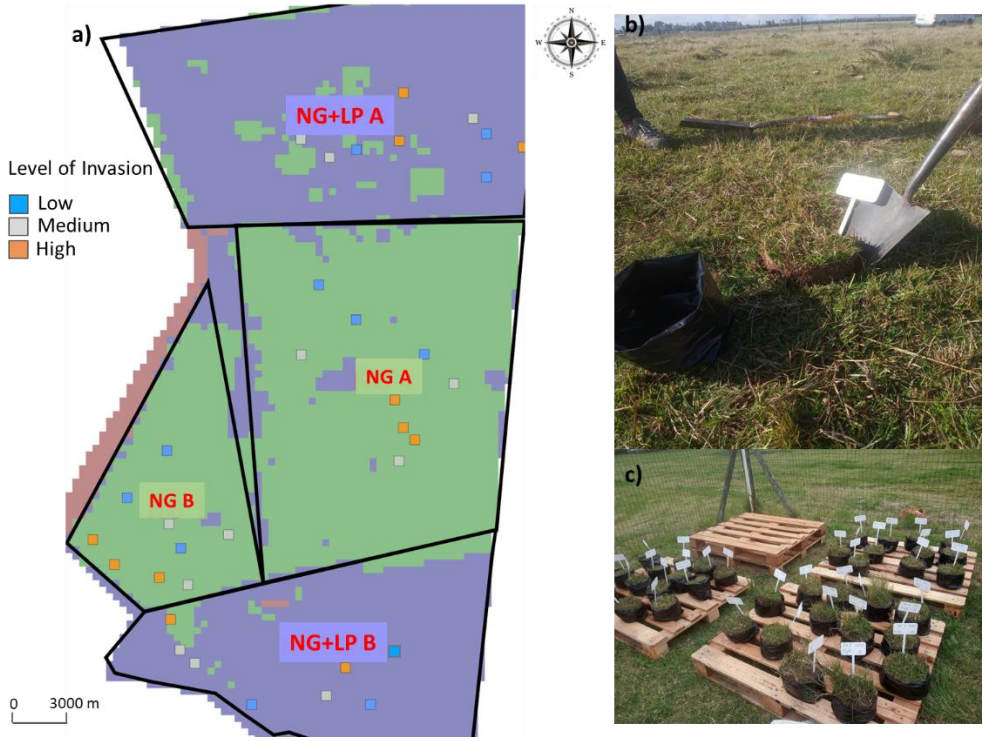


Figure 1. Location of the sampling points. Each square corresponds to monoliths with low (0%–10% in blue), medium (30%–50% in gray), and high (70%–90% in orange) levels of invasion, within the surveyed paddocks. Green and purple background colors correspond to NG (native grasslands extensively managed) and NG + LP (oversown with exotic legumes and fertilized with P, respectively). Letters A and B refer to the two sites sampled (a); photograph of the field sample of monoliths (b) and the experiment at the Faculty of Agronomy (c).

Monoliths were kept on in an open-air garden at the Faculty of Agronomy, in Montevideo, Uruguay (34.83740 °S, 56.22240 °W), where they grew uninterrupted for ten months, *i.e.* one growing season. Irrigation with tap water was provided to prevent water stress, but no nutrients were applied. During winter, plants were watered with a hose. During the warm season, to ensured that water was available as plants needed, the monoliths were placed in flooded trays to absorb water by capillarity.

In each monolith, all species other than *P. notatum* and *C. dactylon* were manually removed to prevent confounding effects of the identity of neighboring species on mycorrhizal colonization of the target species. To assess whether disturbance caused by mechanical removal of neighboring plants affected mycorrhizal status of *P. notatum* (i.e., manipulation artifact), mycorrhizal colonization was measured in six extra monoliths collected from NG (three monoliths from each site A and B) with medium *C. dactylon* cover, in which neighboring plants were not removed (control for removal effect).

4.4.2. Biomass sampling and assessment of arbuscular mycorrhizal colonization in *P. notatum*

In April 2020, after the 10-months period, all above- and belowground biomass of *P. notatum* was harvested and aboveground was dried with forced air at 60°C for 48 to 72 hr until constant weight. Dry samples were weighed and then aboveground and their P and N concentration determined. P concentration was quantified by sulfuric digestion and ammonium molybdate colorimetry, and N concentration was determined by combustion at 900ºC and subsequent detection of N2 by thermal conductivity.

To assess arbuscular mycorrhizal colonization, roots were washed and cleared with KOH solution (10%) and then stained with trypan blue (0.05%), as proposed by Koske and Gemma (1989). We randomly scanned 30 root segments about 1 cm long per monolith and three fields of view per segment. In each scan, hyphae, vesicles, arbuscules and coils of arbuscular mycorrhiza fungi were recorded in a total of 90 observation fields (Olympus BX 43) at 10x magnification. Total colonization was quantified as the proportion of fields bearing any fungal structure.

4.4.3. Statistical analysis

Firstly, for evaluating the effect of removing biomass (i.e., manually removal of non-target species), and thus avoiding manipulation artifacts, mycorrhizal colonization data was analyzed using a generalized linear mixed effect model, assuming a binomial distribution of the data. The fixed effect was the removal (removal and no removal) and the random effect was the site (A and B). *A posteriori* mean comparison was performed with LSD test adjusted by Bonferroni.

Mycorrhizal colonization of *P. notatum* – total, vesicles and arbuscules plus coils (i.e., exchange structures) – was analyzed using a generalized linear mixed effect model, assuming a binomial probability distribution. For analyses of aboveground biomass data, P and N shoot concentration and N:P ratio, linear mixed effect models (i.e. normal probability distribution) were used. In all cases, agronomic management (NG and NG+LP), level of invasion by *C. dactylon* (low, medium and high) and its interaction were considered as fixed effects. Differences between treatments were assessed using the Least Significant Difference (LSD) test adjusted by Bonferroni. In all models, site was specified as a random effect in the models.

Multiple lineal regressions were performed to analyze the relationships between plant nutrients, aboveground biomass and arbuscular mycorrhizal colonization. For this, linear mixed effect models were used, in which aboveground biomass and P shoot content were the responses variables. Agronomic management (NG and NG+LP), level of invasion by *C. dactylon* (low, medium and high) and its interaction were considered as fixed factors. Total mycorrhizal colonization was specified as a co-variable and site was considered as a random effect. Statistical analyses were carried out in INFOSTAT, using the interface with R Studio (Di Rienzo et al. 2014).

## 4.5. Results

### 4.5.1. Arbuscular mycorrhizal colonization of *P. notatum*

Arbuscular mycorrhizae were present in all treatments. Total colonization varied widely, ranging from as low as 3% in some NG+LP monoliths with high levels of invasion by *C. dactylon*, to more than 50% in some NG monoliths with low level of invasion by *C. dactylon* (Figure 2a). There was no effect of the mechanical removal of neighboring species on total mycorrhization ($p$=0.125), vesicles ($p$=0.07) nor exchange structures ($p$=0.662) of *P. notatum* (Appendix S3).

Total mycorrhization of *P. notatum* was smaller as the level of invasion by *C. dactylon* increased, and this magnitude was larger in NG+LP than in NG (Figure 2a; Table 1; Appendix S4). Thus, compared with low levels of *C. dactylon* invasion, the total mycorrhizal colonization in NG decreased in 28% and 43% in medium and high level of invasion, respectively. In NG+LP, however, this reduction was 35% and 85%

at medium and high levels of *C. dactylon* invasion respectively, compared to low level of invasion (Figure 2a; Table 1). When the level of invasion of *C. dactylon* was low, arbuscular mycorrhizal colonization was similar in NG and NG+LP (Figure 2; Table 1; Appendix S4).

Vesicular colonization showed the same response pattern as total colonization (Figure 2b; Table 1; Appendix S4). Conversely, colonization by arbuscles plus coils (exchange structures) was less associated with the studied factors. This variable was negatively associated with the level of invasion by *C. dactylon* in natural grassland, but not in NG+LP (Figure 2c; Table 1; Appendix S4).

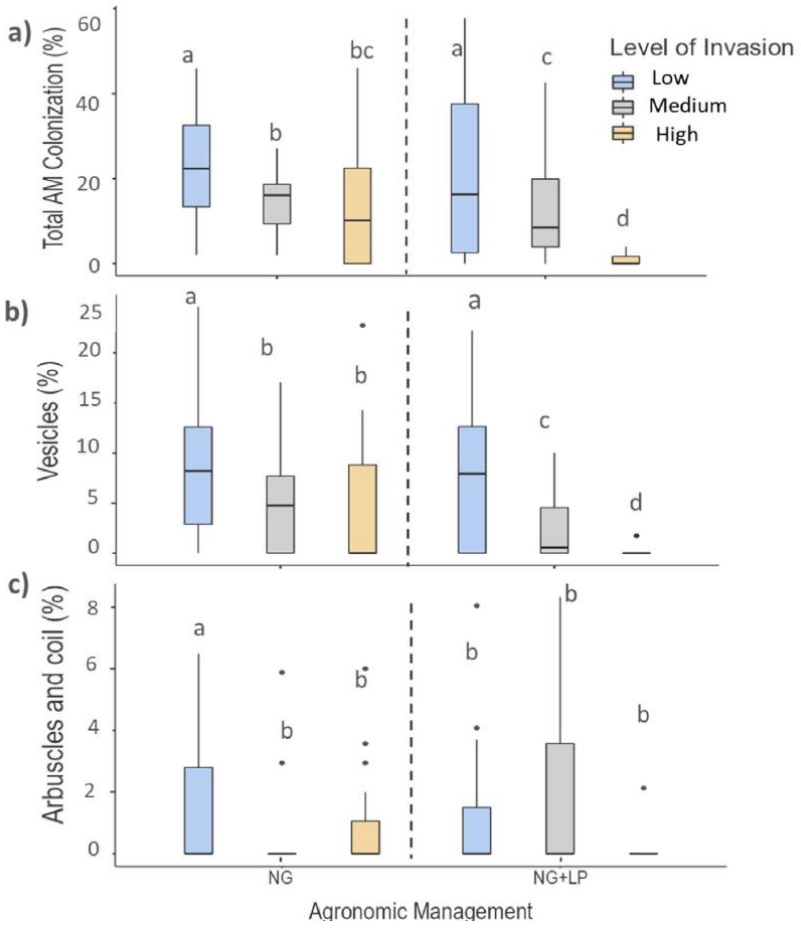


Figure 2 Box plots (n = 6 per treatment) of the percentage of roots with colonization by total (a), vesicles (b), and exchange structures (c) of Paspalum notatum plants growing with low (0%–10% in blue), medium (30%–50% in gray), or high (70%–90% in orange) levels of Cynodon dactylon invasion, in native grasslands managed either extensively (NG) or oversown with exotic legumes and fertilized with

P (NG + LP). Different letters above boxplots indicate a statistically significant difference among treatments ($p < 0.05$) using the Least Significant Difference (LSD) test adjusted by Bonferroni

Table 1. Results of generalized linear mixed effect models (with binomial error distribution) that tested the effects of treatments on mycorrhizal colonization. Treatment contrasts, with the intercept representing the level NG (natural grassland) and 0%–10% (low invasion), are shown. NG + LP refers to native grassland oversown

| Response Variable | Treatment z | Estimate | S.E | z value | Pr(>\|z\|) |
|---|---|---|---|---|---|
| Total colonization | Intercept | -1.28 | 0.26 | -4.88 | <0.001 |
| | NG+LP | -0.04 | 0.10 | -0.44 | 0.656 |
| | 30-50 | -0.45 | 0.13 | -3.46 | 0.005 |
| | 70-90 | -0.68 | 0.13 | -5.20 | <0.001 |
| | NG+LP*30-50 | -0.43 | 0.19 | -2.33 | 0.019 |
| | NG+LP*70-90 | -2.29 | 0.41 | -5.61 | <0.001 |
| Vesicles | Intercept | -2.69 | 0.61 | -4.42 | <0.001 |
| | NG+LP | 0.03 | 0.15 | 0.18 | 0.854 |
| | 30-50 | -0.50 | 0.20 | -2.53 | 0.011 |
| | 70-90 | -0.70 | 0.22 | -3.25 | 0.001 |
| | NG+LP*30-50 | -0.94 | 0.32 | -2.95 | 0.003 |
| | NG+LP*70-90 | -2.48 | 0.75 | -3.32 | 0.009 |
| Arbuscles and Coils | Intercept | 3.71 | 0.36 | -10.30 | <0.001 |
| | NG+LP | 0.32 | 0.80 | -2.55 | 0.010 |
| | 30-50 | -2.33 | 0.73 | -3.19 | 0.001 |
| | 70-90 | -0.96 | 0.40 | -2.38 | 0.017 |
| | NG+LP*30-50 | 2.24 | 0.84 | 2.66 | 0.007 |
| | NG+LP*70-90 | -0.98 | 1.10 | -0.89 | 0.374 |

with exotic legumes and fertilized with phosphorus (intensive management), whereas 30%–50% and 70%–90% refer to medium and high levels of invasion by *Cynodon dactylon*.

4.5.2. Aboveground biomass and nutrient content of *P. notatum*

Aboveground biomass, shoot N and P content were all greater in NG+LP than in NG (Figure 3; Table 2; Appendix S5). On average, *P. notatum* had 48% more biomass, 65% more N and 18% more P in NG+LP than in NG. Conversely, there were no differences associated with the levels of invasion by *C. dactylon* (Figure 3 a-c; Table 2; Appendix 5). The N:P ratio in *P. notatum* shoots ranged between 12 and 19 and it was not affected by any factor (Figure 3d; Table 2; Appendix S5).

The lower values of mycorrhizal colonization that corresponded to situations with high levels of invasion by *C. dactylon* did not correlate with lower biomass or nutritional status of *P. notatum.* In fact, there was a negative relationship between total arbuscular colonization and shoot P content ($p$=0.033; $R^2$=0.150) in *P. notatum*, regardless of the level of invasion or agronomic management (Figure 4a; Table 3; Table 4). The association between total colonization and aboveground biomass was marginally negative ($p$=0.052; $R^2$= 0.051), and did not depend on the level of invasion or the agronomic management (Figure 4b; Table 3; Table 4). The low value of $R^2$ suggests that, although the variables were correlated, arbuscular mycorrhizal colonization did not explain much of the variability in shoot P content or aboveground biomass in *P. notatum*.

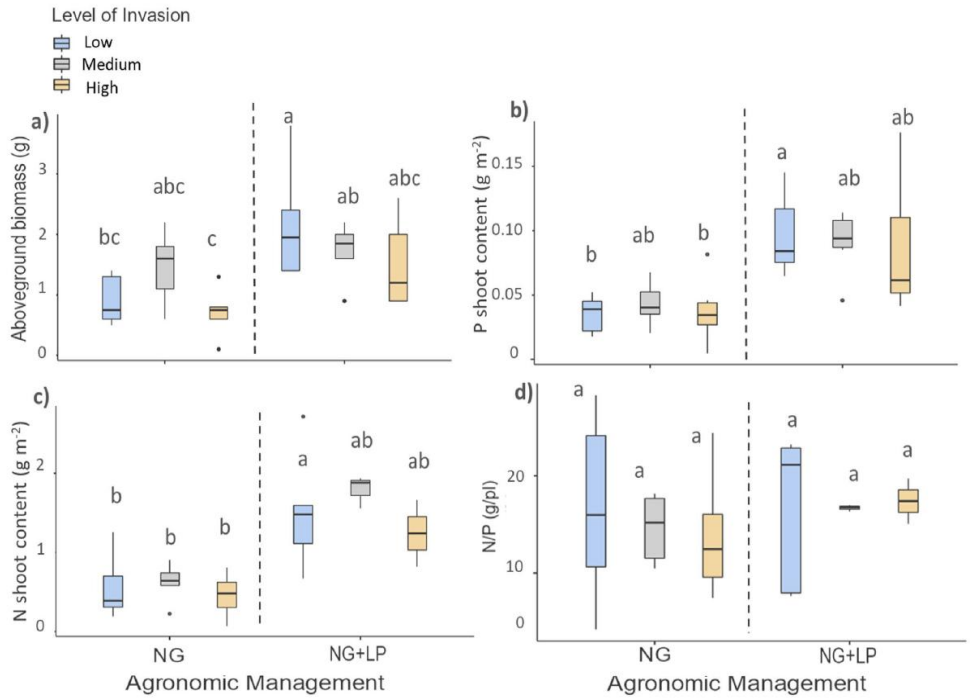


Figure 3. Box plots (n = 6 per treatment) of aboveground biomass (a), P shoot content (b), N shoot content (c), and N:P ratio in above-ground biomass (d) of Paspalum notatum plants growing with low (0%–10% in blue), medium (30%–50% in gray), or high (70%–90% in orange) levels of Cynodon dactylon invasion, in natural grasslands managed either extensively (NG) or oversown with exotic legumes and fertilized with phosphorus (NG + LP). Different letters above boxplots indicate significant statistical difference among treatments ($p < 0.05$) using the Least Significant Difference (LSD) test adjusted by Bonferroni.

Table 2. Results of linear mixed effect models (with normal error distribution) that tested the effects of treatments on Aboveground biomass, P shoot content, N shoot content, and N:P shoot ratio. Treatment contrasts, with the intercept representing the level NG (natural grassland) and 0%–10% (low invasion), are shown. NG + LP refers to native grassland oversown with exotic legumes and fertilized with phosphorus (intensive management), whereas 30%–50% and 70%–90% refer to medium and high levels of invasion by *Cynodon dactylon*.

| Response Variable | Treatment | Estimate | S.E | t value | Pr(>\|t\|) |
|---|---|---|---|---|---|
| | Intercept | 0.88 | 0.21 | 4.15 | 0.003 |

| | | | | | |
|---|---|---|---|---|---|
| Aboveground biomass | NG+LP | 1.14 | 0.32 | 3.60 | 0.001 |
| | 30-50 | 0.28 | 0.30 | 0.94 | 0.354 |
| | 70-90 | -0.17 | 0.30 | -0.55 | 0.584 |
| | NG+LP *30-50 | -0.57 | 0.44 | -1.31 | 0.202 |
| | NG+LP*70-90 | -0.33 | 0.45 | -0.75 | 0.462 |
| P shoot content | Intercept | 0.04 | 0.01 | 2.85 | 0.008 |
| | NG+LP | 0.06 | 0.02 | 3.38 | 0.002 |
| | 30-50 | 0.01 | 0.02 | 0.45 | 0.647 |
| | 70-90 | <0.01 | 0.02 | 0.14 | 0.889 |
| | NG+LP*30-50 | -0.01 | 0.03 | -0.56 | 0.557 |
| | NG+LP*70-90 | -0.01 | 0.03 | -0.44 | 0.662 |
| N shoot content | Intercept | 0.55 | 0.18 | 3.09 | 0.005 |
| | NG+LP | 0.96 | 0.26 | 3.64 | 0.001 |
| | 30-50 | 0.07 | 0.25 | 0.29 | 0.778 |
| | 70-90 | -0.09 | 0.25 | -0.37 | 0.717 |
| | NG+LP*30-50 | 0.20 | 0.41 | 0.50 | 0.619 |
| | NG+LP*70-90 | -0.18 | 0.44 | -0.41 | 0.687 |
| N:P shoot ratio | Intercept | 16.68 | 2.82 | 5.91 | 0.004 |
| | NG+LP | -0.21 | 3.99 | -0.05 | 0.958 |
| | 30-50 | -2.06 | 3.80 | -0.54 | 0.594 |
| | 70-90 | -2.92 | 3.80 | -0.77 | 0.451 |
| | NG+LP*30-50 | 1.82 | 6.16 | 0.30 | 0.770 |
| | NG+LP*70-90 | 3.38 | 6.72 | 0.50 | 0.619 |

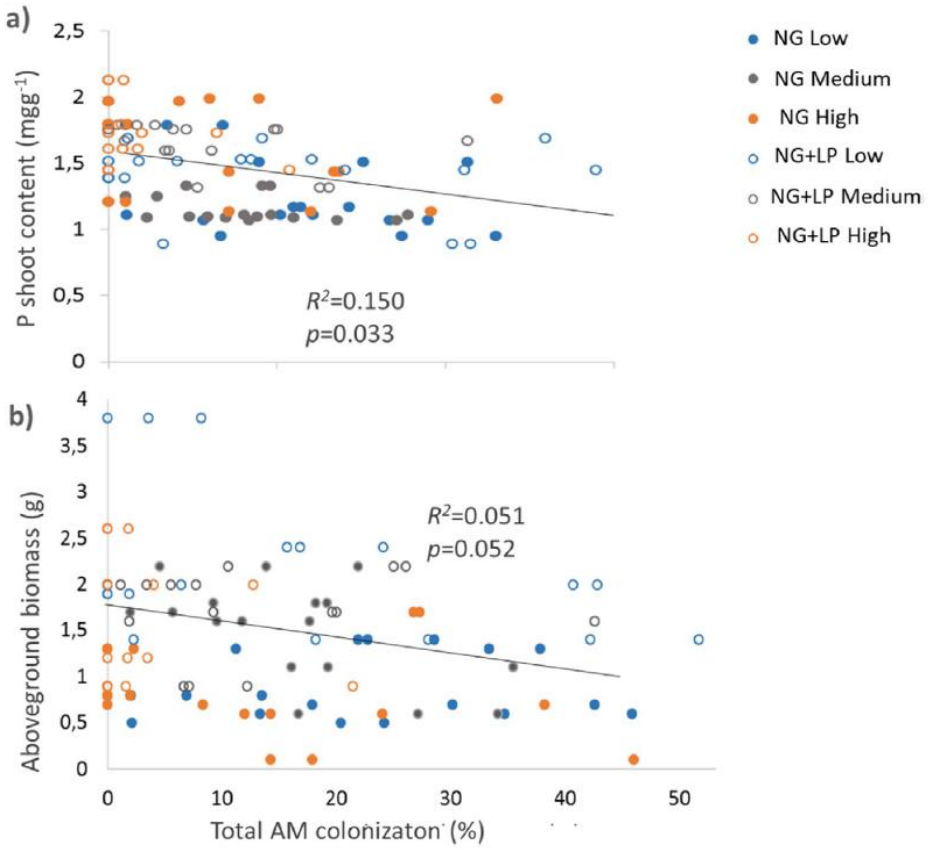


Figure 4. Relationship between P shoot content and total arbuscular mycorrhizal colonization (a), and between aboveground biomass and total arbuscular mycorrhizal colonization (b) in Paspalum notatum plants growing with low (0%–10% in blue), medium (30%–50% in gray), and high (70%–90% in orange) levels of Cynodon dactylon invasion, in natural grasslands managed either extensively (NG, closed circles) and oversown with exotic legumes and fertilized with phosphorus (NG + LP, open circles). See Tables 3 and 4 for statistical details

### 4.6. Discussion

We expected that higher levels of invasion by *C. dactylon* would be associated with a reduction in mycorrhizal colonization of *P. notatum* and that this effect would be more important in NG+LP. As a consequence of this mutualism degradation, we expected to find negative effects on the growth and nutritional status of *P. notatum*. Our results show that the level of invasion by *C. dactylon* was consistently associated to a decline in arbuscular mycorrhizal colonization of *P. notatum*, particularly total

and vesicular colonization. This likely disruptive effect was more pronounced on grasslands that had been oversown with an exotic legume and fertilized with P. However, lower mycorrhizal colonization in *P. notatum* was not associated with lower aboveground biomass or nutrient status. *Paspalum notatum* shows a high presence of arbuscular mycorrhizas, as is typical for species with thick roots (Hartnett and Wilson, 1999; Pezzani et al. 2006; García et al. 2016, 2019; Michelini et al. 2024). Indeed, experimentally induced 4- to 6-fold increases in soil available P have been shown to barely reduce this symbiosis in *Paspalum dilatatum* and *Mnesithea selloana*, two common native grasses that coexist with *P. notatum* (García et al. 2016), without any reduction in arbuscular mycorrhizal fungi diversity (García et al. 2017). Hence, is truly notable that *C. dactylon* invasion was so closely associated with a disruption of such a stable symbiosis between mycorrhizal colonization and a native grass. Questions regarding the actual mechanism underlying this effect and its consequences for the competitiveness of invasive species are still open.

Although our methodological approach cannot disentangle cause-effect relationships between the level of invasion and mycorrhizal colonization, we suggest that *C. dactylon* can likely degrade the mycorrhizal symbiosis of a native species, supporting the hypothesis of mutualism degradation (*c.f.* Vogelsang et al. 2004; Vogelsang and Bever, 2009; Hale and Kalisz, 2012; Traveset and Richardson, 2014; Roche et al. 2023). In this study, we selected patches with different levels of invasion that were contiguous, relatively small and within the same soil type and plant community in paddocks with contrasting long-term management. This has allowed us to suggest relationships between levels of invasion and arbuscular mycorrhization, but an experiment in which levels of invasion are manipulated is essential to demonstrate causality. Mycorrhizal mutualism degradation is proposed as an important strategy of invasive species to establish in new environments, particularly when an invasive species is non-mycorrhizal, or has low dependence on mycorrhizae (Grove et al. 2017).

Worldwide, *C. dactylon* has been reported as non-mycorrhizal or facultative mycorrhizal, as it has shown either no arbuscular mycorrhizal structures (Muthukumar and Udaiyan, 2000; Muthukumar et al. 2006), very few fungal structures (Endresz et al. 2013), or absence of nutrient exchange structures (Lugo et al. 2018). In Uruguayan

grasslands, *C. dactylon* has very low arbuscular mycorrhizal colonization (Michelini et al. 2024), whereas in invaded Uruguayan grasslands we have corroborated that *C. dactylon* is less colonized and has fewer exchanges structures than native species (Michelini et al. 2024.). Lack of arbuscular mycorrhizae in the invasive species could limit the allocation of energy to the mycorrhizal fungi by outcompeting native species that do rely on this interaction (Grove et al. 2017), eventually, leading to loss of fungi that are specific to native species. Furthermore, *C. dactylon* has been reported to be potentially allelopathic (Golparvar et al. 2015; Favaretto et al. 2018; Guido et al. 2020; Bresciano et al. 2022), which can reduce the germination of arbuscular mycorrhizal spores (Roberts and Anderson, 2001; Stinson et al. 2006), spore density (Callaway et al. 2008) and mycorrhizal colonization (Barto et al. 2011). Evidence of allelopathy as an important mechanism disrupting microorganism-plant mutualisms is widespread (Hale and Kalisz, 2012; Grove et al. 2017; Pinzone et al. 2018; Roche et al. 2021, 2023), and is considered an important strategy in plant invasions.

One of the scenarios where arbuscular mycorrhizal disruption is widely reported is in nutrient-enriched environments (Johnson et al. 2010; Chen et al. 2014; Grove et al. 2017; Garcia et al. 2016; Yazici et al. 2021). In these cases, arbuscular mycorrhizae can become a burden for host plant (Grimoldi et al. 2006). Therefore, increases in nutrient availability would reduce C allocation to fungus (Konvalinková et al. 2017) and decrease in root colonization by arbuscular mycorrhizal fungi (Chen et al. 2014; Garcia et al. 2016; Yazici et al. 2021). However, our results indicate that mycorrhizal degradation would not be explained just by increased P availability, but suggest a strong, intrinsic invasion effect.

Degradation of arbuscular mycorrhizal symbiosis due to an increase in invasion level was greater in NG+LP, suggesting that whatever mechanism underlies the effects of invasive species on arbuscular mycorrhizal performance is exacerbated in more intensively managed grasslands. For example, greater production of toxic compounds for soil microorganisms in NG+LP compared to NG may explain the disruption of mutualism. Stronger allelopathy in response to increased nutrient availability has been reported in three Compositae invasive species affecting native plants (Sun et al. 2022). Furthermore, *C. dactylon* plant development might have been greater in NG+LP after

the experimental 10-months period, leading to more intense light competition. A larger *C. dactylon* root system will increase the chance of encountering mycorrhizal spores in soil, making them unavailable to colonize *P. notatum* roots. To test this, it would be interesting to study how the invasive grass affects the arbuscular mycorrhizal spore bank.

Contrary to our expectations, reduced arbuscular mycorrhizal colonization was not associated with reductions in aboveground biomass nor P shoot content of the native species. These results, together with the fact that no differences in mycorrhizal colonization were observed between NG and NG+LP in low-invasion situations, suggest that the success of *C. dactylon* invasion, disrupting the arbuscular mycorrhizal symbiosis in *P.* notatum, would not be mediated by P. Instead, degradation of the mycorrhizal interaction might be primarily due to a direct impact of *C. dactylon* on soil mycorrhizae propagules. Future research is need to verify this possible mechanism of degradation and analyze its effect on the plant community as a whole.

We show that the aboveground biomass of *P. notatum* and its P shoot content were unaffected by the invasion. This could be occurring at the expense of energy allocated to belowground organs or to mycorrhizal symbiosis, as it may be indicated by the decrease in arbuscular mycorrhizae vesicles (reserve structures of the arbuscular mycorrhizae) as the invasion levels increases. In order to test this, it would be necessary to measure other native plant performance variables, particularly belowground organs that could be affected by *C. dactylon* invasion.

### 4.7. Conclusions

This is the first study to assess whether invasion success can be associated to disruption of a stable symbiosis of recipient species under different nutrient conditions linked to different agronomic management. Our results showed that the level of invasion of *C. dactylon* was coupled to a reduction in the mycorrhizal colonization of the dominant native grass *P. notatum,* and that this reduction was exacerbated in more intensively managed grasslands. However, neither competition with the invasive nor the reduction in arbuscular mycorrhizal colonization due to *C. dactylon* invasion were

associated with a decrease in the aboveground biomass or nutritional status of *P. notatum.*

Considering that *P. notatum,* as several other thick rooted native species in Uruguayan grasslands, is highly colonized by arbuscular mycorrhizae (García et al. 2016; Del Pino et al. 2022; Michelini et al. 2024), this symbiosis might be providing plants in native grasslands benefits beyond nutritional ones. For instance, resistance to drought (Kakouridis et al. 2022; Romero-Munar et al. 2024), or protection against pests and diseases (Gange et al. 1994; Newsham et al. 1995; Smith and Read, 2008; Weng et al. 2022). The loss of this stable symbiosis can have individual effects such as reducing native plant growth and community-wide effects such as impacts on native plant diversity (Bever et al. 2001; Castillo et al. 2016), turning the system more vulnerable to plant invasion.

Since our study focused on the interaction between *C. dactylon* and only one native species in a controlled setting, extrapolations of these results should be taken with caution. In order to be able to scale-up conclusions about the role of mycorrhizal disruption in the success of *C. dactylon* invasion, future research would benefit from investigating the response of other (subordinate) native species that may be more dependent on mycorrhizae to compete with this invasive plant in a controlled experiment for disentangle some causal relationships.

Investigating the effect of the invasive plant on other variables related to arbuscular mycorrhizae may help to identify the precise mechanism by which the mutualism is disrupted. For example, studying the colonization rates in the invasive plant could allow us to determine whether mycorrhizae promote it competitive ability. On the other hand, analyzing the changes in fungal community composition of both *C. dactylon* and native plants in different invasion situations could show which fungal taxa are favored or harmed by the invasion.

**Author Contributions**

S.G., F.P., A.G., and F.L. conceived the research idea, collected data, commented on the manuscript, and discussed the results. S.G. performed statistical analyses and wrote the paper.

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The authors declare no conflicts of interest.

**Data Availability Statement**

The data used in this publication are freely available at the Repositorio de datos abiertos de investigación de Uruguay: https:// doi. org/ 10. 60895/ redata/ FQJPDM.Rebeca Gonet, and Lucila Bentancour for helping with field sampling, and Cecilia Bardier and Diego Michellini for supporting the data analysis.

## 4.8. References

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**4.9. Supporting Information to the paper**

Appendix S1. Geographic coordinates of the four sampled paddocks corresponding to two sites (A and B) with two different agronomic management (NG: natural grassland and NG+LP: NG oversown with exotic legumes and fertilized with P; n=6).

| | NG | NG+LP |
|---|---|---|

| | | |
|---|---|---|
| Site A | -33.87186, -55.58325 | -33.87402, -55.58710 |
| Site B | -33.87020,-55.58316 | -33.86631, -55.58411 |

Appendix S2. Soil chemical characteristics (0-10 cm horizon) at the site where monoliths were collected. NG: natural grasslands, NG+LP: NG oversown with exotic legumes and fertilized with P

| | NG | NG+LP |
|---|---|---|
| P Bray, ppm | 3.2 | 5.8 |
| K, cmol kg-1 | 0.4 | 0.34 |
| Ca, cmol kg-1 | 4.7 | 5.3 |
| Mg, cmol kg-1 | 1.8 | 1.7 |
| pH | 5.1 | 5.0 |
| Organic C, % | 2.5 | 2.7 |

Appendix S3. Average (n=6) of the total arbuscular mycorrhizal colonization, colonization by vesicles and colonization by exchange structures (arbuscles and coils) in Paspalum notatum growing in monoliths with neighbors species removal (species removal +) and without neighbors species removal (species removal -). In the study, all species other than P. notatum and C. dactylon were manually removed to prevent confounding effects of the identity of neighboring species on mycorrhizal colonization. To assess whether disturbance caused by mechanical removal of neighboring plants affected mycorrhizal status of P. notatum (i.e., manipulation artifact), mycorrhizal colonization was measured in six extra monoliths collected, without species removal.

Different letters indicate a significant difference among the means of the treatments ($P < 0.05$) according to LSD test.

| | Species removal+ | Species removal - |
|---|---|---|
| Total Colonization (%) | 16.6 ± 9.5a | 17.2 ± 4.4a |
| Vesicles (%) | 5.22 ± 4.9a | 6.09 ± 4.6a |
| Arbuscles and Coils (%) | 0.52 ± 1.5a | 0.37 ± 0.6a |

(n=6 ± SE)

Appendix S4. Results of Generalized Linear Mixed Effect Models (with binomial error distribution) that tested the effects of treatments on mycorrhizal colonization, showing the degree of freedoms. AgrManrefers to the agronomic management (NG and NG+LP) and LI refers to the level of invasion by Cynodon dactylon.

| Variable | Predictors | DF | F value | P value |
|---|---|---|---|---|
| Total colonization | AgrMan | 1 | 42.97 | <0.01 |
| | LI | 2 | 57.65 | <0.01 |
| | AgrMan *LI | 2 | 17.04 | <0.01 |
| Vesicles | AgrMan | 1 | 17.49 | <0.01 |
| | LI | 2 | 29.43 | <0.01 |
| | AgrMan *LI | 2 | 9.02 | 0.01 |
| Arbuscles and Coils | AgrMan | 1 | 0.73 | 0.39 |
| | LI | 2 | 6.81 | 0.01 |
| | AgrMan *LI | 2 | 4.23 | 0.02 |

Appendix S5. Results of multiple linear regression with P shoot content and Aboveground biomass as response variable, agronomic management (AgrMan) and level of invasion (LI) as fixed factors, and total arbuscular mycorrhizal colonization (TC) as a covariable, showing the degree of freedoms.

| Variable | Predictors | DF | F value | P value |
|---|---|---|---|---|
| Abovegroun d biomass | AgrMan | 1 | 21.6 | 0.01 |
| | LI | 2 | 1.44 | 0.25 |
| | AgrMan *LI | 2 | 0.86 | 0.43 |
| P shoot | AgrMan | 1 | 26.24 | 0.01 |
| | LI | 2 | 0.05 | 0.95 |
| | AgrMan *LI | 2 | 0.17 | 0.84 |
| N shoot | AgrMan | 1 | 31.60 | <0.01 |
| | LI | 2 | 1.79 | 0.19 |
| | AgrMan *LI | 2 | 0.34 | 0.71 |
| N:P shoot | AgrMan | 1 | 0.31 | 0.58 |
| | LI | 2 | 0.18 | 0.83 |
| | AgrMan *LI | 2 | 0.13 | 0.88 |

## 5. Discusión general y conclusiones

A través de distintos enfoques experimentales, esta tesis abordó el estudio de las interacciones bióticas entre la principal especie vegetal invasora de los pastizales de Uruguay, *Cynodon dactylon*, y dos especies nativas muy frecuentes en las comunidades vegetales del país. Comprender estas interacciones y los factores que las regulan resulta esencial para identificar los mecanismos ecofisiológicos determinantes del éxito de invasión a escala local. A pesar de la ubicuidad de *C. dactylon* como especie invasora, es notoriamente escasa la información sobre su dinámica en pastizales. De hecho, la mayor parte de la literatura existente sobre esta especie hace foco en su rol como forrajera (cultivares de *bermudagrass*) y como césped (*turfgrass*,

Wu, 2011). Los estudios mecanísticos de su biología como invasora son escasos, mayormente concentrados en sistemas agrícolas en los que no existe una comunidad receptora y con énfasis en respuestas a manejos de control (Soares et al., 2023). En particular, hay muy pocos estudios que busquen comprender la habilidad competitiva de *C. dactylon* en comparación con especies nativas y particularmente qué situaciones promueven su éxito. Por ende, es escasa la información disponible para sustentar el diseño de medidas efectivas tanto de prevención como de control de su invasión en los pastizales de Uruguay.

Los resultados obtenidos muestran que *C. dactylon* tiene una clara superioridad competitiva respecto a dos gramíneas nativas muy frecuentes de los pastizales de Uruguay, tanto en condiciones de baja como de alta disponibilidad de nutrientes. Asimismo, la especie invasora mostró una mayor habilidad competitiva cuando el incremento de nutrientes ocurrió tanto en la etapa incial (siembra) como en etapas posteriores de establecimiento de las plantas (tres meses después). La estrategia competitiva de esta invasora se basa en la capacidad de tolerar la presencia de plantas vecinas, así como de afectar negativamente su crecimiento, debido a su patrón de asignación de biomasa. La gramínea invasora presentó de forma consistente mayor asignación a biomasa aérea, en comparación con las especies nativas, en todas las situaciones evaluadas, comportamiento que ha sido reportado en otras especies invasoras (van Kleunen et al., 2010). Específicamente este patrón estuvo explicado por su alta e invariable asignación a estolones, estructuras eficientes en la propagación vegetativa y la rápida colonización de suelo desnudo (Holm et al., 1977; Horowitz, 1996).

En concordancia con la hipótesis del disturbio y la hipótesis de los recursos fluctuantes, nuestros resultados muestran que la intensificación productiva genera condiciones que favorecen la invasión de *C. dactylon*, al disminuir la resistencia biótica y liberar recursos disponibles. La gran asignación de energía a estolones en la gramínea invasora podría explicar el éxito de invasión en situaciones de sobrepastoreo (Altesor et al., 1998, 2005) y en mejoramientos de campo, en particular los que se localizan en el sur y este del país (Jaurena et al., 2015; Pañella et al., 2022). Aunque previamente se ha reportado una relación positiva entre el nivel de P disponible y la

cobertura de *C. dactylon* en los mejoramientos de campo de las regiones centro sur y sierras (Cáceres, 2019; Jaurena et al., 2015), nuestros resultados muestran que el éxito de *C. dactylon* se debería principalmente a una superioridad competitiva por el espacio, más que por su habilidad de captar nutrientes. En estos sistemas, la baja persistencia de las leguminosas sembradas (Jaurena et al., 2015; Pañella et al., 2022) genera frecuentemente claros en la vegetación que abren una oportunidad para la colonización de *C. dactylon*. Este fenómeno podría acentuarse si se utilizan leguminosas anuales que cumplen su ciclo de vida en una sola estación y luego dejan espacios descubiertos hasta que germinan nuevamente. Debido a su alta inversión en estolones, *C. dactylon* colonizaría de manera eficiente estos espacios y consumiría rápidamente los recursos liberados. Además, los estolones representan órganos de bajo costo energético para la planta (Chapin et al., 1990), lo que constituye una ventaja en presencia de competidores. La alta invasión de *C. dactylon* en situaciones de sobrepastoreo podría estar explicada por una rápida capacidad de rebrote tras la defoliación y por su exitosa propagación vegetativa para ocupar espacios vacíos (Zwerts et al., 2015). Además, parece ser menos preferida por el ganado en comparación con algunas gramíneas nativas (Altesor et al., 1998; 2005), lo que también favorece su proliferación. Por lo tanto, el pastoreo podría mediar el éxito de establecimiento de *C. dactylon* en los mejoramientos de campo, donde la presión del pastoreo suele ser mayor que en los pastizales naturales. Además, el consumo preferencial de leguminosas por parte del ganado podría favorecer la aparición de nuevos espacios vacíos en la vegetación que podrían ser rápidamente ocupados por *C. dactylon*.

La mayor presencia de *C. dactylon* en mejoramientos de campo con respecto a pastizales naturales puede estar relacionada con la resistencia biótica a la invasión que ejercen las comunidades de ambos sistemas. Tal como propone la hipótesis de resistencia biótica, comunidades más diversas mostrarían menor invasibilidad, ya que ocurre una optimización en la utilización de los recursos disponibles y deja menos oportunidades para posibles invasores (Elton, 1958). La menor diversidad vegetal en los mejoramientos, en comparación con los pastizales naturales (Jaurena et al., 2015; Pañella et al., 2022), reduciría la resistencia biótica del sistema, lo que incrementaría su vulnerabilidad a la invasión. En este sentido, Wang et al. (2024) reportan que

especies invasoras clonales en comunidades con baja diversidad logran propagarse y ocupar rápidamente espacios (Wang et al., 2024). El rol de la diversidad en la resistencia biótica de las comunidades de pastizales podría ser uno de los factores que explica por qué ocurre la invasión por *C. dactylo*n en los mejoramientos de campo del sur y no en la región del basalto (Cáceres, 2019; Guido et al., 2024). Los estudios sobre los pastizales naturales de la cuesta basáltica uruguaya revelan una heterogeneidad florística notable, con una alta riqueza local (Lezama et al., 2006). Otro aspecto que estaría explicando la resistencia biótica de los pastizales del basalto es la composición diferencial de grupos funcionales de gramíneas. En esta región, el tapiz vegetal está dominado por especies con metabolismo $C_4$ postradas (Millot et al., 1987), que ejercerían una mayor competencia sobre la invasora por tratarse del mismo grupo funcional (MacArthur y Levins, 1967). Otro de los factores que afectarían la baja invasión en la región de basalto es la menor presión de propágulos de *C. dactylon* en dicha región (Bresciano et al., 2014; Guido et al., 2023), que se encuentra en mayor estado de conservación con respecto al centro-sur y las sierras del este (Altesor et al., 2019). En futuros estudios sería interesante incluir una gramínea nativa estolonífera y de metabolismo $C_4$ que, al igual que la especie invasora, sea capaz de producir estructuras de expansión vegetativa. Dada la capacidad de ocupación espacial que confieren los estolones y considerando la hipótesis de similitud límite, la cual propone que la competencia es mayor en especies funcionalmente similares (MacArthur y Levins, 1967), se espera que una gramínea nativa con este atributo afecte en mayor medida el desempeño de *C. dactylon*.

Nuestros resultados sugieren que la alelopatía no sería el principal mecanismo explicando la habilidad competitiva de *C. dactylon* en nuestros resultados. La alelopatía ha sido ampliamente reportada como un mecanismo de invasión, ya que, tal como propone la hipótesis de las armas novedosas (*novel weapons hypothesis*), los compuestos que resultan relativamente ineficaces frente a especies vecinas adaptadas en el rango nativo pueden ejercer efectos inhibitorios sobre las plantas en el rango invasor (Callaway y Ridenour, 2004; Ridenour y Callaway, 2001). Sin embargo, el estudio de la alelopatía es desafiante y pocas veces puede confirmarse, debido a que los distintos enfoques presentan limitaciones inherentes que dificultan su

extrapolación a campo. Particularmente la metodología empleada en nuestro trabajo cuenta con varias limitaciones ya que nos permite estimar la alelopatía de manera indirecta y no permitiría descartarla completamente.

Por otro lado, otros abordajes metodológicos a nivel nacional y también muestran escasa evidencia de alelopatía en *C. dactylon*. Por un lado, Bresciano et al. (2022) no reportaron un efecto significativo de la producción de aleloquímicos sobre la germinación de especies nativas. Por otro lado, Guido et al. (2020) observaron ciertos efectos inhibitorios, pero estos no fueron diferentes a los que produciría una especie nativa de la comunidad residente. Cabe destacar que nuestro trabajo no evaluó directamente el efecto de la alelopatía sobre las plantas, sino que, mediante el uso de carbón activado, eliminamos los compuestos alelopáticos para estimar su efecto relativo en la interacción competitiva. Futuros experimentos podrían abordar el efecto del agregado de compuestos alelopáticos sobre plantas nativas ya establecidas, como las utilizadas en este estudio, y en condiciones más realistas a las que se presentan en pastizales invadidos.

Un resultado novedoso e importante de resaltar es que *C. dactylon* produjo inflorescencias únicamente en presencia de sus especies vecinas. Esta respuesta sugiere un caso de plasticidad fenotípica por parte de la especie invasora, un rasgo común en plantas exóticas con alta invasividad (Silva et al., 2020; Zhang et al., 2021). Tal plasticidad podría representar una estrategia para asegurar la reproducción cuando compite con otras especies (Aarssen, 2008). Estudios en condiciones controladas verificaron que una de las respuestas de *C. dactylon* al sombreo es la producción de macollos verticales (ortotrópicos), que normalmente terminan en inflorescencias (Dong y De Kroon, 1994; Dong y Pierdominici, 1995). En condiciones de campo, esta respuesta podría conferirle ventajas adaptativas, lo que le permitiría ajustar su inversión reproductiva según la intensidad de la competencia y favorecería su expansión en ambientes heterogéneos. En campos más conservados —como pastizales naturales con baja presión de pastoreo, mayor densidad y altura de vegetación— *C. dactylon* podría incrementar su inversión en reproducción sexual como una estrategia para alcanzar mayores distancias y evitar parches con alta presencia de especies competidoras. Conocer el comportamiento reproductivo de esta especie invasora es

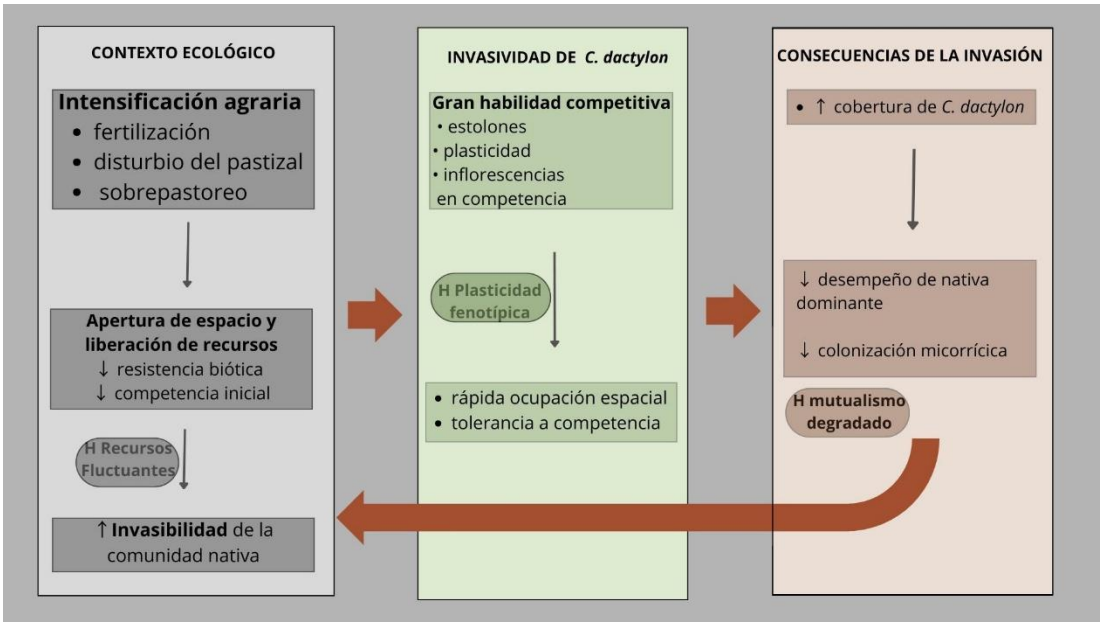
crucial para determinar medidas de restauración y conservación de los pastizales: en campos muy degradados o sobrepastoreados, el desafío es controlar la propagación vegetativa; en campos conservados, el énfasis debe ponerse en evitar la dispersión sexual y mantener la diversidad nativa.

Nuestros resultados sugieren que *C. dactylon* fue capaz de degradar la colonización micorrírica arbuscular de *Paspalum notatum*, una gramínea nativa dominante de los pastizales de Uruguay con alta y estable asociación con micorrizas (García et al., 2025), y podría constituir un mecanismo de retroalimentación positiva en el proceso de invasión por *C. dactylon*, al debilitar interacciones suelo–planta clave para las especies nativas. Este efecto fue exacerbado en situaciones de mejoramientos de campo. El mecanismo y las consecuencias funcionales de esta respuesta son poco claros. El hecho de que perjudique un mutualismo tan conservado en gramíneas nativas sugiere que este mecanismo también podría estar involucrado en el éxito de invasión, por lo que es necesario seguir profundizando en su estudio. Esta simbiosis ha mostrado ser muy estable entre las gramíneas de los pastizales de Uruguay, manteniendo una gran abundancia y diversidad aun en sistemas de pastizales perturbados y enriquecidos en nutrientes (García et al., 2016; 2017). Se abren entonces nuevas preguntas sobre el rol de las micorrizas en gramíneas nativas, ya que, contrariamente a lo esperado, la reducción en la micorrización no se correlacionó con la biomasa ni el contenido de fósforo de la gramínea nativa. Esto sugiere que los hongos micorrícicos en las raíces de *P. notatum* estarían proporcionando beneficios adicionales más allá de la adquisición de nutrientes, tales como resistencia al estrés ambiental. Si bien estos resultados no indican que la degradación del mutualismo micorrícico en *P. notatum* afecte su desempeño, es necesario profundizar en cómo se ve impactada la diversidad de hongos micorrícicos y el banco de esporas de la comunidad de pastizal. El estudio de la diversidad micorrícica en distintos contextos de invasión permitiría identificar taxones fúngicos que podrían estar favoreciendo a la especie invasora. Por otro lado, un banco de esporas negativamente afectado perjudicaría a otras especies del pastizal con mayor dependencia de las micorrizas. En este mismo sentido, resulta fundamental ampliar el estudio a especies subordinadas que dependen más estrechamente de la

simbiosis micorrícica (Michelini et al., 2024) para adquirir nutrientes y competir eficazmente con la invasora.

Los resultados de esta tesis evidencian el rol de las interacciones bióticas en el proceso de invasión de *C. dactylon,* especie que representa una amenaza para los pastizales naturales de Uruguay y que es una problemática ya instalada en pastizales fertilizados y sembrados con leguminosas (Jaurena et al., 2015; Pañella et al., 2022), impactando negativamente su biodiversidad y funcionamiento ecológico. El presente trabajo permitió arrojar luz sobre los mecanismos subyacentes que favorecen la invasión de esta gramínea exótica en diferentes contextos de manejo y disponibilidad de recursos. En particular, para situaciones de mejoramiento de campo, nuestros resultados indican que el incremento de nutrientes no sería el principal factor que determina el éxito de la invasión, ya sea a través de la competencia o mediante la alteración de interacciones mutualistas, como las micorrizas. En su lugar, otros rasgos, como su elevada capacidad para ocupar espacios y ajustar su estrategia reproductiva, tendrían un papel más determinante. En conjunto, tal como se resumen en la figura 2, los resultados de esta tesis indican que la invasión de *C. dactylon* en los pastizales de Uruguay no puede explicarse por un único mecanismo, sino que emerge de la interacción entre la intensificación productiva, el debilitamiento de la resistencia biótica y rasgos funcionales que confieren tolerancia competitiva, plasticidad fenotípica y alteración de los mutualismos micorrícicos. Esta convergencia de mecanismos concuerda con el caracter multicausal y contexto-dependiente de los procesos de invasión propuesto en la literatura.

**Figura 1.** Esquema conceptual que ilustra el efecto de la intensificación agraria sobre la invasibilidad de los pastizales naturales y la invasión por *Cynodon dactylon*. La intensificación (fertilización, disturbio y sobrepastoreo) genera liberación de recursos y disminución de la resistencia biótica, favoreciendo la invasión. La alta capacidad competitiva de *C. dactylon* promueve su expansión, lo que conduce a una reducción del desempeño de la especie nativa dominante y de la colonización micorrícica, generando un proceso de retroalimentación que incrementa la invasibilidad del sistema.




Profundizar en el estudio de las interacciones bióticas entre *C. dactylon* y otras especies nativas, especialmente aquellas funcionalmente similares a *Cynodon dactylon*, como por ejmplo *Axonopus affinis*, es clave para desarrollar estrategias de manejo más eficaces. Sin embargo, aún quedan interrogantes acerca de la habilidad competitiva y el desempeño de una gramínea nativa estolonífera en condiciones de campo, dado que este tipo de órgano representa una proporción considerable de biomasa aérea que queda expuesta a eventos de remoción, como el pastoreo, una perturbación muy frecuente en los pastizales de nuestro país. Comprender las complejas relaciones ecológicas que ocurren en los pastizales —incluidas las interacciones con mutualismos benéficos, como micorrizas u organismos fijadores de

nitrógeno— permitirá diseñar medidas de control integrales y eficaces en reducir la vulnerabilidad de estos agroecosistemas a colonización de especies invasoras. Dado que la liberación de recursos favorece la propagación de *C. dactylon*, aplicar prácticas de manejo que minimicen la generación de espacio disponible (por ejemplo, ajuste estacional de la carga animal) se vuelven fundamentales para disminuir las perturbaciones y, con ello, limitar el avance de la especie invasora.

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# 7. Anexos

## 7.1. Invasion strategies of *Cynodon dactylon*: Competitive ability under low-nutrient conditions[1]

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RESEARCH ARTICLE

# Invasion strategies of *Cynodon dactylon*: Competitive ability under low-nutrient conditions

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**Abstract**

*Cynodon dactylon* is one of the five most important invasive alien species worldwide. It is the invasive alien species with the broadest distribution range in Uruguay, and its expansion is frequently associated with disturbances. Since natural grasslands are facing processes of productive intensification, *C. dactylon* represents a threat as it could displace native species. However, the mechanisms that explain its invasion success remain unclear. The objective of this study was to analyse interspecific interactions under low nutrient conditions between *C. dactylon* and two species that are native to the *Campo* grasslands in Uruguay. Specifically, we assessed differences in the components of competitive ability effects and responses (or tolerance) as possible mechanisms involved in *C. dactylon* invasiveness. We performed a greenhouse experiment in pots with low-nutrient substrate assessing pair-wise interactions between *C. dactylon*, *Mnesithea selloana* and *Paspalum notatum* plus control pots consisting of single individual of each species. The invasive species showed greater competitive ability than both native grasses, as it reduced their below and above-biomass. Conversely, the size of *C. dactylon* plants interacting with native species was similar to that of single *C. dactylon* plants growing alone (controls). This reveals that the greater competitive ability of the invasive species was due to a greater tolerance to grow with neighbouring plants. The reason underlying this tolerance was a marked increase in biomass allocation towards stolons and leaves, at the expense of roots. Conversely, native species barely changed their shoot-root allocation pattern when interacting with neighbours. Furthermore, *C. dactylon* induced reproductive development solely when interacting with neighbours. Along with the fact that the potential growth rate of the invasive and native species was quite similar, these results suggest that sensitive and rapidly triggered shade avoidance responses could be one mechanism involved in the invasion success of *C. dactylon*.

**KEYWORDS**
biological invasions, competitive ability, *Mnesithea selloana*, *Paspalum notatum*, Uruguayan campo grasslands

## INTRODUCTION

Invasive plant species impact terrestrial ecosystems by reducing their biodiversity, changing nutrient pools and altering disturbance regimes (Reichmann et al., 2016), with potentially significant changes in the functioning of, and the services provided by, natural ecosystems (Mack et al., 2000; Pysek et al., 2012; Vila et al., 2011). *Cynodon dactylon* is one of the five most important invasive alien species worldwide, colonizing different types of ecosystems around the world (Holm et al., 1991). It is a perennial, prostrate, fine-leaved and warm season grass, which is native to Africa and Europe. It extends throughout tropical and subtropical

  

areas, well-established into temperate climates and coastal zones (Harlan & de Wet, 1969; Holm et al., 1991). In Uruguay, as in many places worldwide (Holm et al., 1991), *C. dactylon* is the most frequent and extended invasive alien plant species (Bresciano et al., 2014). In agroecosystems dominated by crop-pasture sequences, *C. dactylon* expands during the pasture phase but it is effectively controlled with herbicides during the phase with glyphosate-resistant crops. Currently, *C. dactylon* is also becoming a frequent problem in agroecosystems dominated by native grasslands, particularly when overgrazed (Altesor et al., 1998, 2005) or overseeded with legumes and fertilized with phosphorus (P; Jaurena et al., 2015; Pañella et al., 2022).

*Campo* grasslands, part of the Río de la Plata grasslands and occupying more than 60% of Uruguay (Baeza & Paruelo, 2020; MGAP, 2011), are highly biodiverse, with more than 3000 species of temperate and subtropical plants (Andrade et al., 2018; Bilenca & Minarro, 2004). In Uruguay, five physiognomic types of *Campo* grassland communities are distinguished based on the density and height of the vegetation, but in all cases, it is a heterogeneous mosaic composed of warm season shortgrasses and to a lesser extent of tall grasses warm season shortgrasses (Lezama et al., 2019). *C. dactylon* invasions easily trigger almost irreversible processes of degradation that end up in monospecific stands (e.g. Altesor et al., 2019; Jaurena et al., 2015; Pañella et al., 2022). Undisturbed *Campo* grasslands still show low *C. dactylon* incidence (Bresciano et al., 2014; Lezama et al., 2019; Pañella et al., 2022), but its presence is increasing under the current context of intensification of livestock production (Pañella et al., 2022), threatening its productivity and resilience which are both based on the high biodiversity of this agroecosystem (Jaurena et al., 2021). Furthermore, since it does not exist as a selective herbicide against *C. dactylon*, its chemical control in native grassland would also affect non-target native vegetation.

What underlies the success of invasive alien species is not always clear and often depends on the specific invasive species and invaded community considered (Vila et al., 2011; Vila & Ibañez, 2011), but their ability to out-compete native species from the recipient community is relevant, particularly during early invasion stages (Gioria & Osborne, 2014; Vila & Weiner, 2004). Two components define competitive ability: a 'competitive effect'—that is the capacity of an individual to acquire resources at rates high enough to reduce its availability to neighbouring plants—and a 'competitive response'—that is its capacity to perform with low resources (Goldberg, 1990; Goldberg et al., 1999). Successful invasive alien species have been shown to exhibit traits that allow higher rates of resource acquisition than native species (Callaway & Aschehoug, 2000; Pysek & Richardson, 2007; van Kleunen, Dawson, et al., 2010; van Kleunen, Weber, et al., 2010), as well as the ability to tolerate better the lower availability of resources due to competition (e.g. Craine et al., 2005; Funk, 2013; Weiner, 1993). However, the specific relevance of each component in a given situation depends on the availability of resources (Gioria & Osborne, 2014). For instance, invasive alien plants have been found to be better at acquiring nutrients than natives in nutrient-enriched environments (Burke & Grime, 1996; Harrison, 1999; Heunneke et al., 1990; Liu & van Kleunen, 2017) and to tolerate better low-nutrient availability in ecosystems characterized by their scarcity (Funk, 2013; Groves et al., 2003).

Plant invasions are explained by many factors that operate at different scales in the successive stages of the invasions process: transport, colonization, establishment and landscape spread (Theoharides & Dukes, 2007). In regards to *C. dactylon* in grasslands, the mechanisms that explain its great success at the different stages of the invasion process

remain unclear and little studied. Ultimately, the competitive ability of a species results from traits that determine how plants acquire and use resources, and how it interacts with neighbours. *C. dactylon* can spread rapidly by stolons and rhizomes (Moreira, 1975; Taliaferro et al., 2004), which could confer a high ability to compete for nutrients and light in open ecosystems, as stolons enable it to forage for light while rhizomes serve as organs for storage of resources and as a bud bank (Dong & de Kroon, 1994). Little is known about the role of biomass allocation to leaves, stolons, rhizomes and roots plays in *C. dactylon* in competitive interactions with native species, during the invasion process. In grasses, Reichmann et al. (2016) showed that greater allocation to roots in native species can constrain their relative growth rate (RGR), perhaps due to the trade-off between allocation to roots and specific leaf are (SLA; Reich, 2014). Indeed, alien invasive species are often thought to have inherently higher growth rates than co-occurring native species (Arredondo et al., 1998; James & Drenovsky, 2007).

Traits providing competitive ability may be modulated by convergence or divergence in plant physiology and morphology between invasive and native species. Alternative hypotheses have been put forward about mechanisms of invasion and community resistance that differ in how functional traits are expected to vary in native vs. invasive species (Drenovsky et al., 2012, van Kleunen, Dawson, et al., 2010, van Kleunen, Weber, & Fischer, 2010). On one hand, 'habitat filtering and neutral processes hypotheses' propose that invasive and dominant native species share similar functional traits, which would confer a pre-adaptation in being introduced to new environments (Duncan & Williams, 2002). On the other hand, 'limit similarity' hypotheses (Catford et al., 2009) predict that invasive and native plants should differ in morphological and physiological traits to coexist (Funk, 2008).

The objective of this study was to analyse the interspecific interactions between *C. dactylon* and two native species under low-nutrient conditions, as a possible mechanism for its invasion success in grasslands. For this, we evaluated the competitive ability of *C. dactylon*, *Paspalum notatum* and *Mnesithea selloana* against each other when grown in a low-nutrient substrate. *Paspalum notatum* and *M. selloana* are perennial $C_4$ grasses of high forage value that are native and frequent and/or dominant in *Campo* grasslands of Uruguay (Pizarro, 2000; Rosengurtt, 1979). Specifically, we aim to answer whether, compared to these two natives, *C. dactylon* has (i) a greater competitive effect, (ii) greater tolerance to plant interaction and (iii) what role does above- vs. below-ground allocation of biomass play in regard to the competitive ability of the invasive alien species.

## METHODS

### Experimental design

We performed an experiment using pair-wise interactions between *C. dactylon* (Invasive), *M. selloana* (Native 1) and *P. notatum* (Native 2) growing in a greenhouse from the Instituto Nacional de Investigación Agropecuaria (INIA, Treinta y Tres) over one growing season (180 days). Six treatments resulted from the combination of one individual per species: (i, ii and iii) were the controls (each species alone without neighbour), (iv) *M. selloana* + *P. notatum* (Native 1 + Native 2), (v) *M. selloana* + *C. dactylon* (Native 1 + Invasive) and (vi) *P. notatum* + *C. dactylon* (Native 2 + Invasive). Four replicates (i.e. pots) per treatment were arranged in a completely randomized design. As a result, the experiment involved a total of 24 pots.

## Experiment management

Pots (15 cm diameter, 15 cm height) were filled with a nutrient-low substrate obtained by mixing sand with white peat (2:1 volume ratio), that had 2.9% organic carbon, 0.09% total N. The C:N ratio was greater than 30, 1.7 ppm of plant available P (Bray) and less than 20 ppm of plant available K. These nutrient levels are lower than that of natural grasslands soils in Uruguay, which are typically greater than 0.20% total N, C:N ratios of 10–11, 4 ppm P Bray, and 150 ppm plant available K (Altamirano et al., 1976).

In November 2018, at the beginning of the growing season of $C_4$ species (mid-spring), four seeds of each species were sown in each pot. We used commercial seeds of *P. notatum* cv INIA Sepé, a cultivar developed from a local ecotype, and commercial seeds of *C. dactylon*. For *M. selloana*, we collected seeds from *Campo* grasslands in Treinta y Tres department located in the east of Uruguay, in September and October 2018. One month later, pots were thinned to either one seedling per pot in controls (treatments i–iii), or to one seedling of each species in interaction (treatments iv–vi). Irrigation was automated, and pots were randomly rotated weekly inside the greenhouse so that light, water and nutrient availability were the same for all pots during the experiment.

## Measurements and calculations

After 6 months, in May 2019, at the end of the growing season of $C_4$ species (late autumn), we recorded the number of inflorescences in each plant and then we harvested all individuals. Each plant was separated into above (shoot, stolons, leaves and inflorescences) and below-ground organs (roots and rhizomes). Samples were dried at 60°C until constant weight (48 to 72 h with forced air) and then weighed. Shoot N concentration was measured by Kjeldhal (Sommers & Nelson, 1972) but due to small sample sizes, all replicates of each treatment had to be pooled.

The relative interaction intensity index (RII) was used to evaluate the result of each interaction treatment for each target species (Armas et al., 2004). As it quantifies the proportional change in plant biomass due to interaction, it allows the comparison of the interaction effect between interacting species. Thus, for each target species, RII was estimated from total biomass at the end of the experiment as

$$RII = (Bc - Bo) / (Bc + Bo)$$

where Bc is the total biomass of the target plant when competing with a neighbour (for treatments iv–vi), and Bo is the biomass of the target plant without competition (the average biomass of the corresponding species in treatment i). Thus, RII ranges from –1 to 1 and is symmetrical around zero; and more negative values indicate higher competition effect (Armas et al., 2004).

## Statistical analyses

We compared plant biomass (above and belowground) and the number of inflorescences of each target species (i.e. *M. selloana, P. notatum* and *C. dactylon*) between neighbouring interaction treatments using ANOVAs. Similarly, the RII values were compared between neighbouring interaction treatments within and between each target species using ANOVAs, including neighbour species identity and target species identity as the factors in the model.

Biomass allocation to above- vs. below-ground organs of each target species was assessed through ANOVA of shoot: root ratios and via allometric relationships of the following general form:

$$\log(MY) = \log(\beta) + \alpha \log(MX)$$

where MY and MX represent the biomass of organs Y and X, respectively, $\alpha$ reflects the RGR ratio between organs X and Y (Ledig & Perry, 1966; Poorter et al., 2012) and $b$ is a scaling parameter. We compared these relationships between species by combining data from all treatments using model II linear regression (Falster et al., 2006).

## RESULTS

### Nutrient deficiency

N concentration in shoots was very low in all species, with values between 0.46% and 0.80%, reflecting the limited nutrient availability of the substrate. An inverse relationship with shoot mass was apparent across species, which indicated more N concentration in small plants (Figure 1).

### Competitive ability: Competitive effects and competitive responses

The three species differed in their competitive ability. The relative interaction intensity index (RII) based on whole-plant biomass was always lower than zero for both native grasses (Figure 2), indicating *M. selloana* and *P. notatum* were negatively affected by neighbouring species (neighbour effect). However, there were no differences in the

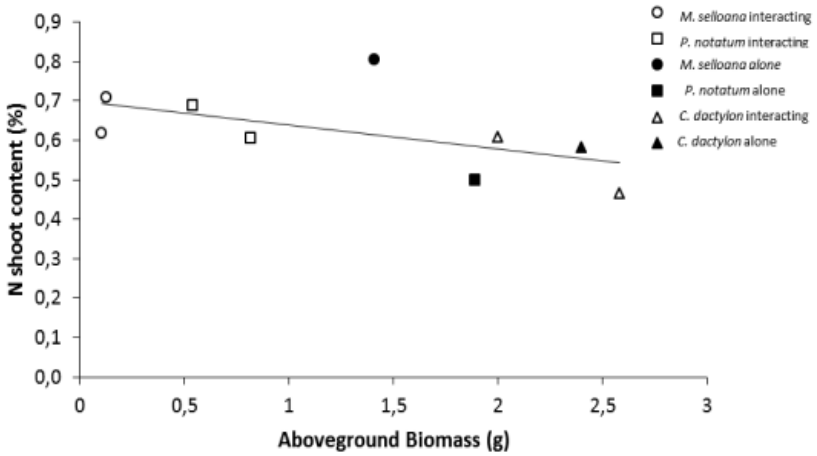


**FIGURE 1** Relationship between N concentration in shoots and aboveground biomass of native grasses *Mnesithea selloana* (circles) and *Paspalum notatum* (squares) and the invasive species *Cynodon dactylon* (triangles), when grown alone (closed symbols) or interacting with other species (open symbols). Each data point is a composed sample obtained from the mean of four replications from each treatment.

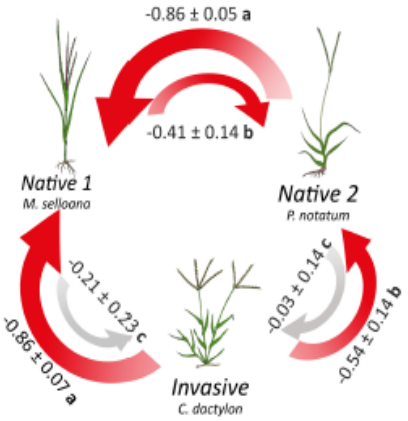


**FIGURE 2** Effect of neighbouring interaction treatments on the relative interaction intensity index (RII) for the three species: *Cynodon dactylon* (Invasive), *Mnesithea selloana* (Native 1) and *Paspalum notatum* (Native 2). The RII values (mean ± SD; $n = 4$) are based on the total plant biomass of individual plants at the end of the 6-month experimental period (Armas et al., 2004). Arrows (scaled to RII value) indicate RII that differs (red) or not from zero (grey). Different letters indicate statistically significant differences ($p < 0.05$) between different species, and for any given species, in the different treatments. Images: invasive (*C. dactylon*) and Native 2 (*P. notatum*), adapted from free vectors from Depositphotos and Shutterstock, respectively. Native 1 (*M. selloana*), own.

identity of the neighbour, as we observed similar effects when the neighbour was the invasive *C. dactylon* or one of the natives (Figure 2). *M. selloana* (Native 1) was negatively and equally affected when interacting with either *C. dactylon* (Invasive) or *P. notatum* (Native 2), as indicated by the similar RII values (−0.86). *Paspalum notatum* (Native 2) was less affected than Native 1 when interacting with neighbours, and again these effects were similar when the neighbour was the *Invasive* or *Native 1* (Figure 2). Conversely, RII was not statistically different from zero for *C. dactylon* (Invasive) when interacting with either of both native species, which indicated no neighbour effect (Figure 2).

The negative impact of the invasive *C. dactylon* on native's biomass was due to effects on both above- and below-ground biomass. Likewise, negative effects between the native species were also due to effects on both shoot and roots (Figure 3), since both native plant biomass fractions were reduced by plant interaction. For *C. dactylon*, none of the biomass fractions were affected by neighbour interaction (Figure 3). The biomass of *M. selloana* (Native 1) decreased by 90% when interacting with either *C. dactylon* (Invasive) or *P. notatum* (Native 2; Figure 3), whereas *P. notatum* showed a 70% biomass reduction when interacting with either Invasive or Native 1 (Figure 3).

Notably, when grown alone (control) total biomass production was lowest for the Invasive (3.4 g plant$^{-1}$; Figure 3a), intermediate for *M. selloana* (4.0 g plant$^{-1}$; Figure 3c) and had the highest values for *P. notatum* (6.0 g plant$^{-1}$; Figure 3b). When comparing Invasive and both native grasses when grown alone (control), there were major differences in root biomass with greater root biomass in native plants; shoot biomass of control plants was similar for the three species. (Figure 3).

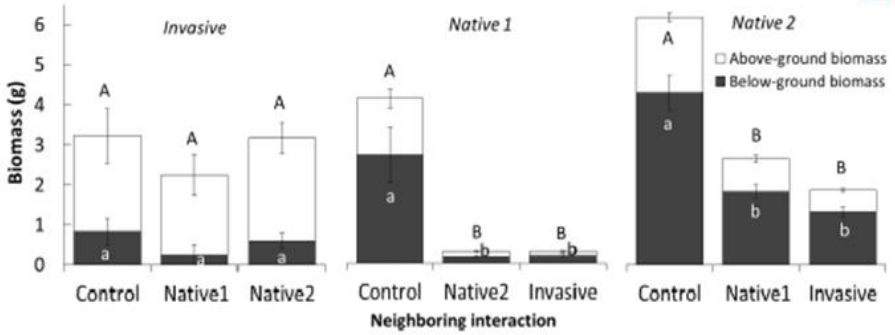


**FIGURE 3** Above (light bars)—and below-ground (dark bars) biomass of Native 1 (*Mnesithea selloana*), Native 2 (*Paspalum notatum*) and invasive (*Cynodon dactylon*) species when grown alone (Control) or in neighbouring interaction treatments. Different capital and lowercase letters indicate statistically significant differences ($p < 0.05$) in above and below-ground biomass, respectively, between neighbour treatments for each species. Bars indicate SE ($n = 4$).

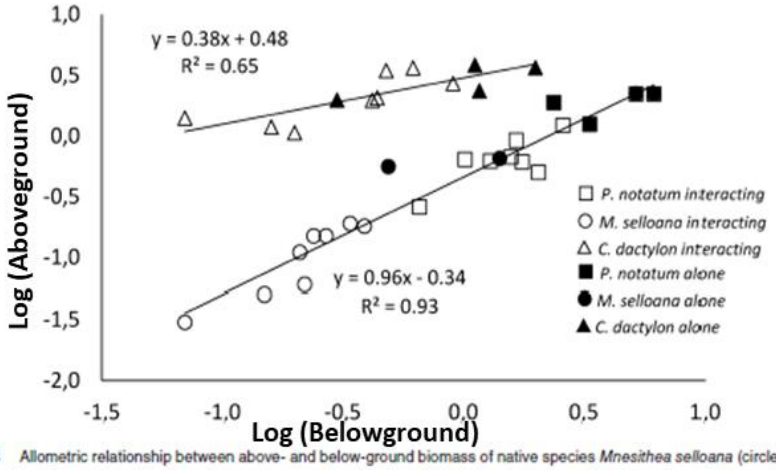


**FIGURE 4** Allometric relationship between above- and below-ground biomass of native species *Mnesithea selloana* (circles) and *Paspalum notatum* (squares) and the invasive species *Cynodon dactylon* (triangles), when grown alone (closed symbols, $n = 4$) or in neighbouring interaction treatments (open symbols, $n = 8$). Each data point is a replicate.

## Above- and below-ground biomass allocation patterns

The invasive species had a higher allocation of biomass to aboveground organs compared to both natives (+0.48 vs. −0.34 shoot per unit root; $p = 0.006$). Furthermore, this preferential allocation to shoot was far less affected by neighbouring interactions compared with native species (0.38 vs. 0.96 change in shoot per unit change in root; $p = 0.006$). Native species showed a similar pattern of biomass allocation (Figure 4; $p = 0.830$). Both native species allocated biomass to rhizomes at a constant proportion versus roots and this pattern did not change due to neighbouring interaction treatments; meanwhile, *C. dactylon* did not produce rhizomes (Figure 5).

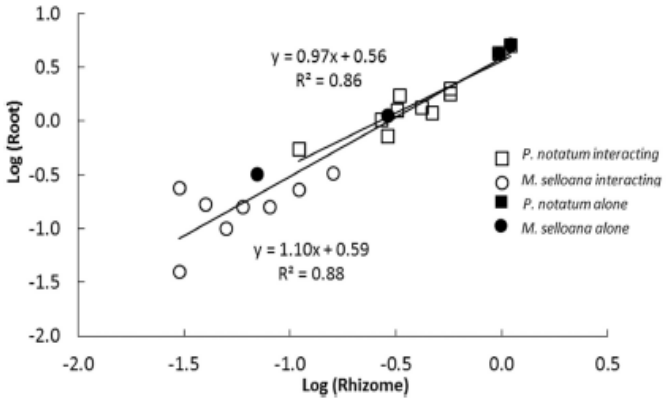


**FIGURE 5** Allometric relationship between roots and rhizomes of native species *Mnesithea selloana* (circles) and *Paspalum notatum* (squares) when grown alone (closed symbols, $n=4$) or in neighbouring interaction treatments (open symbols, $n=8$). Each data point is a replicate.

**TABLE 1** Mean number of inflorescences for each target species when grown alone (control) or when interacting with each neighbouring species.

| | Neighbouring interaction | | | |
|---|---|---|---|---|
| **Target species** | **Control** | **Invasive** | **Native 1** | **Native 2** |
| Invasive | 0 a | - | 5.5±1.84 b | 1.5±0.86 ab |
| Native 1 | 4±1.29 a | 0.25±0.28 b | - | 0.5±0.25 b |
| Native 2 | 0 | 0 | 0 | - |

*Note*: Invasive: *Cynodon dactylon*; Native 1: *Mnesithea selloana* and Native 2: *Paspalum notatum*. Different letters indicate statistically significant differences ($p<0.05$) between neighbouring interactions within target species ($n=4\pm$SE).

### Reproductive effort

After 6 months, *P. notatum* (Native 2) did not allocate energy to sexual reproduction (Table 1). For *M. selloana* (Native 1), the number of inflorescences was significantly reduced when interacting with *P. notatum* (Native 2) or the invasive *C. dactylon*. For *C. dactylon* the opposite response was observed, as the inflorescences developed only when interacting with either native species (Table 1).

## DISCUSSION

The invasive alien species *C. dactylon* showed greater competitive ability than both native grasses. Nutrient availability was scarce in the present study, and this was reflected in plant N concentration (0.46%–0.8%), which was lower than sufficiency values for $C_4$ grasses (e.g. 2%–5% Greenwood et al., 1990; 2%–4% Mathews et al., 2016). The competitive ability of a species is explained by the combination of its effects on neighbour species and by its response to the conditions of lower resource availabile due to neighbours (Goldberg et al., 1999). In the present study, the invasive species negatively affected native plant biomass; however, this effect was similar to that observed in native-native interactions.

Rather, the greater competitive ability of *C. dactylon* emerged as a consequence of its capacity to tolerate the conditions created by the presence of natives, as it was not affected by any of the neighbouring plants. The invasive alien plant preferentially allocated biomass to aboveground organs (which included stolons), and when interacting with natives its allocation to roots decreased. Conversely, both natives had a greater allocation to below-ground organs, which included storage organs (rhizomes), and did not modify this pattern when interacting with the invasive species.

The invasive grass showed negative effects on native species by decreasing their below- and above-biomass, as was evidenced by negative values of RII for both native grasses. However, competitive effects of the invasive species were not a relevant component explaining its greater competitive ability, because the inclusion of native-native treatment in our experimental design allowed us to show that the invasive effect was not greater than a co-occurring native. If an invasive alien species is competitively superior to co-occurring natives, we would expect a greater suppression of target species (greater effect of the invader) and more ability to tolerate competition with neighbouring plants (weaker response of the invader; Guido et al., 2019; Vila & Weiner, 2004). Effects in plant competition are explained by the capacity of an individual to acquire resources at rates high enough to reduce their availability to neighbouring plants (Goldberg et al., 1999; Leishman et al., 2007). Traits associated with competitive effects such as the growth rate (GR) are strongly habitat-dependent and are most important in high-resource environments (Gioria & Osborne, 2014). In fact, we found that *C. dactylon* did not show intrinsically higher GR than the natives under low -nutrient availability, since total plant biomass (a proxy for GR in this experiment) was similar in all three species grown alone.

Interaction with native species did not affect the plant biomass of *C. dactylon* (RII not statistically different from zero). This indicated a high tolerance to lower availability of resources resulting from competition with neighbours (Goldberg et al., 1999). Such an ability has been found to be relevant in explaining invasive plant success (Craine et al., 2005; Funk, 2013). Under poor resource environments, invasive alien species can display traits indicative of both resource acquisition and resource conservation strategies to tolerate neighbouring interaction (Funk, 2013). This author examined the physiological and morphological traits of native and invasive species in low-resource environments and found that the main trait explaining the success of invasive plants was their efficiency of use of nutrients (EUN). Indeed, 12 out of 19 studies reported greater EUN in invasive than native species (Drenovsky et al., 2008; Firn et al., 2012; Funk & Vitousek, 2007; Godoy et al., 2011; Matzek, 2011). A greater EUN can be reached due to physiological mechanisms like the ability to resorb nutrients from senescing tissues or constructing cheaper tissues (Drenovsky et al., 2012). Although we did not analyse variables related to EUN, we suggest that traits explaining *C. dactylon* invasiveness may be related to resource conservation strategies such as EUN.

Another mechanism that could explain tolerance in plant competition is biomass allocation to different organs, as a strategy to cope with limited resources. In our experiment, *C. dactylon* allocated more biomass to aerial organs (including stolons) than both native species. Furthermore, preferential biomass allocation to shoots was actually exacerbated by interaction with native grasses. Conversely, above- and below-ground biomass of native grasses were equally affected by the presence of neighbours. Our results about biomass allocation patterns suggested that invasive and native species showed divergence in this growth strategy. Previous studies show that there may exist convergence (Drenovsky

et al., 2012) or divergence (Leishman et al., 2007) in traits between natives and invasive species. This is in line with the hypothesis based on limit similarity, which predicts invasive and native plants should differ in their morphological and physiological traits to coexist, as a biotic resistance mechanism in the recipient community (Catford et al., 2009). Considering that this experiment was carried out in low-nutrient conditions and that native grasses allocated more to below-ground organs than *C. dactylon*, it would be expected that native species would cope better with the nutrient competition. Yet, the invasive plant showed a superior competitive ability over native plants. This response suggests that other mechanisms than nutrient competition may be underlying plant–plant interaction. Instead, *C. dactylon* responses are consistent with shade avoidance syndrome. Previous studies have shown that this species is indeed highly sensitive to shading (Adamipour et al., 2016; Guglielmini & Satorre, 2002). It is well known that when neighbour proximity changes light quality, plants activate responses leading to elongation growth and also to the acceleration of flowering (Ballaré & Pierik, 2017; Smith, 1995). Taking into account that invasive grass did not show greater GR than natives, we suggest that in a stoloniferous species like *C. dactylon*, shade avoidance could be one of the mechanisms explaining the higher allocation to aboveground biomass when interacting with natives. Indeed, shade avoidance syndrome has already been mentioned as a putative reason underlying the success of invasive herbaceous plants (Du et al., 2017; van Kleunen et al., 2011) when invading native systems; however, a specific study should be performed to confirm that. Another factor explaining invasive success in our research may be allelopathy since it has been reported that this invasive grass produces allelopathic compounds that would displace recipient species (Bresciano et al., 2022; Favaretto et al., 2018; Golparvar et al., 2015; Guido et al., 2019). For *M. selloana*, the number of inflorescences was significantly reduced due to plant interaction, while, *P. notatum* did not produce inflorescences, which could be due to the short term of the experiment. On the other hand, invasive grass flowered only when interacting with both native species, which could be another response related to shade avoidance syndrome in *C. dactylon* (Adamipour et al., 2016; Guglielmini & Satorre, 2002).

## CONCLUSIONS

*Cynodon dactylon*, an invasive alien plant worldwide, had a superior competitive ability than two co-occurring native grasses that are frequent in Uruguayan natural Campo grasslands. This was mostly explained by *C. dactylon* tolerance to the presence of neighbours under low-nutrient conditions. The invasive grass allocated more biomass to above-ground organs than both native species, especially when interacting with them. Such a response, consistent with classic shade avoidance syndrome, could be one of the mechanisms explaining greater tolerance in *C. dacylon* when interacting with natives. Furthermore, it did not produce below-ground storage organs (rhizomes), as both native species did, and solely induced sexual reproduction when interacting with natives. These results suggest that the main driver underlying plant–plant interaction in this experiment may not be the ability to acquire nutrients, although higher nutrient use efficiency in the generation of aboveground tissue could have played a role as well.

Biomass allocation patterns observed in *C. dactylon* should be considered in management plans for its control. Since this invasive grass

presented greater biomass allocation to above-ground parts, and lower to below-ground organs, decreasing its aerial biomass would imply a limitation for its recovery. In this sense, management practices focused on removing *C. dactylon* above biomass, could be effective to control it.

Since our results suggest that *C. dactylon* success would not be explained by higher competitive effects mediated by nutrient acquisition, future research would need to focus on other mechanisms behind, such as allelopathy and traits related to nutrient use efficiency.

The present research focuses on plant–plant interactions, therefore scaling up inferences must be done with caution. In future studies, it would be necessary to consider population or community variables, as well as to take into account direct and indirect interactions involving native and invasive species. On the other hand, the outcomes of biotic interactions are context dependent, so it would be crucial to expand research to different environmental conditions to assess the driven factors of this invasion process. In this sense, due to *C. dactylon* invading disturbed Uruguayan grasslands, it is necessary to explore its competitive ability at high nutrient levels.

## AUTHOR CONTRIBUTIONS

**Silvina Garcia:** Conceptualization (equal); data curation (lead); formal analysis (lead); investigation (lead); software (equal); visualization (equal); writing – original draft (lead); writing – review and editing (equal). **Anaclara Guido:** Conceptualization (equal); funding acquisition (lead); investigation (equal); methodology (equal); project administration (lead); supervision (lead); validation (equal); writing – review and editing (equal). **Fabiana Pezzani:** Conceptualization (equal); investigation (equal); supervision (lead); validation (equal); writing – review and editing (equal). **Fernando Lattanzi:** Conceptualization (equal); funding acquisition (lead); investigation (equal); supervision (lead); validation (equal); visualization (equal); writing – review and editing (equal).

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## DATA AVAILABILITY STATEMENT

The data will be available in supplemental information for online version.

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## 7.3 Degradation of arbuscular mycorrhizal symbiosis: A mechanism underlying *Cynodon dactylon* invasion in grasslands?

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SPECIAL ISSUE: INVASIONS IN PLANT COMMUNITIES

# Degradation of Arbuscular Mycorrhizal Symbiosis: A Mechanism Underlying *Cynodon dactylon* Invasion in Grasslands?

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**Keywords:** arbuscular mycorrhizae | bermudagrass | biological invasions | *Cynodon dactylon* | mutualism degradation | nutrient availability | *Paspalum notatum* | Río de la Plata grasslands

## ABSTRACT

**Questions:** Degradation of facilitative interactions of native species can play an important role in the establishment and expansion of invasive plants in communities. We evaluated the relationship between the level of invasion of *Cynodon dactylon* and the arbuscular mycorrhizal colonization of the native *Paspalum notatum* in Uruguayan grasslands, which were either extensively managed (natural vegetation [NG]) or oversown with exotic legumes and fertilized with phosphorus (NG + LP). Specifically, we investigated whether increasing invasion levels were associated with reductions in *P. notatum* mycorrhizal colonization, growth, and nutrient content.

**Location:** Uruguayan grasslands of Río de la Plata grasslands region.

**Methods:** Two paddocks with 19 and 27 years under NG + LP management were selected contiguous to two paddocks with NG management. In each paddock, we collected nine monoliths (0.2 m diameter × 0.3 m depth) that had *P. notatum* and increasing percentages of *C. dactylon* cover, classified as low (0%–10%), medium (30%–50%), or high (70%–90%) invasion levels. After 10 months of uninterrupted growth, shoot mass, phosphorus and nitrogen shoot concentration, and arbuscular mycorrhizal colonization were assessed in *P. notatum* plants.

**Results:** Mycorrhization decreased with increasing *C. dactylon* invasion. This was greater in more intensively managed grasslands (NG + LP). However, lower arbuscular mycorrhizal colonization was not associated with either lower aboveground growth or phosphorus or nitrogen shoot concentration. Furthermore, at low invasion levels, mycorrhizal colonization was similar between NG and NG + LP, despite their contrasting soil P availability.

**Conclusions:** The presence of arbuscular mycorrhizae in the target native grass *P. notatum* was negatively associated with the invasion level of *C. dactylon*. Therefore, mutualism degradation might be a mechanism underlying the success of *C. dactylon* invading grasslands, particularly those intensively managed, albeit probably not via phosphorus nutritional effects.

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## 1 | Introduction

Biological invasions are a leading cause of global biodiversity loss, impacting the structure and functioning of ecosystems (Mack et al. 2000; Vila et al. 2011; IPBES 2023). At the local scale, the mechanisms involved in invasion processes are primarily linked to biotic interactions between the invasive species and native species from the recipient community (Richardson, Allsopp, et al. 2000; Mitchell et al. 2006; Blackburn et al. 2011; Traveset and Richardson 2020).

Most studies have focused on the role of negative interactions between native and invasive species during the invasion process, such as competition and predation, as mechanisms underlying invasion success (Elton 1958; Mitchell et al. 2006; Traveset and Richardson 2020). However, increasing evidence suggests that facilitative interactions can also play an important role in the establishment and spread of invasive species (Traveset and Richardson 2014, 2020; Pyšek et al. 2019). In some cases, non-native plants may form mutualistic relationships with native soil microbes, enhancing their ability to compete with native species (Shah, Reshi, and Khasa 2009). Whereas in other cases, invasive species negatively affect mutualistic interactions essential for some native species of the recipient community, which is referred to as the "degraded mutualism" or "mutualism disruption" hypothesis (Vogelsang et al. 2004; Vogelsang and Bever 2009; Hale and Kalisz 2012; Traveset and Richardson 2014; Roche et al. 2023).

Among mutualisms, arbuscular mycorrhizae stand out because they play a critical role in determining patterns of abundance and invasiveness (Pringle et al. 2009; Traveset and Richardson 2020). Arbuscular mycorrhizal mutualisms derive from the association between fungi belonging to the phylum *Glomeromycota* and the roots of most terrestrial plants (Smith and Read 2008). Chief among the benefits that plants get from mycorrhizae is the enhanced absorption of slowly mobile nutrients, such as P (Grimoldi et al. 2005; Smith and Read 2008). Improved water uptake (Kakouridis et al. 2022; Romero-Munar et al. 2024) and protection against pests and diseases have also been documented (Newsham, Fitter, and Watkinson 1995; Schausberger et al. 2012; Weng et al. 2022). Furthermore, arbuscular mycorrhizae may also interact with other symbionts, such as rhizobia and epichloe endophytes (García-Parisi et al. 2017). Not surprisingly, this symbiosis is very conspicuous in natural ecosystems (Smith and Read 2008; Brundrett 2009).

The precise mechanisms underlying mutualism degradation during invasions are yet unclear (Grove et al. 2017) but can be indirectly via the host plant or due to a direct effect on the mycorrhizal interaction (Grove et al. 2017). Indirect effects occur, for example, when invaders can affect mycorrhizae by competitively reducing or excluding suitable plant hosts, and this mechanism is particularly important when the invader is not mycorrhizal (Vogelsang and Bever 2009; Zubek et al. 2016). In these cases, low dependence on mycorrhizae causes a reduced bank of arbuscular mycorrhizal spores in invaded communities (Vogelsang and Bever 2009; Wilson, Hickman, and Williamson 2012; Traveset and Richardson 2014; Zubek et al. 2016; Roche et al. 2023). On the other hand, an invasive plant can directly affect the mycorrhizal community in the soil, for example, by increasing soil N content (Castro-Díez et al. 2014), which can affect the composition of the fungal community (Jumponen et al. 2005) or reduce mycorrhizal structures associated with nutrient uptake (i.e., arbuscules; Johnson et al. 2010). Another direct effect is due to allelopathy, as there is evidence that secondary metabolites inhibit spore germination and the establishment of the symbiosis (Roberts and Anderson 2001; Hale and Kalisz 2012; Pinzone et al. 2018; Roche et al. 2021, 2023). Moreover, since arbuscular mycorrhizae benefits are more important in nutrient-poor environments, increasing nutrient availability may act as a selection pressure favoring less efficient fungal species (Johnson, Graham, and Smith 1997; Grove et al. 2017). Some situations of increased soil promote mycorrhizal disruption via a phytocentric control because plants take up nutrients directly rather than via mycorrhizae (Paries and Gutjahr 2023). In other cases, mutualism can turn into parasitism (Johnson, Graham, and Smith 1997). Finally, invasive plants are capable of selecting more efficient arbuscular mycorrhizal symbionts, which can generate positive feedback that enhances their performance (Bever 2002; Bever et al. 2009; Zhang et al. 2010; Wilson, Hickman, and Williamson 2012).

Arbuscular mycorrhizal colonization is widespread among native species in the highly biodiverse Río de la Plata grasslands, a temperate-subhumid region in southern South America (Parodi and Pezzani 2011; García et al. 2016, 2019; Del Pino et al. 2021), particularly in thicker-rooted species (Michelini et al. 2024). In Uruguay, grasslands persist either as natural vegetation (NG) or subject to overseeding with legumes coupled with phosphorus (P) fertilization, to increase forage productivity and nutritive value (NG + LP; Jaurena et al. 2021). However, the invasion of *Cynodon dactylon* was frequently reported as a result of agronomic practices such as legume and P addition on NG (Jaurena et al. 2015; Pañella et al. 2022). *Cynodon dactylon* is a perennial warm-season grass native to eastern Africa and southern Europe that has become one of the five most important invasive alien species worldwide (Holm et al. 1991).

The mechanisms underlying the success of *C. dactylon* invasion of NG + LP are unclear. It exhibits successful clonal propagation through rhizomes and stolons, as well as reserve organs that enhance its survival (Horowitz 1996; Taliaferro, Rouqutte, and Mislevy 2004). Moreover, it produces allelopathic compounds capable of inhibiting the growth of other plants (Golparvar et al. 2015; Favaretto, Scheffer-basso, and Perez 2018; Guido et al. 2020; Bresciano, Glison, and Lezama 2022). The competitive ability of *C. dactylon* is greater than that of the dominant native species of the Río de la Plata grasslands, which seems to be related to greater plasticity associated with root: shoot allocation (García et al. 2023). Among the native grasses, *Paspalum notatum* stands out for its abundance and forage value (Pizarro 2000) and, together with *Axonopus affinis*, represent the most frequent species in grassland communities of Uruguay (Lezama et al. 2019). It is a perennial species with a prostrate habit, summer cycle (Rosengurtt 1979), and $C_4$ photosynthetic metabolism that coexists with the invasive *C. dactylon*. *Paspalum notatum* showed high levels of arbuscular mycorrhizal colonization, which is expected in plants with thick roots (Hartnett and Wilson 1999; Pezzani, Montaña, and Guevara 2006; García et al. 2019; Michelini et al. 2024). In this species, mycorrhizae play a crucial role in water and nutrient acquisition due to the

lack of fine roots. Another strategy explaining the success of *C. dactylon* in NG + LP could be the degradation of arbuscular mycorrhizal fungi mutualism due to greater *C. dactylon* coverage than in NG (García et al. 2016). Although arbuscular mycorrhizae would not be as needed in high nutrient availability, they may develop another function as protection against noxious organisms in NG + LP. *Cynodon dactylon* is capable of forming arbuscular mycorrhizal interactions, but it develops a very low presence of nutrient exchange structures (arbuscles and coils) between symbionts (Endresz, Somodi, and Kalapos 2013; Lugo et al. 2018), so it has been considered non-mycorrhizal or facultative mycorrhizal (Muthukumar and Udaiyan 2000; Muthukumar et al. 2006). This could suggest that this invasive grass would be allocating less energy to mycorrhizal fungi than native plants. However, experimental studies that examine the role of arbuscular mycorrhizae on *C. dactylon* invasion are lacking.

The aim of this study was to explore the relationship between the level of invasion of *C. dactylon* and the mycorrhizal colonization of a conspicuous native species from the recipient community. Specifically, we addressed the following questions: (i) what is the association between the levels of *C. dactylon* invasion and the arbuscular mycorrhizal colonization of a dominant native grass (*P. notatum*)?; (ii) is this association modified by the addition of legumes and P on natural grasslands (NG + LP)?; and (iii) does a reduction of mycorrhizal colonization in *P. notatum* involve a decline in its growth and nutrient content? We expected that higher levels of invasion would be associated with a reduction in mycorrhizal colonization of *P. notatum*, that this effect would be more marked in NG + LP, and that mutualism degradation would be negatively associated with the growth and nutritional status of *P. notatum*.

## 2 | Methods

### 2.1 | Collection and Maintenance of Monoliths

In June 2019, at the "*Centro de Investigación y Experimentación Dr. Alejandro Gallinal*" of the *Secretariado Uruguayo de la Lana*, located in the south-central region of Uruguay (CIEDAG-SUL 33.86932° S, 55.57273° W), we selected two paddocks with 19 (site A) and 27 (site B) years under NG + LP management contiguous to two paddocks with NG communities (Appendix S1). The study area is framed within Community IV "Densely vegetated grasslands of the Eastern Hills, Northeastern Sedimentary Basin and South Central Region", in accordance with the classification of NG communities of Uruguay (Lezama et al. 2019).

NG + LP agronomic management consisted in broadcasting 5 kg/ha of *Lotus subbiflorus* cv El Rincón upon native grasslands, plus annual fertilization with 20 kg P/ha as either superphosphate (until the year 2000) or hyperphosphate (after 2000) in sites A and B, respectively. In contrast, native grassland paddocks under NG management received no fertilizer or legume addition.

All sampled paddocks had similar soil type and topographical position and were continuously stocked with cattle and sheep, although at seasonally varying stocking rates. Soil analysis showed that NG and NG + LP paddocks had similar pH and organic carbon concentration, but plant-available P was 80% higher in NG + LP (Appendix S1).

In each paddock, we selected a small area (radius < 200 m) where *P. notatum* had a similar plant cover but coexisted with different

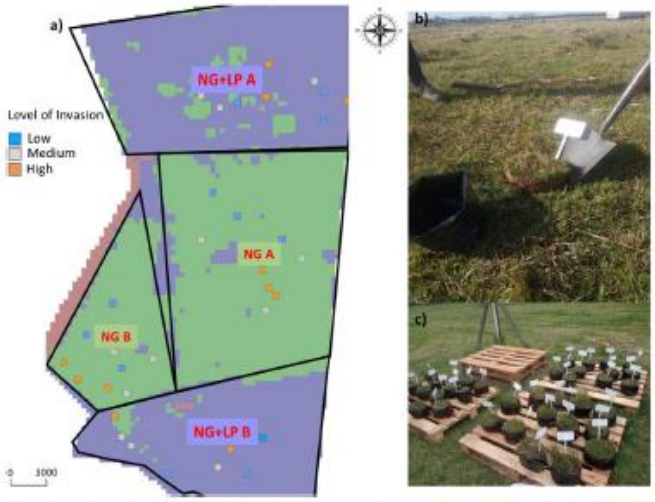


**FIGURE 1** | Location of the sampling points. Each square corresponds to monoliths with low (0%–10% in blue), medium (30%–50% in gray), and high (70%–90% in orange) levels of invasion, within the surveyed paddocks. Green and purple background colors correspond to NG (native grasslands extensively managed) and NG + LP (oversown with exotic legumes and fertilized with P, respectively). Letters A and B refer to the two sites sampled (a); photograph of the field sample of monoliths (b) and the experiment at the Faculty of Agronomy (c).

levels of invasion by *C. dactylon*: low (0%–10%), medium (30%–50%), or high (70%–90%) cover. This ensured homogeneous edaphic conditions, topographical position, and grazing regime, as well as minimized the possibility of initial differences between invasion levels (Figure 1). At each invasion level, we collected three monoliths (depth 0.20 m and diameter 0.20 m), at least 5 m apart from each other. Monoliths were placed in plastic boxes and immediately transported to a controlled garden for maintenance.

Monoliths were kept on in an open-air garden at the Faculty of Agronomy, in Montevideo, Uruguay (34.83740° S, 56.22240° W), where they grew uninterrupted for 10 months, i.e., one growing season. Irrigation with tap water was provided to prevent water stress, but no nutrients were applied. During winter, plants were watered with a hose. During the warm season, to ensure that water was available as plants needed, the monoliths were placed in flooded trays to absorb water by capillarity.

In each monolith, all species other than *P. notatum* and *C. dactylon* were manually removed to prevent confounding effects of the identity of neighboring species on mycorrhizal colonization of the target species. To assess whether disturbance caused by mechanical removal of neighboring plants affected mycorrhizal status of *P. notatum* (i.e., manipulation artifact), mycorrhizal colonization was measured in six extra monoliths collected from NG (three monoliths from each site A and B) with medium *C. dactylon* cover, in which neighboring plants were not removed (control for removal effect).

### 2.2 | Biomass Sampling and Assessment of Arbuscular Mycorrhizal Colonization in *P. notatum*

In April 2020, after the 10-month period, all above- and belowground biomass of *P. notatum* was harvested and aboveground was dried with forced air at 60°C for 48–72 h until constant weight. Dry samples were weighed and then aboveground and their P and N concentration determined. P concentration was quantified by sulfuric digestion and ammonium molybdate

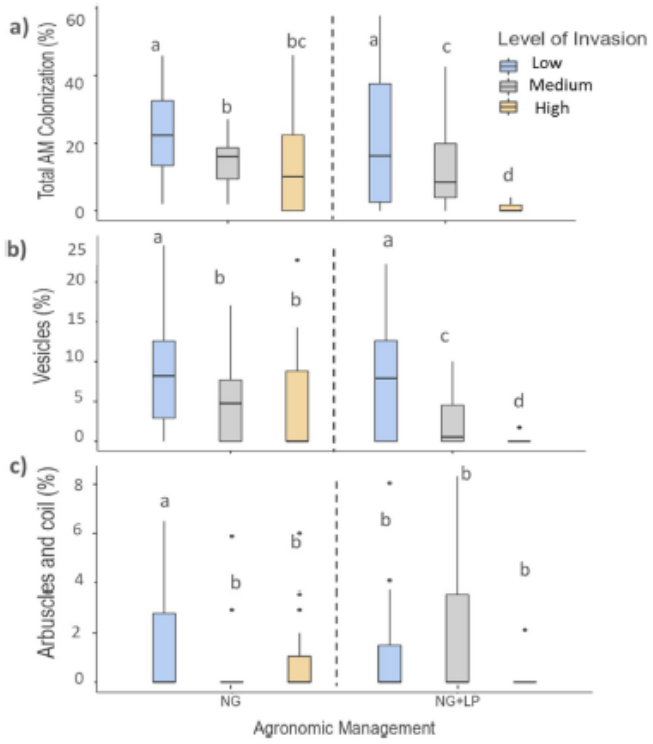


**FIGURE 2** | Box plots ($n = 6$ per treatment) of the percentage of roots with colonization by total (a), vesicles (b), and exchange structures (c) of *Paspalum notatum* plants growing with low (0%–10% in blue), medium (30%–50% in gray), or high (70%–90% in orange) levels of *Cynodon dactylon* invasion, in native grasslands managed either extensively (NG) or oversown with exotic legumes and fertilized with P (NG + LP). Different letters above boxplots indicate a statistically significant difference among treatments ($p < 0.05$) using the Least Significant Difference (LSD) test adjusted by Bonferroni.

colorimetry, and N concentration was determined by combustion at 900°C and subsequent detection of N2 by thermal conductivity.

To assess arbuscular mycorrhizal colonization, roots were washed and cleared with KOH solution (10%) and then stained with trypan blue (0.05%), as proposed by Koske and Gemma (1989). We randomly scanned 30 root segments about 1 cm long per monolith and three fields of view per segment. In each scan, hyphae, vesicles, arbuscules, and coils of arbuscular mycorrhiza fungi were recorded in a total of 90 observation fields (Olympus BX 43) at 10× magnification. Total colonization was quantified as the proportion of fields bearing any fungal structure.

### 2.3 | Statistical Analysis

First, for evaluating the effect of removing biomass (i.e., manual removal of non-target species), and thus avoiding manipulation artifacts, mycorrhizal colonization data were analyzed using a generalized linear mixed effect model, assuming a binomial distribution of the data. The fixed effect was the removal (removal and no removal) and the random effect was the site (A and B). A posteriori mean comparison was performed with the Least Significant Difference (LSD) test adjusted by Bonferroni.

Mycorrhizal colonization of *P. notatum*—total, vesicles, and arbuscules plus coils (i.e., exchange structures)—was analyzed using a generalized linear mixed effect model, assuming a binomial probability distribution. For analyses of aboveground biomass data, P and N shoot concentration and N:P ratio, linear mixed effect models (i.e., normal probability distribution) were used. In all cases, agronomic management (NG and NG + LP), level of invasion by *C. dactylon* (low, medium, and high), and its interaction were considered as fixed effects. Differences between treatments were assessed using the LSD test adjusted by Bonferroni. In all models, the site was specified as a random effect in the models.

Multiple linear regressions were performed to analyze the relationships between plant nutrients, aboveground biomass, and arbuscular mycorrhizal colonization. For this, linear mixed effect models were used, in which aboveground biomass and P shoot content were the response variables. Agronomic management (NG and NG + LP), level of invasion by *C. dactylon* (low, medium, and high), and its interaction were considered as fixed factors. Total mycorrhizal colonization was specified as a covariable and site was considered as a random effect. Statistical

**TABLE 1** | Results of generalized linear mixed effect models (with binomial error distribution) that tested the effects of treatments on mycorrhizal colonization. Treatment contrasts, with the intercept representing the level NG (natural grassland) and 0%–10% (low invasion), are shown. NG + LP refers to native grassland oversown with exotic legumes and fertilized with phosphorus (intensive management), whereas 30%–50% and 70%–90% refer to medium and high levels of invasion by *Cynodon dactylon*.

| Response variable | Treatment | Estimate | SE | z | Pr(>\|z\|) |
|---|---|---|---|---|---|
| Total colonization | Intercept | −1.28 | 0.26 | −4.88 | <0.001 |
| | NG + LP | −0.04 | 0.10 | −0.44 | 0.656 |
| | 30–50 | −0.45 | 0.13 | −3.46 | 0.005 |
| | 70–90 | −0.68 | 0.13 | −5.20 | <0.001 |
| | NG + LP*30–50 | −0.43 | 0.19 | −2.33 | 0.019 |
| | NG + LP*70–90 | −2.29 | 0.41 | −5.61 | <0.001 |
| Vesicles | Intercept | −2.69 | 0.61 | −4.42 | <0.001 |
| | NG + LP | 0.03 | 0.15 | 0.18 | 0.854 |
| | 30–50 | −0.50 | 0.20 | −2.53 | 0.011 |
| | 70–90 | −0.70 | 0.22 | −3.25 | 0.001 |
| | NG + LP*30–50 | −0.94 | 0.32 | −2.95 | 0.003 |
| | NG + LP*70–90 | −2.48 | 0.75 | −3.32 | 0.009 |
| Arbuscles and coils | Intercept | 3.71 | 0.36 | −10.30 | <0.001 |
| | NG + LP | 0.32 | 0.80 | −2.55 | 0.010 |
| | 30–50 | −2.33 | 0.73 | −3.19 | 0.001 |
| | 70–90 | −0.96 | 0.40 | −2.38 | 0.017 |
| | NG + LP*30–50 | 2.24 | 0.84 | 2.66 | 0.007 |
| | NG + LP*70–90 | −0.98 | 1.10 | −0.89 | 0.374 |

analyses were carried out in INFOSTAT, using the interface with R Studio (Di Rienzo et al. 2014).

## 3 | Results

### 3.1 | Arbuscular Mycorrhizal Colonization of *P. notatum*

Arbuscular mycorrhizae were present in all treatments. Total colonization varied widely, ranging from as low as 3% in some NG + LP monoliths with high levels of invasion by *C. dactylon*, to more than 50% in some NG monoliths with a low level of invasion by *C. dactylon* (Figure 2a). There was no effect of the mechanical removal of neighboring species on total mycorrhization ($p = 0.125$), vesicles ($p = 0.07$), or exchange structures ($p = 0.662$) of *P. notatum* (Appendix S3).

Total mycorrhization of *P. notatum* was smaller as the level of invasion by *C. dactylon* increased, and this magnitude was larger in NG + LP than in NG (Figure 2a; Table 1; Appendix S4). Thus, compared with low levels of *C. dactylon* invasion, the total mycorrhizal colonization in NG decreased by 28% and 43% in medium and high level of invasion, respectively. In NG + LP, however, this reduction was 35% and 85% at medium and high levels of *C. dactylon* invasion, respectively, compared with a low level of invasion (Figure 2a; Table 1). When the level of invasion of *C. dactylon* was low, arbuscular mycorrhizal colonization was similar in NG and NG + LP (Figure 2; Table 1; Appendix S4).

Vesicular colonization showed the same response pattern as total colonization (Figure 2b; Table 1; Appendix S4). Conversely, colonization by arbuscles plus coils (exchange structures) was less associated with the studied factors. This variable was negatively associated with the level of invasion by *C. dactylon* in natural grassland, but not in NG + LP (Figure 2c; Table 1; Appendix S4).

### 3.2 | Aboveground Biomass and Nutrient Content of *P. notatum*

Aboveground biomass, shoot N and P content were all greater in NG + LP than in NG (Figure 3; Table 2; Appendix S5). On average, *P. notatum* had 48% more biomass, 65% more N, and

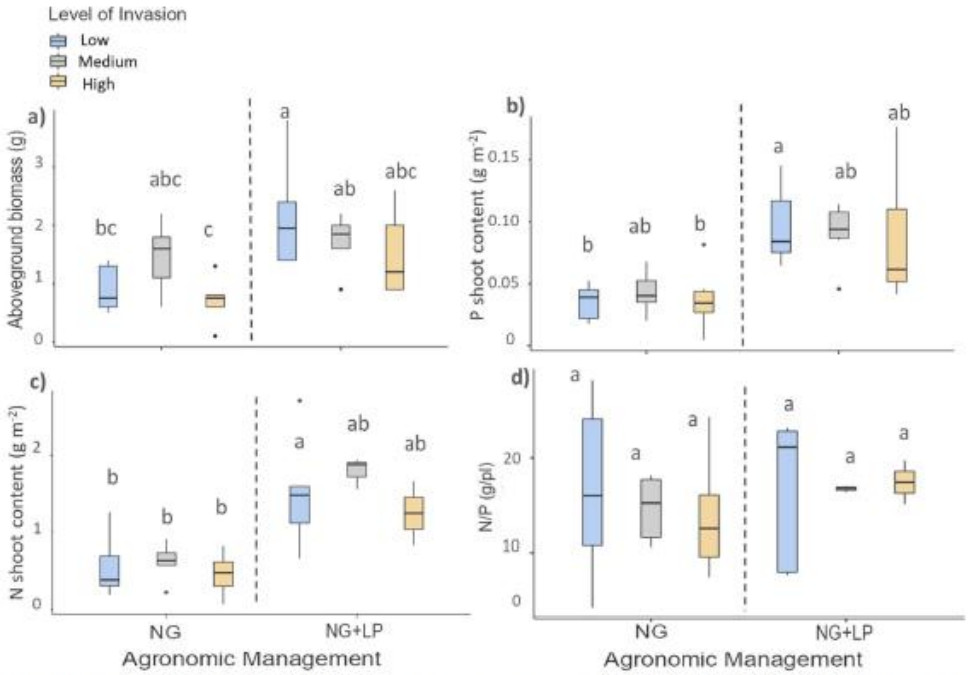


**FIGURE 3** | Box plots ($n = 6$ per treatment) of aboveground biomass (a), P shoot content (b), N shoot content (c), and N:P ratio in above-ground biomass (d) of *Paspalum notatum* plants growing with low (0%–10% in blue), medium (30%–50% in gray), or high (70%–90% in orange) levels of *Cynodon dactylon* invasion, in natural grasslands managed either extensively (NG) or oversown with exotic legumes and fertilized with phosphorus (NG + LP). Different letters above boxplots indicate significant statistical difference among treatments ($p < 0.05$) using the Least Significant Difference (LSD) test adjusted by Bonferroni.

18% more P in NG + LP than in NG. Conversely, there were no differences associated with the levels of invasion by *C. dactylon* (Figure 3a–c; Table 2; Appendix S5). The N:P ratio in *P. notatum* shoots ranged between 12 and 19 and it was not affected by any factor (Figure 3d; Table 2; Appendix S5).

The lower values of mycorrhizal colonization that corresponded to situations with high levels of invasion by *C. dactylon* did not correlate with lower biomass or nutritional status of *P. notatum*. In fact, there was a negative relationship between total arbuscular colonization and shoot P content ($p = 0.033$; $R^2 = 0.150$) in *P. notatum*, regardless of the level of invasion or agronomic management (Figure 4a; Tables 3 and 4). The association between total colonization and aboveground biomass was marginally negative ($p = 0.052$; $R^2 = 0.051$) and did not depend on the level of invasion or the agronomic management (Figure 4b; Tables 3 and 4). The low value of $R^2$ suggests that, although the variables were correlated, arbuscular mycorrhizal colonization did not explain much of the variability in shoot P content or aboveground biomass in *P. notatum*.

## 4 | Discussion

We expected that higher levels of invasion by *C. dactylon* would be associated with a reduction in mycorrhizal colonization of *P. notatum* and that this effect would be more important in NG + LP. As a consequence of this mutualism degradation, we expected to find negative effects on the growth and nutritional status of *P. notatum*. Our results show that the level of invasion by *C. dactylon* was consistently associated with a decline in arbuscular mycorrhizal colonization of *P. notatum*, particularly total and vesicular colonization. This likely disruptive effect was more pronounced on grasslands that had been oversown with an exotic legume and fertilized with P. However, lower mycorrhizal colonization in

**TABLE 2** | Results of linear mixed effect models (with normal error distribution) that tested the effects of treatments on Aboveground biomass, P shoot content, N shoot content, and N:P shoot ratio. Treatment contrasts, with the intercept representing the level NG (natural grassland) and 0%–10% (low invasion), are shown. NG + LP refers to native grassland oversown with exotic legumes and fertilized with phosphorus (intensive management), whereas 30%–50% and 70%–90% refer to medium and high levels of invasion by *Cynodon dactylon*.

| Response variable | Treatment | Estimate | SE | *t* | Pr(>\|*t*\|) |
|---|---|---|---|---|---|
| Aboveground biomass | Intercept | 0.88 | 0.21 | 4.15 | 0.003 |
| | NG + LP | 1.14 | 0.32 | 3.60 | 0.001 |
| | 30–50 | 0.28 | 0.30 | 0.94 | 0.354 |
| | 70–90 | −0.17 | 0.30 | −0.55 | 0.584 |
| | NG + LP*30–50 | −0.57 | 0.44 | −1.31 | 0.202 |
| | NG + LP*70–90 | −0.33 | 0.45 | −0.75 | 0.462 |
| P shoot content | Intercept | 0.04 | 0.01 | 2.85 | 0.008 |
| | NG + LP | 0.06 | 0.02 | 3.38 | 0.002 |
| | 30–50 | 0.01 | 0.02 | 0.45 | 0.647 |
| | 70–90 | <0.01 | 0.02 | 0.14 | 0.889 |
| | NG + LP*30–50 | −0.01 | 0.03 | −0.56 | 0.557 |
| | NG + LP*70–90 | −0.01 | 0.03 | −0.44 | 0.662 |
| N shoot content | Intercept | 0.55 | 0.18 | 3.09 | 0.005 |
| | NG + LP | 0.96 | 0.26 | 3.64 | 0.001 |
| | 30–50 | 0.07 | 0.25 | 0.29 | 0.778 |
| | 70–90 | −0.09 | 0.25 | −0.37 | 0.717 |
| | NG + LP*30–50 | 0.20 | 0.41 | 0.50 | 0.619 |
| | NG + LP*70–90 | −0.18 | 0.44 | −0.41 | 0.687 |
| N:P shoot ratio | Intercept | 16.68 | 2.82 | 5.91 | 0.004 |
| | NG + LP | −0.21 | 3.99 | −0.05 | 0.958 |
| | 30–50 | −2.06 | 3.80 | −0.54 | 0.594 |
| | 70–90 | −2.92 | 3.80 | −0.77 | 0.451 |
| | NG + LP*30–50 | 1.82 | 6.16 | 0.30 | 0.770 |
| | NG + LP*70–90 | 3.38 | 6.72 | 0.50 | 0.619 |

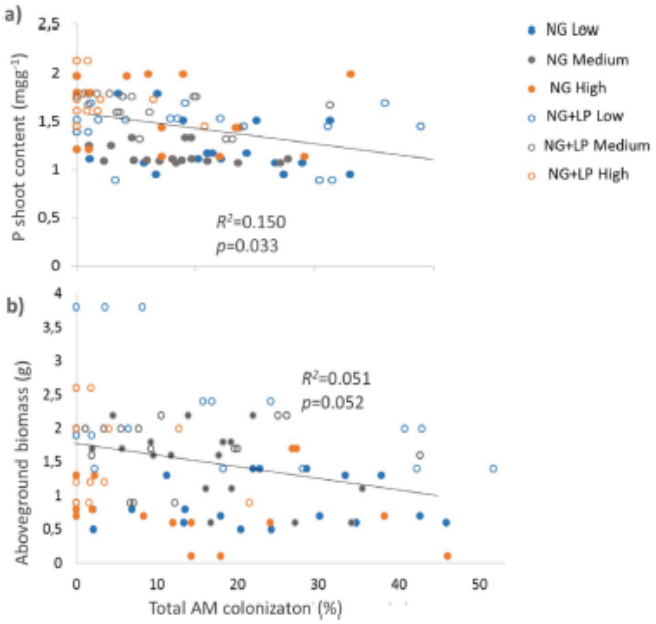


**FIGURE 4** | Relationship between P shoot content and total arbuscular mycorrhizal colonization (a), and between aboveground biomass and total arbuscular mycorrhizal colonization (b) in *Paspalum notatum* plants growing with low (0%–10% in blue), medium (30%–50% in gray), and high (70%–90% in orange) levels of *Cynodon dactylon* invasion, in natural grasslands managed either extensively (NG, closed circles) and oversown with exotic legumes and fertilized with phosphorus (NG + LP, open circles). See Tables 3 and 4 for statistical details.

*P. notatum* was not associated with lower aboveground biomass or nutrient status. *Paspalum notatum* shows a high presence of arbuscular mycorrhizas, as is typical for species with thick roots (Hartnett and Wilson 1999; Pezzani, Montaña, and Guevara 2006; García et al. 2019; Michelini et al. 2024). Indeed, experimentally induced 4- to 6-fold increases in soil available P have been shown to barely reduce this symbiosis in *Paspalum dilatatum* and *Mnesithea selloana*, two common native grasses that coexist with *P. notatum* (García et al. 2016), without any reduction in arbuscular mycorrhizal fungi diversity (García et al. 2017). It is therefore truly remarkable that the invasion of *C. dactylon* was so closely associated with the disruption of such a stable symbiosis between mycorrhizal colonisation and a native grass. Questions regarding the actual mechanism underlying this effect and its consequences for the competitiveness of invasive species are still open.

Although our methodological approach cannot disentangle cause-effect relationships between the level of invasion and mycorrhizal colonization, we suggest that *C. dactylon* can likely degrade the mycorrhizal symbiosis of a native species, supporting the hypothesis of mutualism degradation (*c.f.* Vogelsang et al. 2004; Vogelsang and Bever 2009; Hale and Kalisz 2012; Traveset and Richardson 2014; Roche et al. 2023). In this study, we selected patches with different levels of invasion that were contiguous, relatively small, and within the same soil type and plant community in paddocks with contrasting long-term management. This has allowed us to suggest relationships between levels of invasion and arbuscular mycorrhization, but an experiment in which levels of invasion are manipulated is essential to demonstrate causality. Mycorrhizal mutualism degradation is proposed as an important strategy of invasive species to establish in new environments, particularly when an invasive species is non-mycorrhizal, or has low dependence on mycorrhizae (Grove et al. 2017).

Worldwide, *C. dactylon* has been reported as nonmycorrhizal or facultative mycorrhizal, as it has shown no arbuscular mycorrhizal structures (Muthukumar and Udaiyan 2000; Muthukumar et al. 2006), very few fungal structures (Endresz, Somodi, and Kalapos 2013), or the absence of nutrient exchange structures (Lugo et al. 2018). In Uruguayan grasslands, *C. dactylon* has very low arbuscular mycorrhizal colonization (Michelini et al. 2024), whereas in invaded Uruguayan grasslands, we have corroborated that *C. dactylon* is less colonized and has fewer exchange structures than native species (Michelini et al. 2024). Lack of arbuscular mycorrhizae in the invasive species could limit the allocation of energy to the mycorrhizal fungi by outcompeting native species that rely on this interaction (Grove et al. 2017), eventually leading to the loss of fungi that are specific to native species. Furthermore, *C. dactylon* has been reported to be potentially allelopathic (Golparvar et al. 2015; Favaretto, Scheffer-basso, and Perez 2018; Guido et al. 2020; Bresciano, Glison, and Lezama 2022), which can reduce the germination of arbuscular mycorrhizal spores (Roberts and Anderson 2001; Stinson et al. 2006), spore density (Callaway et al. 2008), and

**TABLE 3** | Results of multiple linear regression with P shoot content and aboveground biomass as response variable, agronomic management and level of invasion as fixed factors, and total arbuscular mycorrhizal colonization (TC) as a covariable. Treatment contrasts, with the intercept representing the level NG (natural grassland) and 0%–10% (low invasion), are shown. NG + LP refers to native grassland oversown with exotic legumes and fertilized with phosphorus (intensive management), whereas 30%–50% and 70%–90% refer to medium and high levels of invasion by *Cynodon dactylon*.

| Response variable | Treatment | Estimate | SE | *t* | Pr(>\|*t*\|) |
|---|---|---|---|---|---|
| P shoot content | Intercept | 1.41 | 0.11 | 13.03 | <0.001 |
| | NG + LP | 0.08 | 0.13 | 0.68 | 0.510 |
| | 30–50 | −0.14 | 0.13 | −1.23 | 0.268 |
| | 70–90 | 0.25 | 0.13 | 1.87 | 0.053 |
| | TC | −0.01 | 4.0E-03 | −1.57 | 0.138 |
| | NG + LP*30–50 | 0.35 | 0.13 | 2.87 | 0.006 |
| | NG + LP*70–90 | 0.01 | 0.15 | 0.10 | 0.917 |
| | NG + LP*TC | <0.01 | 4.5E-03 | 0.32 | 0.747 |
| | 30–50*TC | <0.01 | 0.01 | 0.07 | 0.917 |
| | 70–90*TC | <0.01 | 0.01 | 0.29 | 0.837 |
| Aboveground biomass | Intercept | 0.97 | 0.27 | 3.62 | 0.005 |
| | NG + LP | 1.42 | 0.28 | 5.11 | <0.001 |
| | 30–50 | 0.66 | 0.28 | 2.33 | 0.022 |
| | 70–90 | −0.19 | 0.28 | −0.66 | 0.510 |
| | TC | <0.01 | 0.01 | −0.45 | 0.655 |
| | NG + LP*30–50 | −1.12 | 0.27 | −4.08 | 0.001 |
| | NG + LP*70–90 | −0.67 | 0.32 | −2.07 | 0.040 |
| | NG + LP*TC | −0.01 | 0.01 | −0.75 | 0.454 |
| | 30–50 *TC | <0.01 | 0.01 | −0.38 | 0.705 |
| | 70–90*TC | <0.01 | 0.01 | −0.13 | 0.893 |

mycorrhizal colonization (Barto et al. 2011). Evidence of allelopathy as an important mechanism disrupting microorganism-plant mutualisms is widespread (Hale and Kalisz 2012; Grove et al. 2017; Pinzone et al. 2018; Roche et al. 2021, 2023), and is considered an important strategy in plant invasions.

One of the scenarios where arbuscular mycorrhizal disruption is widely reported is in nutrient-enriched environments (Johnson et al. 2010; Chen et al. 2014; Grove et al. 2017; García et al. 2016; Yazici et al. 2021). In these cases, arbuscular mycorrhizae can become a burden for the host plant (Grimoldi et al. 2006). Therefore, increases in nutrient availability would reduce C allocation to fungus (Konvalinková et al. 2017) and decrease in root colonization by arbuscular mycorrhizal fungi (Chen et al. 2014; García et al. 2016; Yazici et al. 2021). However, our results indicate that mycorrhizal degradation would not be explained just by increased P availability, but suggest a strong, intrinsic invasion effect.

Degradation of arbuscular mycorrhizal symbiosis due to an increase in invasion level was greater in NG + LP, suggesting that whatever mechanism underlies the effects of invasive species on arbuscular mycorrhizal performance is exacerbated in more intensively managed grasslands. For example, greater production of toxic compounds for soil microorganisms in NG + LP compared with NG may explain the disruption of mutualism. Stronger allelopathy in response to increased nutrient availability has been reported in three Compositae invasive species affecting native plants (Sun et al. 2022). Furthermore, *C. dactylon* plant development might have been greater in NG + LP after the experimental 10-month period, leading to more intense light competition. A larger *C. dactylon* root system will increase the chance of encountering mycorrhizal spores in soil, making them unavailable to colonize *P. notatum* roots. To test this, it would be interesting to study how the invasive grass affects the arbuscular mycorrhizal spore bank.

Contrary to our expectations, reduced arbuscular mycorrhizal colonization was not associated with reductions in aboveground biomass nor P shoot content of the native species. These results, together with the fact that no differences in mycorrhizal colonization were observed between NG and NG + LP in low-invasion situations, suggest that the success of *C. dactylon* invasion, disrupting the arbuscular mycorrhizal symbiosis in *P. notatum*, would not be mediated by P. Instead, degradation of the mycorrhizal interaction might be primarily due to a direct impact of

**TABLE 4** | Results of multiple linear regression with P shoot content and aboveground biomass as response variable, agronomic management (AgrMan) and level of invasion (LI) as fixed factors, and total arbuscular mycorrhizal colonization (TC) as a covariable.

| Variable | Predictors | df | *F* | *p* |
|---|---|---|---|---|
| P shoot content | Intercept | 1 | 1553 | <0.001 |
| | AgrMan | 1 | 6.91 | 0.010 |
| | LI | 2 | 5.16 | 0.007 |
| | TC | 1 | 4.69 | 0.033 |
| | AgrMan*LI | 2 | 5.15 | 0.008 |
| | AgrMan*TC | 1 | 0.27 | 0.601 |
| | LI*TC | 2 | 0.04 | 0.965 |
| Aboveground biomass | Intercept | 1 | 105.71 | <0.001 |
| | AgrMan | 1 | 22.43 | <0.001 |
| | LI | 2 | 5.96 | 0.004 |
| | TC | 1 | 3.85 | 0.052 |
| | AgrMan*LI | 2 | 8.33 | 0.005 |
| | AgrMan*TC | 1 | 0.46 | 0.454 |
| | LI*TC | 2 | 0.07 | 0.930 |

*C. dactylon* on soil mycorrhizae propagules. Future research is needed to verify this possible mechanism of degradation and analyze its effect on the plant community as a whole.

We show that the aboveground biomass of *P. notatum* and its P shoot content were unaffected by the invasion. This could be occurring at the expense of energy allocated to belowground organs or mycorrhizal symbiosis, as it may be indicated by the decrease in arbuscular mycorrhizae vesicles (reserve structures of the arbuscular mycorrhizae) as the invasion levels increase. To test this, it would be necessary to measure other native plant performance variables, particularly belowground organs that could be affected by *C. dactylon* invasion.

## 5 | Conclusions

This is the first study to assess whether invasion success can be associated with the disruption of a stable symbiosis of recipient species under different nutrient conditions linked to different agronomic management. Our results showed that the level of invasion of *C. dactylon* was coupled to a reduction in the mycorrhizal colonization of the dominant native grass *P. notatum*, and that this reduction was exacerbated in more intensively managed grasslands. However, neither competition with the invasive nor the reduction in arbuscular mycorrhizal colonization due to *C. dactylon* invasion was associated with a decrease in the aboveground biomass or nutritional status of *P. notatum*.

Considering that *P. notatum*, as several other thick-rooted native species in Uruguayan grasslands, is highly colonized by arbuscular mycorrhizae (García et al. 2016; Del Pino et al. 2021; Michelini et al. 2024), this symbiosis might be providing plants in native grasslands benefits beyond nutritional ones, for instance, resistance to drought (Kakouridis et al. 2022; Romero-Munar et al. 2024) or protection against pests and diseases (Gange and West 1994; Newsham, Fitter, and Watkinson 1995; Smith and Read 2008; Weng et al. 2022). The loss of this stable symbiosis can have individual effects, such as reducing native plant growth, and community-wide effects, such as impacts on native plant diversity (Bever et al. 2001; Castillo et al. 2016), turning the system more vulnerable to plant invasion.

Since our study focused on the interaction between *C. dactylon* and only one native species in a controlled setting, extrapolations of these results should be taken with caution. To be able to scale-up conclusions about the role of mycorrhizal disruption in the success of *C. dactylon* invasion, future research would benefit from investigating the response of other (subordinate) native species that may be more dependent on mycorrhizae to compete with this invasive plant in a controlled experiment for disentangle some causal relationships.

Investigating the effect of the invasive plant on other variables related to arbuscular mycorrhizae may help to identify the precise mechanism by which the mutualism is disrupted. For example, studying the colonization rates in the invasive plant could allow us to determine whether mycorrhizae promote its competitive ability. On the other hand, analyzing the changes in fungal community composition of both *C. dactylon* and native plants in different invasion situations could show which fungal taxa are favored or harmed by the invasion.

### Author Contributions

S.G., F.P., A.G., and F.L. conceived the research idea, collected data, commented on the manuscript, and discussed the results. S.G. performed statistical analyses and wrote the paper.


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### Conflicts of Interest

The authors declare no conflicts of interest.

### Data Availability Statement

The data used in this publication are freely available at the Repositorio de datos abiertos de investigación de Uruguay: https://doi.org/10.60895/redata/FQJPDM.

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**Supporting Information**

Additional supporting information can be found online in the Supporting Information section.