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Short Communication

Emergence and establishment of native and non-native species in soils of remnant and converted highland grasslands – southern Brazil

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Abstract: Native grasslands in the Campos de Cima da Serra, Brazil, are being converted at speed for exotic tree plantations and cropland. The impact of modified and novel soil conditions on the establishment of native grassland species is unknown; establishment of non-native species, deliberately or accidentally introduced, could be favoured. In a common garden composed of fully randomized replicate samples of soils collected from remnant grassland, former cropland and pine plantations, we tested emergence and establishment of five cold-season species: Native low-tussock grass *Piptochaetium montevidense* (Spreng.) Parodi; native legume *Trifolium riograndense* Burkart; naturalized low-tussock grass *Vulpia bromoides* (L.) Gray; low-tussock grass *Holcus lanatus L.,* cultivated and naturalized in Brazil; and a cultivar of non-native *Trifolium repens*. Other than expected, soil type and species*soil type interactions had no significant effect on seedling emergence after 132 days in the field. Species effect on seedling emergence, however, was highly significant. *Vulpia bromoides* emergence was significantly highest in all soil types. *Holcus lanatus* and *Trifolium riograndense* both achieved second highest emergence rates and did not differ significantly from each other. Lowest overall emergence rates were found in the non-native clover cultivar. Lab germination tests failed for *Piptochaetium*, although it showed reasonable emergence in the field. Good performance of the native clover is encouraging for future grassland restoration, but the value of highly germinable *Vulpia* as a forage remains to be tested. *Holcus* tolerates a wide range of soil conditions and its life history traits may promote naturalization, or even invasiveness. Native grasslands of the region should be monitored for this species. Studies like these, but set up on a larger geographical scale and with a wider array of native species, will be essential in developing ecological restoration methods for southern Brazilian grasslands.

Keywords: *Seedling emergence, degraded soil, native forage species, invasive species.*

Emergência e estabelecimento de espécies nativas e exóticas em solos de campos convertidos e nativos remanescentes do Planalto - Sul do Brasil

Resumo: Uma acelerada conversão do campo nativo em plantações de espécies florestais exóticas e lavouras tem ocorrido nos Campos de Cima da Serra, Sul do Brasil. Ainda é desconhecido o impacto que as condições edáficas, em solos alterados ou preservados, exercem no estabelecimento de espécies nativas do campo; as espécies exóticas, introduzidas deliberada ou acidentalmente, talvez sejam favorecidas. Em um *common garden* composto por repetições aleatórias de amostras de solo, coletadas em áreas de antigas plantações de pinus, de antigas lavouras ou em áreas com campo nativo preservado, foram testados a emergência e o estabelecimento de cinco espécies hibernais: *Piptochaetium montevidense* (Spreng.) Parodi, gramínea cespitosa nativa; *Trifolium riograndense* Burkart, leguminosa nativa; *Vulpia bromoides* (L.) Gray, gramínea cespitosa naturalizada no sul do Brasil; *Holcus lanatus L.,* gramínea cespitosa exótica, cultivada e disseminada no Brasil; e um cultivar de *Trifolium repens,* leguminosa exótica largamente utilizada. Diferentemente do esperado, o tipo de solo e interações espécie*tipo de solo não tiveram efeito significativo na emergência de plântulas após 132 dias de teste a campo. O efeito da espécie na emergência das plântulas, entretanto, foi altamente significativo. A emergência de *Vulpia bromoides* foi significativamente superior em qualquer tipo de solo. Ambos *Holcus lanatus* e *Trifolium riograndense* apresentaram as segundas maiores taxas de emergência, não diferindo significativamente entre si; as taxas mais baixas foram apresentadas pelo cultivar de trevo não nativo. O Teste de Germinação em laboratório falhou para o *Piptochaetium*, apesar de este ter demonstrado razoável emergência a campo. O bom desempenho do trevo nativo é encorajador para futuras restaurações de pastagens nativas; o valor forrageiro de *Vulpia*, que apresentou alta germinação, ainda precisa ser testado. *Holcus* tolera uma ampla faixa de condições de solo, e suas características adaptativas podem vir a torná-lo naturalizado ou mesmo invasivo. Os campos da região devem ser monitorados em função dessa espécie. Estudos como esse, mas configurados em escala geográfica maior e com maior variedade de espécies nativas, serão essenciais no desenvolvimento de métodos de restauração para os Campos Sulinos Brasileiros.

Palavras-chave: *Emergência de plântulas, solo degradado, espécie campestre nativa, espécie invasora.*

Introduction

In the past decade, considerable changes in the landscape and in land use have occurred in the highlands of southern Brazil. Agricultural and silvicultural practices modify biotic and abiotic ecosystem components through liming, application of N-P-K fertilizer, deliberate and inadvertent introduction of non-native species. There is an increasing need for restoration of native grasslands after grassland conversion even inside designated conservation units (Hermann et al. 2016). However, for most native forages, routines for seed sourcing, testing and propagation have not been established. Revegetation of these areas relies on colonization from the regional species pool which contains both native and non-native species.

A major concern is that non-native taxa might turn invasive in these modified areas as "passengers of change" (see e.g. MacDougall & Turkington 2009). The Eurasian-origin tussock grass *Holcus lanatus*, for example, is being deliberately seeded in winter pastures of the *Campos de Cima da Serra* - which are often established in rotation with intensely cultivated crops such as potato and maize - and has expanded outside these areas in recent years. Cultivars of Eurasian-origin legumes *Trifolium pratense* and *Trifolium repens* are also used for winter forage and establish spontaneously, principally in roadsides and lawns in settlement areas. Native grassland species are presumably well adapted to the highly acidic grassland soils, but their performance in altered soil, relative to that of non-native species, is unknown.

The aim of this pilot-project is to determine the effect of original and novel soil conditions, modified by the expansion of agriculture and silviculture in the region, on the emergence of native and non-native plant species. Consequences for grassland restoration seeding are discussed.

Experimental setup and monitoring

The soils to be tested are sourced from a subset of areas investigated within the research project KO1741/3-1: 5 remnant grazed and burnt native grasslands, 5 pine plantations on former grassland, logged and re-grazed ("Ex-pine-plantation"), and 5 croplands on former grassland, re-grazed after cessation of arable land use ("Ex-Cropland"; see Table 1 and Koch et al. 2016 for further information on land use history and vegetation composition). All are located in the municipalities of São Francisco de Paula and Cambará do Sul, between 50°31'25.18"W and 50° 6'57.74"W, 29° 4'45.88"S and 29°23'2.54"S, Rio Grande do Sul state. From 24th to 27th January 2015 soil samples were taken from 0 – 10 cm depth in random locations on each site. We used this depth instead of the usual 0 – 20 cm because this superficial soil layer has most impact on the germination of the seeded seeds, and the principal aim of this study was not to observe the seed bank of the soil.

Five cold-season species were tested: Native low-tussock grass *Piptochaetium montevidense* (Spreng.) Parodi; native legume *Trifolium riograndense* Burkart; naturalized low-tussock grass *Vulpia bromoides* (L.) Gray; low-tussock grass *Holcus lanatus L.,* cultivated and naturalized in Brazil; and a cultivar of non-native *Trifolium repens*. Seeds of the first four were collected in the study region in mid-summer 2013 and 2014. *T. repens* did not produce sufficient seeds in the field and the seeds were store-bought. For details on harvest, seed cleaning and storage see Table S1, Appendix. This species set does not optimally represent grasslands of the region as it does not contain, for example, characteristic large-tussock species of the genera *Andropogon* and *Sorghastrum*. Unfortunately, these species often produce infertile seed (see also Overbeck et al. 2006) and in spite of repeated harvests across the region, not enough viable seeds could be obtained.

In March 2015, the seeds were subjected to germination tests in the Seed Analysis Laboratory of the Faculty of Agriculture at UFRGS – Porto Alegre according to the Rules for Seed Testing (Brasil 2009). The treatment to overcome seed dormancy was manual scarification with sandpaper number 180 for 20 seconds for both *Trifolium* species; for the other species we used KNO_3 (0.2%) solution. For the germination test, we used 4 repetitions with 100 seeds for each species. *Piptochaetium montevidense*, *Trifolium riograndense* and *Vulpia bromoides*, as many other native species, are not included in these Rules. Thus, we decided to use similar parameters for them as are given for other species from the same genus by various authors (Brasil 2009; Suñé & Franke 2006; Fochesato et al. 2000). Seeds were tested in a germination chamber with 8 hours light and 16 hours dark cycle. Type and duration of treatments are detailed in Table S2, Appendix. 1,000 seeds of each species were weighed at UERGS - São Francisco de Paula (more results, see Table 2). Median germination percentages per species are given in Table 2.

The field experiment was performed in a common garden in an open area (park lawn) belonging to the Public Agency of Transport (DAER), São Francisco de Paula. Soil was filled into in perforated 20 ^{x} 13 ^{x} 5 cm aluminium trays. There were 9 trays for each donor site: 8 repetitions of untreated soil and one tray with sterilized soil in order to assess and delete from analyses emergence from soil bank and seed rain. Land use type and donor site were noted on the bottom of the trays to enable a blinded monitoring. Trays were placed in a fully randomized setup on white shading fabric to reduce weed growth, with sufficient space between them to reduce contamination by soil splashing, and covered with tulle to minimize contamination by seed rain.

Table 1. Characterisation of soil source sites: Median time of conversion and of recovery; four indicators of soil fertility (median (min-max)); per-tray biomass produced in 4.25 months in the common garden (median (min-max)) as indicator of productivity. Same letter: no significant difference between soil types. Detailed statistics results for soil in Table S4, Appendix, and for biomass in Table S5, Appendix.

	Converted	In recovery	pH_{H2O}	Base sat. [%]	Al [cmolc/dm^3]	P [mg/dm^3]	Total dry biomass [g]
Native pasture	Never	-	4.3 (4.2 - 4.8) ^{a}	10 (7-38) ^{a}	4.8 (1.2 - 7.6) ^{ab}	2.1 (1.8 - 7.3) ^{a}	6.9 (4.8-10.2) ^{a}
Ex-Pine plantation	20 ys ago	3 ys	4.1 (3.8 - 4.3) ^{b}	6 (3-12) ^{a}	6.2 (4.0 - 10.7) ^{a}	4.0 (2.0 - 7.3) ^{a}	5.8 (5 - 15.5) ^{a}
Ex-Cropland	5.5. ys ago	5 ys	5.3 (4.5 - 5.4) ^{c}	64 (16-69) ^{b}	0.3 (0.1 - 4.5) ^{b}	12 (2.6 - 41.0) ^{a}	13 (7.4 - 20.1) ^{a}

Table 2. Thousand-seed-weight, lab germination and field emergence across all soil types of tested species. Median (min-max); emergence column, same letter: no significant difference between species. Detailed statistics results in Table S3, Appendix.

	TSW [g]	Lab germin. [%]	Germinable/sown	Emergence[1] [%]	Soil effect on emergence
Trifolium riograndense	0.5399	35	18/50	29 (9-40) [a]	n.s.
Vulpia bromoides	0.6691	79	20/25	100 (60-128*) [b]	n.s.
Holcus lanatus	0.2630	79	40/50	30 (18-49) [a]	n.s.
Trifolium repens	0.6145	78	39/50	6 (1-14) [c]	n.s.
				Emergence[2] [%]	
Piptochaetium montevidense	0.5403	0.75	n.a./50	13 (4-21)	n.s.

Emergence[1]: Median (min-max) % of germinable seed; Emergence [2]: Median (min-max) % of sown seed; *Values >100 due to higher germination success in the field than in the lab.

On 9th February 2015, each tray was subdivided in 6 squares (subplots): one unseeded control, five sown with seeds of one species (25 seeds of *Vulpia*, 50 seeds of the other species; *Vulpia* seeds were too large, including awns, to seed the same amount), randomly assigned to subplots. A thin soil layer was spread over the seeds and lightly pressed down. Trays were watered in the first week after setup and afterwards exposed to ambient climate.

In five of eight trays, all emerged seedlings, seeded and spontaneous, were counted and harvested once per month after sowing, on 11th March, 10th April, 8th May and 21st June (29, 60, 88 and 132 days after setup). Three of eight trays were left to grow undisturbed, and censused and harvested on 21st June; these were originally intended to monitor establishment and biomass production of seeded species. In counts we distinguished between the five seeded species, unseeded graminoids, and unseeded herbs. Biomass of each tray was dried at 105°C for 1-2 days and weighed in the lab of UERGS-São Francisco de Paula; at the final harvest, biomass was separated into seeded and unseeded grasses and herbs. In order to facilitate identification of seedlings, study species had also been seeded into two trays with commercial potting soil where their development could be observed. Pictures of each tray were taken every month for additional control and monitoring.

In the second half of June 2015, the common garden site was vandalized by stray dogs and the experiment closed after a total of 132 days. However, emergence of all species in all soils peaked between day 60 and day 88; we are therefore confident to have captured most of the potential total emergence.

Soil chemical data presented in Table 1 were analysed by the Soil Analysis Laboratory of the Faculty of Agriculture at UFRGS - Porto Alegre from one composite sample per site, collected in southern winters 2013/14 and 2014/15.

Analysis

For analysis, for each species and tray, we subtracted background emergence in unseeded subplots from number of seedlings in seeded subplots (two seedlings total in controls in April and May, 16 total in June, not more than 5% of total emergence of a species in a given tray; zero emergence in sterilized trays). For all species except *Piptochaetium*, we expressed emergence as number of seedlings related to number of germinable sown seed per subplot; *Piptochaetium* emergence is number of seedlings related to number of sown seed per subplot (see Table 2).

Due to partial destruction of the experiment, not enough replicate trays remained to analyse cumulative emergence (five seeded trays per donor site) and undisturbed establishment (three seeded trays per donor site) as originally planned. Instead, we analysed overall emergence by pooling emergence data from undisturbed and regularly harvested trays, summing data from all four surveys for the latter, in order to obtain a minimum of five replicate trays per donor site. A given site is represented by the median of these replicates in the analyses.

Due to the low number of replicate source sites and heterogeneous variance between them, we calculated and graphed medians of emergence, and we employed nonparametric univariate tests to test for significant differences in soil chemical parameters and in biomass production between soil types. We ran a generalised linear mixed model with emergence as dependent variable, soil type as fixed and species as random factor. In a second step, we again employed nonparametric univariate tests to test for significant differences in seedling emergence between species across soil types and per soil type (Kruskal-Wallis-test, Mann-Whitney U-test for pairwise post-hoc comparisons). Soil impact on *Piptochaetium* emergence was analysed separately. The data were analyzed and graphed with SPSS 24.0.

Results

Viable seeds were obtained for all five species, although with varying success. More than three quarters of *Holcus*, *Vulpia* and *T. repens* seeds and one third of *T. riograndense* seeds germinated in two weeks under laboratory conditions. For *Piptochaetium* this test failed. Lab germination was practically zero (Table 2), while median 13% of sown seeds emerged in 132 days in the field.

With five replicate sites per soil type, we were able to represent the original grassland soil conditions as well as significant alterations by land use change (Table 1 and Table S4, Appendix). pH and base saturation of grasslands are low (median 4.3, and 10%), and are further lowered by conversion to pine plantation, significantly in the case of pH. Liming and fertilization for agriculture significantly increase pH and also reduce aluminium toxicity, and this effect persisted up to five years after areas were taken out of agricultural use. Higher bioavailability of nutrients of former cropland soils was reflected by ca. two times higher total biomass production than in grassland and ex-pine plantations (Table 1), although only herb biomass showed a weakly significant difference between ex-cropland and ex-pine plantation soils, $U(8) = 2.000$, $Z = -2.193$, $p = 0.032$ (Table S5, Appendix). Herbs and grasses emerging spontaneously from the seed bank made up 55-65% of total biomass in grassland and ex-cropland soil, while seeded species made up more than half of biomass in ex-pine plantation soils.

Nonetheless, other than expected, soil type and species*soil type interactions had no significant effect on seedling emergence after 132 days in the field ($F(6,46) = 1.58$, $MSE = 110.23$, $p = 0.18$). This preliminary result of the GLM was supported by further, nonparametric tests insofar as seedling emergence did not differ significantly between pairs of soil types for any of the five species, with one exception: Native and non-native *Trifolium* both tended to have the lowest emergence in ex-pine plantation soils and the highest emergence on former cropland soils, and this difference was weakly significant (*Trifolium repens* $U(8) = 2.000$, $Z = -2.220$, $p = 0.032$; *Trifolium riograndense* $U(8) = 2.500$, $Z = -2.128$, $p = 0.032$).

Species effect on seedling emergence was highly significant ($F(3,6) = 119.34$, $MSE = 173.77$, $p = 0.00$; Table 2, Figure 1). *Vulpia bromoides* emergence was significantly highest of all species across soil types, and per soil types, in spite of seeds (like *Piptochaetium* seeds) being 15 months old at the start of the experiment. Across all soil types, *Vulpia* differed significantly a) from *Holcus lanatus*, U(26) = 0.000, Z = -4.496, p = 0.000, b) from *Trifolium repens*, U(26) = 0.000, Z = - 4.500, p = 0.000, c) from *Trifolium riograndense*, U(26) = 0.000, Z = -4.504, p = 0.000. These differences were only weakly significant ($p<0.05$) in native pasture soil, and in ex-pine plantation soil, and more pronounced in ex-cropland soil ($p<0.01$); detailed statistics results per soil type are given in Table S3, Appendix.

Holcus lanatus and *Trifolium riograndense* both achieved second highest emergence rates and did not differ significantly from each other, e.g. across all soil types U(28) = 77.000, Z = -1.476, p = 0.148.

Lowest overall emergence rates were found in the non-native clover cultivar. Across soil types, *Trifolium repens* differed significantly not only from *Vulpia* but from a) *Holcus lanatus*, U(28) = 0.000, Z = -4.674, p = 0.000 and b) from *Trifolium riograndense*, U(28) = 4.000, Z = -4.514, p = 0.000.

Discussion and conclusions

There was little indication that novel soils (ex-pine plantation, ex-cropland soil) favoured emergence of non-native species, as hypothesized. Emergence of *Vulpia bromoides*, originally a Eurasian species and now naturalised in Brazil, was higher than that of the other species and this difference was more pronounced in ex-cropland soil than in the other two soils with lower pH and nutrient status. This underlines its characterisation as a "ruderal" species by Brazilian field ecologists (G. Overbeck, pers. comm.). Non-native *Holcus lanatus* was not favoured by novel soil characteristics. *Trifolium repens*, also formerly a Eurasian species, did perform best in cropland soil but the same applied to its native congeneric, *Trifolium riograndense*. Both Fabaceae were probably favoured by the raised pH.

Non-native species do not yet play a major role on logged, re-grazed pine plantations in the study region, but they are an important component of secondary grassland communities on former cropland, accounting for 20% of vegetation cover on average (but making up less than 1% cover in remnant grassland; Koch et al. 2016). This is a globally reported phenomenon that even inspired development of the "novel ecosystem" concept (Cramer et al. 2008). Our study suggests that soil alteration alone does not account for non-native species success but that other factors such as disturbance and deliberate seeding must contribute to their establishment. This underlines the crucial role of restoration seeding to guide secondary grassland development to desired states.

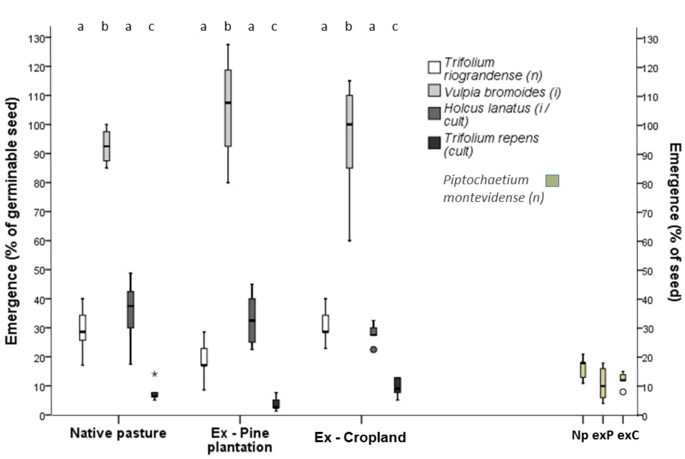

Figure 1. Emergence of five seeded cold-season species in three types of soil over 132 days in late summer – early winter 2015. n: native, i: introduced/naturalized, cult: cultivated. Soil type abbreviations correspond to types in left of graph.

Field emergence success of the native clover, *T. riograndense* is encouraging for future restoration projects, including use of restored grasslands for pasture. Increasing forage value of native grasslands has been a major incentive for introducing and overseeding legume cultivars; yet in this experiment the cultivar – by definition passed through selective enhancement - did not express best potential for emergence.

Piptochaetium montevidense has value as native winter and spring-growing forage grass that is also tolerant of elevated grazing pressure (Boldrini 2009; Maraschin 2009). In our experiment its emergence was lower than that of the other grasses. It is not clear whether this was due to higher age of the seeds, or whether processing and storage were not adequate. Laboratory germination protocols developed for other species from the same genus did not prove suitable.

Vulpia bromoides is not a cultivated forage species in its original range and was not deliberately introduced. It has naturalized spontaneously in Brazil and this experiment gave some indication for the reasons for this success. Seeds retained high germination potential for more than one year, and emergence was high irrespective of significant differences in soil chemistry. Its potential value as a forage species remains to be tested.

Non-native, naturalized *Holcus lanatus* demonstrated a good adaption to all site conditions, even to the remnant grasslands. Whether negative ecological and economic impacts are to be anticipated is not clear. A large body of literature exists for this species; key features that promote naturalization and invasiveness were summarized by Gucker 2008: *H. lanatus* produces large amounts of seeds, is dispersed by a variety of factors (water, animals, wind) and forms an abundant seed bank (notably, *Holcus* seed weight determined in this study was half as low or lower than that of the other species, Table 2). In its original range, adult plants with their velvety stem bases are not preferred cattle forage and it should therefore be grazed when young. Once established at undesired levels, the species cannot be easily removed as it tolerates both grazing and frequent fire (Gucker 2008). Summing up, it might be a potential forage species in novel soils and a potential invasive plant in native soils of our study region. Continuous monitoring of this species in the *Campos de Cima da Serra* is recommended.

Nonetheless, the results of this pilot study must be interpreted with caution and should principally serve to guide future, more extensive and better designed studies of non-native and native species emergence and establishment in remnant sites and under altered site conditions. 1) The range of tested species should be increased to include characteristic, summer-flowering tussock and prostrate grass species as well as representatives of further diverse plant families, e.g. Asteraceae and Cyperaceae. Both summer and winter vegetation period must be tested. 2) Improved replication of soil donor sites, or controlled manipulation of soil fertility, might reveal the range of soil conditions under which native species are at an advantage over non-natives, or at a disadvantage. 3) At present, germinability tests under laboratory conditions are tailored to commercial varieties (in which rapid germination is desirable) and must be adjusted to wild or semi-wild genotypes with prolonged germination periods. Current standard pre-treatments in the lab may also not be adequate to reveal emergence potential in the field.

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Table S1. Appendix: Information on studied species, seed acquisition and processing. The first three species are Poaceae, the Trifolium species are of the Fabaceae family.

Species name [1]	Species origin	Status in Brazil	Harvest site	Harvest and cleaning procedure
Piptochaetium montevidense (Spreng.) Parodi	Southern America	Native	Fertilized native pasture, 29°24'33.52"S, 50°21'17.10"W	Ca. 10 flower stalks harvested off 20 tussocks 15.12.2013, stored at room temperature ca. 3 and in fridge ca. 9 months; seed and chaff rubbed off inflorescences by hand, separated by repeated rubbing and sieving 30.12.2014 (yield ca. 10,000 seeds), fridge-stored until sowing 9.2.2015
Vulpia bromoides (L.) Gray	Eurasia	Naturalized [1]	Fertilized native pasture, 29°24'33.52"S, 50°21'17.10"W	1 handful (10-20) flower harvested off 10 tussocks 15.12.2013; stored at room temperature ca. 3 and in fridge ca. 9 months; spikelets rubbed off stems and between fingers to obtain single florets/seed 30.12.2014 (yield ca. 5,000 single seeds; do not separate easily), awns not removed, fridge-stored until sowing 9.2.2015
Holcus lanatus L.	Eurasia	Cultivated; naturalized [1]	Roadside grassland, 29°23'35.56"S, 50°18'31.82"W	Seed and chaff stripped off ca. 50 tussocks in field 15.12.2014, separated by sieving 10.-13.1.2015 (yield ca. 180,000 seeds), fridge-stored until sowing 9.2.2015
Trifolium riograndense Burkart	Southern America	Native	Fertilized native pasture, 29° 0'47.95"S, 50°25'21.76"W	Transect walks through area, >1000 entire flower heads collected 19.12.2014; stored at room temperature until dry; flower heads plucked apart, rubbed between hands and through metal sieve to break pods, then through plastic sieves to separate seed and chaff 10.-13.1.2015 (yield ca. 10,000 seeds); fridge-stored until sowing 9.2.2015.
Trifolium repens cultivar	Eurasia	Cultivated	Commercial source: Associação Rural, São Francisco de Paula-RS	Cultivar of unknown name produced in Uruguay, clean seed bought in January 2015, fridge-stored until sowing 9.2.2015

[1] Flora do Brasil 2020, under construction (see references)

Table S2 - Appendix: Lab germination testing procedures.

Species	Temperature (^{0}C)	Pre-treatment	First count	Last count
T. riograndense	20 – 30	Mechanical scarification; Pre-cooling, 8^{0}C, 5 days	7 days	14 days
V. bromoides	20 – 30	KNO_3(0,2%); Pre-cooling, 8^{0}C, 5 days	-	14 days
H. lanatus	20 – 30	KNO_3(0,2%); Pre-cooling, 8^{0}C, 5 days	6 days	14 days
T. repens	20	Mechanical scarification; Pre-cooling, 8^{0}C, 5 days	4 days	10 days
P. montevidense	20 – 30	KNO_3(0,2%); Pre-cooling, 8^{0}C, 5 days	7 days	14 days

Table S3 - Appendix: Statistics results for comparison of seedling emergence between soil types and species. Dependent variable: Percent of germinable sown seed emerged after four months in the field. Exception Piptochaetium datasets: Percent of sown seed emerged after four months in the field

GLM, fixed factor: soil type; random factor: species						
	df (effect)	**df (error)**	**F**	**MSE**	**p**	
Intercept	1	3	4.618	20674.267	0.121	
Soiltype	2	6.028	0.022	173.601	0.978	
Species	3	6.007	119.340	173.771	0.000	***
Soiltype*species	6	46	1.577	110.234	0.175	

Kruskal-Wallis-Test, factor: species				
Dataset	**df**	**Chi-Square**	**p**	
Soil types pooled	3	47.586	0.000	***
Native pasture	3	15.317	0.002	***
Ex-Pine plantation	3	16.089	0.001	***
Ex-Cropland	3	16.340	0.001	***

Mann-Whitney U-tests on differences between species (exact asymptotic significance)						
Species 1	**Species 2**	**df**	**U**	**Z**	**p**	
Dataset: Soil types pooled						
Holcus lanatus	*Trifolium repens*	28	0.000	-4.674	0.000	***
Trifolium riograndense	*Holcus lanatus*	28	77.000	-1.476	0.148	
Trifolium riograndense	*Trifolium repens*	28	4.000	-4.514	0.000	***
Trifolium riograndense	*Vulpia bromoides*	26	0.000	-4.504	0.000	***
Vulpia bromoides	*Holcus lanatus*	26	0.000	-4.496	0.000	***
Vulpia bromoides	*Trifolium repens*	26	0.000	-4.500	0.000	***
Dataset: Native pasture						

Level of significance: * P<0.05, ** P<0.01, *** P<0.001

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Table S3 - Appendix (cont.):

GLM, fixed factor: soil type; random factor: species						
	df (effect)	**df (error)**	**F**	**MSE**	**p**	
Holcus lanatus	*Trifolium repens*	8	0.000	-2.619	0.008	**
Trifolium riograndense	*Holcus lanatus*	8	7.000	-1.149	0.130	
Trifolium riograndense	*Trifolium repens*	8	0.000	-2.619	0.008	**
Trifolium riograndense	*Vulpia bromoides*	7	0.000	-2.449	0.016	*
Vulpia bromoides	*Holcus lanatus*	7	0.000	-2.449	0.016	*
Vulpia bromoides	*Trifolium repens*	7	0.000	-2.460	0.016	*
Dataset: Ex-Pine plantation						
Holcus lanatus	*Trifolium repens*	8	0.000	-2.619	0.008	**
Trifolium riograndense	*Holcus lanatus*	8	3.000	-1.991	0.056	
Trifolium riograndense	*Trifolium repens*	8	0.000	-2.627	0.008	**
Trifolium riograndense	*Vulpia bromoides*	7	0.000	-2.460	0.016	*
Vulpia bromoides	*Holcus lanatus*	7	0.000	-2.449	0.016	*
Vulpia bromoides	*Trifolium repens*	7	0.000	-2.460	0.016	*
Dataset: Ex-Cropland						
Holcus lanatus	*Trifolium repens*	8	0.000	-2.627	0.008	**
Trifolium riograndense	*Holcus lanatus*	8	8.000	-0.946	0.421	
Trifolium riograndense	*Trifolium repens*	8	0.000	-2.627	0.008	**
Trifolium riograndense	*Vulpia bromoides*	8	0.000	-2.619	0.008	**
Vulpia bromoides	*Holcus lanatus*	8	0.000	-2.619	0.008	**
Vulpia bromoides	*Trifolium repens*	8	0.000	-2.619	0.008	**

Kruskal-Wallis-Test, factor: soil type			
Dataset	**df**	**Chi-Square**	**p**
Species pooled	2	0.876	0.645
Holcus lanatus	2	1.516	0.469
Trifolium repens	2	5.937	0.051
Trifolium riograndense	2	5.141	0.076
Vulpia bromoides	2	1.304	0.521
Piptochaetium montevidense	2	3.047	0.218

Mann-Whitney U-tests on differences between soil types (exact asymptotic significance)						
Soil type 1	**Soil type 2**	**df**	**U**	**Z**	**p**	
Dataset: Species pooled						
Native pasture	Ex-Pine plantation	36	157.000	-0.686	0.506	
Native pasture	Ex-Cropland	37	187.500	-0.070	0.945	
Ex-Pine plantation	Ex-Cropland	37	157.500	-0.914	0.365	
Dataset: *Holcus lanatus*						
Native pasture	Ex-Pine plantation	8	11.000	-0.313	0.841	
Native pasture	Ex-Cropland	8	6.500	-1.261	0.222	
Ex-Pine plantation	Ex-Cropland	8	9.000	-0.738	0.548	
Dataset: *Trifolium repens*						
Native pasture	Ex-Pine plantation	8	4.000	-1.798	0.095	
Native pasture	Ex-Cropland	8	9.000	-0.740	0.548	
Ex-Pine plantation	Ex-Cropland	8	2.000	-2.220	0.032	*
Dataset: *Trifolium riograndense*						
Native pasture	Ex-Pine plantation	8	4.500	-1.697	0.095	
Native pasture	Ex-Cropland	8	11.000	-0.319	0.841	
Ex-Pine plantation	Ex-Cropland	8	2.500	-2.128	0.032	*
Dataset: *Vulpia bromoides*						
Native pasture	Ex-Pine plantation	6	4.000	-1.155	0.343	
Native pasture	Ex-Cropland	7	8.000	-0.494	0.730	
Ex-Pine plantation	Ex-Cropland	7	7.500	-0.615	0.556	
Dataset: *Piptochaetium montevidense*						
Native pasture	Ex-Pine plantation	8	6.000	-1.586	0.151	
Native pasture	Ex-Cropland	8	6.000	-1.366	0.222	
Ex-Pine plantation	Ex-Cropland	8	11.000	-0.314	0.841	

Level of significance: * P<0.05, ** P<0.01, *** P<0.001

Table S4 - Appendix: Comparison of selected soil chemistry traits between soil types

Kruskal-Wallis-Test, factor: soil type			
Dependent var.	**df**	**Chi-Square**	**p**
pH [H2O]	2	10.272	0.006
P [mg/dm^3]	2	4.837	0.089
Al [cmolc/dm^3]	2	7.348	0.025
Base saturation [%]	2	10.158	0.006

Mann-Whitney-U-Test on differences between soil types (exact asymptotic significance)						
Soil type 1	**Soil type 2**	**df**	**U**	**Z**	**p**	
Dataset: pH [H2O]						
Native pasture	Ex-Pine plantation	8	2.500	-2.128	0.032	*
Native pasture	Ex-Cropland	8	2.500	-2.108	0.032	*
Ex-Pine plantation	Ex-Cropland	8	0.000	-2.627	0.008	**
Dataset: P [mg/dm^3]						
Native pasture	Ex-Pine plantation	8	7.000	-1.156	0.310	
Native pasture	Ex-Cropland	8	3.000	-1.984	0.056	
Ex-Pine plantation	Ex-Cropland	8	6.000	-1.358	0.222	
Dataset: Al [cmolc/dm^3]						
Native pasture	Ex-Pine plantation	8	7.500	-1.048	0.310	
Native pasture	Ex-Cropland	8	3.000	-1.984	0.056	
Ex-Pine plantation	Ex-Cropland	8	1.000	-2.402	0.016	*
Dataset: Base saturation [%]						
Native pasture	Ex-Pine plantation	8	3.000	-1.991	0.056	
Native pasture	Ex-Cropland	8	2.000	-2.193	0.032	*
Ex-Pine plantation	Ex-Cropland	8	0.000	-2.619	0.008	**

Level of significance: * P<0.05, ** P<0.01.

Table S5 - Appendix: Comparison of harvested dried biomass between soil types

Kruskal-Wallis-Test, factor: soil type				
Dependent var.	**df**	**Chi-Square**	**p**	
Total biomass [g]	2	0.430	0.112	
Herb biomass [g]	2	6.540	0.038	*
Graminoid biomass [g]	2	3.860	0.145	

Mann-Whitney-U-Test on differences between soil types						
Soil type 1	**Soil type 2**	**df**	**U**	**Z**	**p**	
Dataset: Total biomass [g]						
Native pasture	Ex-Pine plantation	8	10.000	-0.522	0.690	
Native pasture	Ex-Cropland	8	3.000	-1.984	0.056	
Ex-Pine plantation	Ex-Cropland	8	5.000	-1.567	0.151	
Dataset: Herb biomass [g]						
Native pasture	Ex-Pine plantation	8	6.000	-1.358	0.222	
Native pasture	Ex-Cropland	8	4.000	-1.776	0.095	
Ex-Pine plantation	Ex-Cropland	8	2.000	-2.193	0.032	*
Dataset: Graminoid biomass [g]						
Native pasture	Ex-Pine plantation	8	10.000	-0.522	0.690	
Native pasture	Ex-Cropland	8	3.000	-1.984	0.056	
Ex-Pine plantation	Ex-Cropland	8	6.000	-1.358	0.222	

Level of significance: * P<0.05.

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