Lessons on direct seeding to restore Neotropical savanna

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1. Introduction

Open vegetation such as grasslands and savannas covered large areas of the tropics and have been disproportionately converted to large-scale farming (e.g., Veldman et al., 2015a). In contrast, most restoration projects and research in the tropics are centered on forests, mostly tree seedling planting, in these environments (Andrade et al., 2015; Ferreira et al., 2017). As a result, applying restoration techniques developed for forests, mostly tree seedling planting, in these environments leads to inappropriate, non-savanna endpoints, inadequate afforestation, unsuccessful restoration or the establishment of non-recruiting tree stands (Suding, 2011; Veldman et al., 2015b). There is an urgent need to develop effective restoration techniques for tropical savannas, where research and restoration practice may inform each other and lead to improved regulations (Chaves et al., 2015). National legislation and international agreements set targets to restore degraded areas, driving practitioners to undertake restoration efforts irrespective of information availability.

In savannas, as in other fire-prone ecosystems, natural plant populations persist under nutrient and water restrictions mostly through clonal reproduction and resprouting after fire and herbivory (Pausas et al., 2017). As common trade-offs, Neotropical savanna woody species have slow aboveground growth rates (Castro and Kaufman, 1998; Dantas and Pausas, 2013) and often produce a small number of viable seeds (Andrade et al., 2014; Salazar et al., 2012). Therefore, once the seed and especially bud bank are depleted following conversion to pastures or agricultural fields, natural regeneration is slow and episodic (e.g., Ferreira et al., 2017). In addition, plowing, fertilization and liming alter...
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Table 1

<table>
<thead>
<tr>
<th>Experiment name</th>
<th>Year</th>
<th>Site</th>
<th>soil type</th>
<th>Factors tested</th>
<th># of soil plowings</th>
<th>Seeding density of ground cover species</th>
<th>Seeding density of native spp. established</th>
<th># of native spp.</th>
<th>Mean RNC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st direct seeding</td>
<td>2012</td>
<td>CVNP</td>
<td>Plinthosol</td>
<td>Seeding</td>
<td>200</td>
<td>185 ± 38 (seed in m⁻²)</td>
<td>185 ± 38 (seed in m⁻²)</td>
<td>185 ± 38</td>
<td>24.6 ± 1.9</td>
</tr>
<tr>
<td>2nd direct seeding</td>
<td>2013</td>
<td>CVNP, CBR, Plinthosol</td>
<td>Seeding at 200 m²</td>
<td>3</td>
<td>185 ± 38 (seed in m⁻²)</td>
<td>185 ± 38 (seed in m⁻²)</td>
<td>185 ± 38</td>
<td>24.6 ± 1.9</td>
<td></td>
</tr>
<tr>
<td>3rd direct seeding</td>
<td>2014</td>
<td>CVNP, CBR, Plinthosol</td>
<td>Soil preparation intensity</td>
<td>4 &amp; 6</td>
<td>68 ± 2 (seed in m⁻²)</td>
<td>68 ± 2 (seed in m⁻²)</td>
<td>68 ± 2</td>
<td>24.6 ± 1.9</td>
<td></td>
</tr>
<tr>
<td>4th direct seeding</td>
<td>2015</td>
<td>CVNP, CBR, Plinthosol</td>
<td>Soil type of ground cover plant guild</td>
<td>6</td>
<td>953 ± 38 (seed in m⁻²)</td>
<td>953 ± 38 (seed in m⁻²)</td>
<td>953 ± 38</td>
<td>24.6 ± 1.9</td>
<td></td>
</tr>
</tbody>
</table>

Soil interventions were planting and tillage. Average RNC: relative native cover = native cover/total plant cover, the mean values reported do not include control (unseeded) areas. Soil types are rocky, well-drained, and seasonally waterlogged Plinthosols; ground cover plant guilds are native grasses, legumes, forbs, Asteraceae shrubs, palms (P), and trees (T). The experiments were established in four consecutive years across a total restoration area of 55 ha. Experiments in subsequent years were ed based on the results of previous experiments. Following an adaptive management framework, techniques to restore savannas are being improved and applied simultaneously in large-scale restoration practices required by law (Schmidt et al., 2018).

We designed the experiments to evaluate success of direct seeding grasses, forbs, shrubs and trees in reducing invasive grass cover and increasing native plant cover. Specifically, we aimed to answer the following questions:

1. Does seeding density affect native plant cover?
2. Can invasive grasses be controlled successfully by repeated soil plowing?
3. Does soil type affect native species establishment and invasive grass dominance?
4. Which plant guilds – grass, shrub or forb – are most effective in establishing native ground cover in different soil types?

We expected to find greater native species cover in areas restored with a higher density of seeds, since many past studies show that higher seeding rates favor restoration success (Adams and Galatowitsch, 2007). We also expected that native species cover would be greater in harsher soils, since native species are often better adapted than invasive species to naturally infertile soils or to those soils that impose stressful conditions, such as water or root-space limitation (Bustamante et al., 2012; Daehler, 2003). Plowing the soil (25–40 cm deep) damages invasive grass root systems and exposes the seed bank, promoting seed germination. Subsequent plowing then kills the seedlings, depleting the exotic seed bank (Carmona, 1992; Cavers and Benoit, 1989; Tu et al., 2001), so we anticipated that a greater number of soil plowings before restoration would shift the balance toward native species. Finally, we expected that native grasses would establish higher initial cover.
compared to the shrub and forb species tested since functional simi-
larity between native and invasive plants may allow native species to
outcompete invasive plants (Funk et al., 2008).

2. Methods

2.1. Study area

We conducted experiments in abandoned pasturelands in three areas
in Central Brazil: Contagem Biological Reserve, CBR (15°38′07″S
47°52′05″W, 1100 m elevation), Entre Rios Farm, ERF (15°57′31″S
47°27′27″W, 1060 m elevation) and Chapada dos Veadeiros National
Park, CVNP (14°07′03″S 47°38′30″W, 1240 m elevation, Table 1). This mesic
savanna region has a mean annual precipitation of 1500 mm, 90% of
which falls between October and April (Ribeiro and Walter, 2008). The
soils vary across the study site (ERF – cambisols; CBR – latosols; CVNP –
plinthosols) but are all nutrient-poor and acidic (pH ± 5) (Brasil,
1982).

All areas were originally open savannas, i.e. open cerrado and typical
cerrado (sensu Ribeiro and Walter, 2008) characterized by a continuous
grassy layer with scattered trees. There are more than 12,000 vascular
plant species known to the Neotropical savanna, of which around 65%
are non-woody (herb-shrub species). Among non-woody species, Fa-
baceae, Asteraceae and Poaceae are the most species-rich families
(Amaral et al., 2017). The natural vegetation in the study areas had
been converted and the soil had been plowed, fertilized and limed
several times to enrich soil, neutralize acidity and enhance produc-
tivity. The area in CBR was planted with soybeans for a few years
before it was converted to pastureland planted with exotic grasses.
In ERF and CVNP the natural vegetation was converted directly to exotic
pastures. Prior to restoration efforts, African C4 grasses (most abundant
Urochloa decumbens, U. humidicola and Andropogon gayanus and less
common Hyparrhenia rufa, Urochloa brizantha, Melinis minutiflora) ac-
counted for nearly 100% of ground cover. Cattle grazing had been
eliminated from all areas for at least two years prior to the start of the
experiments.

2.2. Experimental design

We replicated direct seeding experiments within and across study
sites, when possible, aiming to identify and overcome restoration bot-
tlenecks (Table 1). We selected species for seeding based on literature
and practitioners’ information on likely establishment and growth rates,
seed availability, abundance in conserved and disturbed areas, and
ability to coexist with invasive grasses. There was no prior information
on these species’ ability to establish through direct seeding in resta-
oration areas. We collected seeds from species of different life forms –
grass, forb, shrub and tree – aiming to increase plant functional group
diversity in restoration areas. We selected common species that pro-
duced sufficiently large quantities of seeds without overharvesting in
dividual plants, which is essential for large-scale applicability. Among
the non-woody species, we also harvested seeds from native ruderal
species assuming that species able to colonize disturbed areas would be
more likely to establish in the early stages of the restoration process.
Species lists can be found in Appendix A; details on species dispersal
period, seed characteristics and field establishment rates were de-
scribed by Pellizzaro et al. (2017).

In the Neotropical savanna, woody species fruit production is con-
centrated late in the dry season (August–October) (Oliveira and Gibbs,
2000), whereas non-woody plants tend to fruit during early dry season
(May–July) (Batalha and Martins, 2004). Seeds from each species were
harvested during the seed production period and stored in woven
polypropylene bags in dry conditions under ambient temperature until
seeding. Most of the harvested seeds are orthodox, and no treatment
was applied to break seed dormancy.

Due to environmental regulations related to federal protected areas
in Brazil (CVNP and CBR sites), we could not use herbicides to control
invasive grasses. The sites were prepared using agricultural techniques
and equipment. All experimental areas were plowed at least one time
(Table 1) during the dry season prior to seeding to decompose the soil
and mechanically control invasive grasses, which have deep roots and
form persistent seed banks.

Restoration experiments with 20 × 20 m experimental plots sepa-
rated by ≥ 20 m were located within larger restoration projects, which
ranged from three hectares in 2012 to 36 ha in 2015. Native seeds were
mixed and broadcasted by hand during the early rainy season
(November). In 2015, when a larger area was restored, the restoration
area was seeded mechanically and the restoration experiments (1.1 ha)
were seeded by hand. We monitored vegetation cover within plots
yearly, during the subsequent two to three rainy seasons
(December–May) using the line-point intercept method (Canfield,
1941), taking at least 200 samples per plot (two 20-m transects with
sample points at 20-cm intervals). At each sample point, we identified
the plant intercepting the line at the tallest height, recorded its origin
(native or exotic), and used this information to calculate the relative
native cover (RNC), where RNC = (native cover/total plant cover).
RNC results are presented as mean ± standard error.

The first direct seeding experiment was performed in November
2012 to test which of 24 native species of grasses, forbs, shrubs and
trees established successfully from seed (Table 1). We seeded the same
seeding mix in 27 20 × 20 m-plots spread across three hectares in one
study area (CVNP). In November 2013, we conducted a seeding density
experiment at all three study areas. We tested three seeding densities of
six ground cover species, with mean seeding density of 185, 318 and
584 seeds.m–2. Each seeding density was replicated in five blocks, each
one with three 20 × 20 m plots, i.e. with 15 experimental plots within a
total of nine hectares seeded, three hectares in each study area
(Table 1).

Due to security and logistical issues, the 2014 and 2015 experiments
were performed only at the CVNP site. Fire experiments carried out in
2013 (data not shown) indicated that burning the areas before plowing
helped to control invasive grasses and increased relative native plant
cover. As a result, we conducted controlled burns in the early dry
season (May), before plowing as part of the soil preparation treatment
for the 2014 and 2015 experiments. In collaboration with local seed
harvesters, we improved seed harvesting, processing and storage,
without significant cost increases (Schmidt et al., 2018); this allowed
for increases in seeding densities in the following experiments (Table 1).
In November 2014, we restored seven hectares in total and we set up the soil preparation experiment with four treatments ac-
cording to soil preparation intensities: T1: plowing soil 4 times to 25-cm
depth during the dry season; T2: plowing soil 6 times; T3: plowing soil 5
times + 1 inversion tillage (40 cm deep); and control (CN): which re-
ceived no soil preparation or seeding. We established and monitored six
20 × 20-m plots for each of the four treatment levels (Table 1). We also
established two other types of control: (i) soil plowing four times (as in
T1) with no seeding and (ii) seeding of native species with no soil
plowing, i.e. seeding into the invasive grass cover. In both these ex-
perimental controls, no native species established, and invasive grasses
represented 100% of the ground cover after the first rainy season
(Cordeiro, 2018); these treatments are not included in analyses pre-
vented here.

The early results of 2014 experiment (data not shown) suggested
better restoration results in more intensively prepared areas. Thus, for
the restoration of 36 ha in 2015, we plowed the soil six times. In
November 2015, we set up a soil type × ground cover plant guild ex-
periment comparing three Plinthic soil types: rocky, well-drained and
seasonally waterlogged. The rocky soil is characterized by > 10% grav
elaterite) in the surface layer, which impairs root growth, and < 20% clay, which results in good drainage but low water storage
capacity. The well-drained soil differs by the absence of gravel. The
seasonally (2–3 months) waterlogged soil has higher clay content
ferences were highly in
or seeding control treatment (ANOVA, F 3,26 = 18.2, p < < 0.01,
6 plowing and 5 plowing + 1 soil inversion tillage) were similarly ef-

z=

paration level; soil type or plant guild) as
considered site and experimental treatment (seeding density; soil pre-
ments. Each soil type by seeding mix combination was replicated in
same species composition and seed densities across the three treat-
 Each soil type by seeding mix combination was replicated in three 20 × 20 m plots in a total of 27 experimental plots (Table 1).

2.3. Data analysis

We analyzed the experiments separately and for each analysis, we considered site and experimental treatment (seeding density; soil preparation level; soil type or plant guild) as fixed factors and evaluated their effects on relative native cover. To better understand the effects of soil preparation, we compared relative native cover at the end of the second rainy season grouping sites according to soil preparation intensity: low (3 or 4 plowing before seeding) vs. high intensity plowing (5 or 6 plowings/inversion tillage). For this analysis, we combined data from 2013 to 2015 experiments and considered seeding year, site and seeding density as independent variables. We focused on relative native cover as a response variable since it is the most common criteria for judging grassland restoration success. We do not analyze the relative cover of invasive grasses as they generally cover any area that is not occupied by natives, so the results would just be the inverse of relative native cover.

For models considering repeated measures or multiple sites, we used general mixed effect models (lme function in ‘nlme’ package) (Pinheiro et al., 2011; Pinheiro and Bates, 2000) and for simpler models we performed ANOVA. We calculated general linear models using Tukey’s all-pair post hoc comparisons (glht function in ‘multcomp’ package). All analyses were performed in R statistical environment v 3.4.3 (R Foundation for Statistical Computing, Vienna, AT). Summarized model results are available in Appendix B.

3. Results

In first direct seeding experiment, the relative native cover increased significantly over the first three rainy seasons after seeding (F2.52 = 11.2, p < 0.01, Fig. 1). The seeding density experiment indicated that, overall, the two higher seeding densities of ground cover species (318 seeds.m⁻² and 584 seeds.m⁻²) resulted in significantly greater relative native cover (25.0 ± 6.9% and 25.6 ± 7.6%, respectively) than the lower seeding density (185 seeds.m⁻² –14.3 ± 6.4%; z = −2.6, p < 0.01) after the third rainy season. However, these differences were highly influenced by the experimental site (Table 2).

In the 2014 soil preparation experiment, all treatments (4 plowing, 6 plowing and 5 plowing + 1 soil inversion tillage) were similarly effective in increasing the native cover relative to the no soil preparation or seeding control treatment (ANOVA, F3,26 = 18.2, p < < 0.01, Fig. 2). However, when all sites and seeding densities from the 2013 to 2015 experiments were analyzed together, we found that higher intensity soil preparation (plowing/inversion tillage five or six times) resulted in significantly higher relative native cover (n = 39) than plowing only three or four times (n = 30, F1,59 = 19, p < 0.01, Fig. 3).

Both soil type (ANOVA, F2,18 = 12.6, p < 0.01) and plant guild
(F2,18 = 13.3, p < 0.01) significantly influenced relative native cover after one growing season. After two rainy seasons, there was a significant interaction between soil type and plant guild (F4,18 = 3.5, p = 0.03). For grasses and shrubs, native cover was highest on rocky soils, intermediate on well-drained soils, and lowest in waterlogged soils (soil F2,18 = 11.5, p < 0.01), whereas forbs did equally well in all soil types by the second rainy season. Relative native cover declined from the first to the second rainy seasons for both grasses in waterlogged soil and for forbs in rocky soil (Figs. 4 and 5).
Across all four studies, we found that 57 of the 67 seeded species established, which included nine of the 11 grass species; all three forb species and the one palm species; eight out of the nine shrub species and 36 of the 43 tree species (Appendix A).

4. Discussion

To our knowledge, these were the first experiments testing direct-seeding techniques to restore open-vegetation areas in the Neotropical savanna. Large scale restoration through this technique has been successfully implemented to restore more than 5000 ha of riparian forests in the Amazon basin and can involve local communities in conservation and restoration efforts, thereby generating income (Campos-Filho et al., 2013; Schmidt et al., 2018). Our results showed that direct seeding is an effective technique to establish a large number of native Cerrado species of different life forms.

In face of the limited information on native species phenology, germination and early growth, we selected species for direct seeding with high frequency and seed production, and the ability to resist and coexist with invasive grasses. Of the 67 species seeded, 85% successfully established suggesting that these criteria could be useful to select species for restoration in other regions. A few of the species seeded did not recruit in field conditions, even though seeds were viable and germinated in greenhouse conditions (Pellizzaro et al., 2017). This indicates that further study of germination requirements in field conditions is essential to increase the species pool available for restoration. At least two of the species that did not establish in restoration areas are known to have dormant seeds (Annona crassaflora and Guazuma ulmifolia) (Da Silva et al., 2007; Ribeiro et al., 2013). For these and other dormant seeds, treatments for breaking seed dormancy are recommended. It is important to note that even though we included a large portion of non-woody species in our seed mixes, we only tested 22 of the over 5000 potential ground cover species in the Neotropical savanna (Amaral et al., 2017), due to lack of information and seed availability. Future studies should continue to experiment with additional species, particularly from under-represented guilds (Lesage et al., 2018).

Savanna trees established well in the experiments with 36 species establishing, but they have an extremely low growth rate in all treatments with most species growing less than 30 cm in height after three rainy seasons (Pellizzaro et al., 2017). Among the tree species, two Fabaceae were the fastest growing species: Tachigali vulgaris and Hy-

menaea stigonocarpa (mean height of 36 cm and 21 cm, respectively, after two rainy seasons) (Pellizzaro et al., 2017). These results reinforce the importance of using plants of different life forms to restore grassland and savannas. Related work at our study sites show that the composition of early establishing herbaceous species strongly influences the recovery trajectory (Coutinho et al., 2019). The native species that comprised the most cover in the first two years of our studies (5–25% depending on the year, site, and experiment) were a mix of the
annual grass *Andropogon fastigatus*, perennial grasses *Schizachyrium sanguineum* and *Trachypogon festuca*gus, along with the shrub *Lepidaploa aurea* (Asteraceae) and the forb *Stylosanthes* spp. (Fabaceae) (Coutinho et al., 2019; Pellizzaro et al., 2017). To increase native species cover, we recommend seeding fast growing grasses, forbs, and shrubs combined, since they tend to perform complementary functional roles (Funk et al., 2008).

Direct seeding is more cost effective than seedling outplanting and allows for the re-introduction of diverse life forms in mixed stands (Silva and Vieira, 2017). The restoration experiments described here, with higher soil preparation intensity and seeding density, cost less than USD 3500.ha$^{-1}$ compared to USD 7000.ha$^{-1}$ for planting tree seedlings (Schmidt et al., 2018). Approximately 40% of the costs are related to soil preparation and 60% to seed harvesting, storing and seeding (Sampaio et al., 2007). We recommend using the higher seeding densities of ground cover species we tested (> 900 seeds.m$^{-2}$), since they tend to result in higher relative native cover (see Table 1), were not cost prohibitive, and are much lower than seeding densities that are effective for reducing invasive grass biomass in other regions. For example, Adams and Galatowitsch (2007) used 3000–15,000 seeds.m$^{-2}$ to restore wetlands in Minnesota, US and Kiehl et al. (2010), recommended seeding 2000–5000 seeds.m$^{-2}$ to restore grasslands in Europe. Higher seeding densities may also contribute to the native soil seed bank and further germination in the following years after seeding (Kolb et al., 2016).

Soil type, determined by physical and chemical properties, is a strong determinant for restoration success and should be considered when prioritizing areas to be restored and planning restoration efforts. Native grassland and savanna species tend to have higher establishment rates in poorer soils when compared to C4 African grasses used in pastures in the tropics (Daehler, 2003; Funk and Vitousek, 2007; Silva et al., 2015). These C4 grasses tend to be more nutrient-demanding and respond more efficiently to fertilization than savanna grasses (Bustamante et al., 2012; Williams and Baruch, 2009) or C3 forbs (Rao et al., 1996), so their growth tends to be reduced in nutrient-poor soils (Funk, 2013; Rao et al., 1997). Likewise, our results showed higher relative native cover on rocky soils than in soils more favorable to invasive grass growth. The well drained non-rocky soils impose less restrictions to invasive grasses and the waterlogged soils are more available to the invasive grass *Urochloa humidicola* that is adapted to waterlogged soils (Dias-Filho and Carvalho, 2000) and is abundant in the study areas. Competition with *U. humidicola* could explain the decline in native grasses in waterlogged soils that we observed in the second year (Coutinho et al., 2019). We compared three general soil types but did not collect nutrient or soil moisture data so we cannot tease out the relative effects of those factors.

As in other regions (e.g. Brooks et al., 2010), invasive grass dominance is the most important limitation for restoration success in Neotropical savannas (Coutinho et al., 2019; Silva and Vieira, 2017). In our experiments, mechanical control of these grasses through repeated soil plowings comprised 40% of restoration costs and reduced the cover of, but did not eliminate, these invasive grasses. Soil plowing has negative outcomes such as decreasing native species resprouting ability (Sampaio et al., 2007), decreases soil organic matter, increases greenhouse gas emission from the release of soil decomposition/organic compounds (Lal, 2004). However, it is a common practice among the farmers in the Neotropical savanna and it has been utilized the land prior to restoration, so the impact of additional plowing to control grasses should be limited compared with this past usage. Furthermore, the native savanna vegetation occurs in soils with lower fertility, higher acidity, and lower organic matter than pastures and agricultural land, which are fertilized and treated with lime application to increase pH. Hence, these repeated soil plowings may reduce the resources for invasive grasses. Currently, the main invasive grass control alternative is herbicides, which decrease restoration costs and cause less disturbance to the soil but are currently prohibited in protected areas in Brazil. These techniques should be considered to improve invasive grass control in savannas and grasslands restoration, including in Protected Areas given the current lack of alternatives.

5. Conclusion

Direct seeding is increasingly being promoted as a more cost-effective method to reintroduce native species to meet large-scale commitments to restore grassland, savanna, and forest ecosystems worldwide (Barr et al., 2017; Campos-Filho et al., 2013; De Vitis et al., 2017; Gibson-Roy et al., 2010; Schmidt et al., 2018). Our results from a series of experiments embedded in larger-scale restoration projects indicated that it is possible to reintroduce a considerable number of native species of diverse life forms by direct seeding in the Neotropical savanna. Effective restoration will likely depend on improved methods for invasive grass control because mechanical control of invasive species before seeding and the establishment of native species cover are not sufficient to eliminate invasive grasses. Moreover, areas with nutrient-poor, shallower soils, thereby less suitable to invasive grasses, should be prioritized for restoration until techniques to restore natural soil properties are developed.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecoleng.2019.07.025.

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