Check for updates

Ecological Applications, 0(0), 2021, e02472
© 2021 by the Ecological Society of America

A comprehensive experimental assessment of glyphosate ecological impacts in riparian forest restoration

Flávia G. Florido,[1] Jussara B. Regitano,[2] Pedro A. M. Andrade,[3] Fernando D. Andreote,[2] and Pedro H. S. Brancalion [1,4]

[1]*Department of Forest Sciences, "Luiz de Queiroz" College of Agriculture, University of São Paulo, Avenida Pádua Dias, 11, Piracicaba, Sao Paulo 13418-900 Brazil*
[2]*Department of Soil Sciences, "Luiz de Queiroz" College of Agriculture, University of São Paulo, Avenida Pádua Dias, 11, Piracicaba, Sao Paulo 13418-900 Brazil*
[3]*Department of Genetics, "Luiz de Queiroz" College of Agriculture, University of São Paulo, Avenida Pádua Dias, 11, Piracicaba, Sao Paulo 13418-900 Brazil*

Citation: Florido, F. G., J. B. Regitano, P. A. M. Andrade, F. D. Andreote, and P. H. S. Brancalion. 2021. A comprehensive experimental assessment of glyphosate ecological impacts in riparian forest restoration. Ecological Applications 00(00):e02472. 10.1002/eap.2472

Abstract. Competition with invasive grasses is one of the most important drivers of tree planting failures, especially in tropical forests. A widely disseminated weeding approach has been glyphosate spraying, the most used herbicide globally in forestry and ecosystem restoration. However, glyphosate use in restoration is highly controversial and requires further studies to elucidate its effects on restoration processes and the environment. We evaluated the use of glyphosate in riparian forest restoration and its impacts on tree planting costs, weed control efficiency, planted seedling performance, herbaceous and woody species regeneration, soil bacteria, and environmental contamination, using mowing treatments as a reference and based on a controlled experiment established in the Brazilian Atlantic Forest. Glyphosate spraying reduced by one-half and one-third the accumulated aboveground biomass of, respectively, weeds in general and of the invasive grass *Urochloa decumbens* compared to mowing treatments, and it reduced the cost by half. The performance of planted tree seedlings was markedly favored by glyphosate spraying compared to mowing treatments, as expressed by improved seedling height (~twice higher), crown area (~5× higher), and basal area (~5× higher); the regeneration of both native woody and ruderal herbaceous plants were also enhanced. Neither glyphosate nor its metabolite Aminomethylphosphonic acid (AMPA) residues were detected in either water runoff or soil samples, but they were found at relatively high concentrations in the runoff sediments (from 1.32 to 24.75 mg/kg for glyphosate and from 1.75 to 76.13 mg/kg for AMPA). Soil bacteria communities differed before and after glyphosate spraying in comparison to mowing plots (without glyphosate). Glyphosate spraying was far more cost effective than mowing for controlling *U. decumbens* and greatly improved the performance of planted tree seedlings and natural regeneration, while not leaving residues in soil and water. However, the changes in the structure of bacterial communities and high concentration of glyphosate and AMPA residues in runoff sediments highlight the need for caution when using this herbicide in riparian buffers. We present alternatives for reducing glyphosate use and minimizing its risks in tree planting initiatives.

Key words: forest regeneration; forestry; invasive grasses; reforestation; restoration costs; soil microbial diversity; tree planting; tropical forests; Urochloa decumbens.

Introduction

Tree planting programs have expanded globally as a means to achieve multiple socio-ecological benefits, but many of these programs have failed due to poor plantation maintenance (Holl and Brancalion 2020). One of the main causes of tree planting failure is the competition with ruderal and invasive plants, which can reduce tree seedlings' survival and performance (Sweeney et al. 2002, Rodrigues et al. 2011, Weidlich et al. 2020). Under competition, seedlings may not grow enough to outcompete ruderal plants through shading and, therefore, tree plantings may never achieve some of their most targeted benefits, such as carbon sequestration, timber production, watershed and soil protection, and biodiversity conservation (Chazdon and Brancalion 2019).

One cost-effective, widely disseminated, weed control approach involves the use of herbicides. Glyphosate, in particular, is the most heavily used herbicide globally in forestry (Rolando et al. 2017) and ecosystem restoration

Manuscript received 25 November 2020; revised 15 April 2021; accepted 4 June 2021; final version received 4 June 2021. Corresponding Editor: Yude Pan.
[4] E-mail: pedrob@usp.br

(Weidlich et al. 2020), due to its low cost, reduced residual effects, and high efficiency in weed control (Wagner et al. 2017). Its use in restoration is expected to grow following the implementation of the several-million-hectare forest and landscape restoration commitments and trillion-tree planting programs planned for the next decade, which was recognized by the United Nations as the Decade on Ecosystem Restoration (Brancalion and Holl 2020). However, glyphosate use in environmental programs is highly controversial, especially in riparian areas, which are ecologically sensitive and may led to drinking water contamination (Gregoire et al. 2010). A growing number of scientific publications and judicial actions contest glyphosate safety for the human health and the environment (Helander et al. 2012, Maggi et al. 2020), which have motivated some restoration practitioners to quit using this herbicide. However, the challenge of controlling competing plants, especially exotic alien grasses in tropical forest restoration, remains and alternatives to herbicides have just started to be developed (Little et al. 2006).

In spite of the general assumption that glyphosate has both financial and practical advantages over non-chemical control methods, this assumption has rarely been tested. Understanding the pros and cons of using glyphosate in restoration is fundamental since costs and field performance cannot be the only references to take decisions on the adoption of new technologies with potential environmental hazards. In addition, reforestation practitioners may use glyphosate cost effectiveness as a reference to decide whether to use non-chemical weeding approaches, so critically comparing glyphosate spraying with mowing can help to support the development of alternative methods with higher chances of adoption. Here, we evaluated the use of glyphosate in riparian forest restoration and its impacts on tree planting costs, weed control efficiency, planted seedling performance, herbaceous and woody species regeneration, soil bacteria, and environmental contamination, using mowing treatments as reference. To the best of our knowledge, this is the first study to conduct such a comprehensive assessment under controlled experimental conditions. Our overarching goal was to obtain scientifically sound evidence of the potential advantages and emerging risks of glyphosate use in restoration, thus contributing to decision making in tree planting programs.

Methods

Experiment set-up

The experiment was set in the Atlantic Forest region of southeastern Brazil, at the Forest Restoration Center of the environmental NGO SOS Mata Atlântica, located in Itu-SP (23.256780° S, 47.418804° W; Appendix S1: Fig. S1). This region has humid subtropical climate (Cwa climate, Köppen classification), with mean annual temperature of 21.5°C and precipitation of 1,279 mm (detailed information about soil is presented on Appendix S1: Table S1). The relief is undulated, with rocky outcrops and slopes varying between 7° and 12°. We selected a study site (1) within a riparian area (borders of a water reservoir), (2) previously occupied by a planted pasture of the invasive African fodder grass *Urochloa decumbens* Stapf., (3) distant from forest remnants, and (4) with no regenerating seedlings of native tree species to represent the predominant restoration condition in the region (Rodrigues et al. 2011).

We initially mowed the grasses with a tractor across the whole experimental area and established 20 × 20 m experimental plots set 5 m apart from each other, and controlled leaf-cutter ants with insecticide baits. We employed a randomized design with eight blocks (24 plots total). We planted 70 nursery-grown seedlings of 20 native tree species in each plot (Appendix S1: Table S2), with one-half of the species classified as pioneer and one-half as non-pioneer, employing a regular spacing of 3 × 2 m and fertilization with NPK 20-05-20, which represents the most used restoration planting approach in the region (Rodrigues et al. 2011). Weeds were mowed in the five meters strip between plots throughout the experiment. This distance was defined to reduce the superficial movement of glyphosate and AMPA residues among plots, as a 4–5 m strip of grasses at water course borders was demonstrated to reduce glyphosate loads by 39–78% (Reichenberger et al. 2007, Lin et al. 2011, Lerch et al. 2017).

We established three types of treatment areas: (1) glyphosate, (2) low-frequency mowing, and (3) mowing. Glyphosate treatment involved spraying glyphosate (6 L/ha) all over the plot area before planting tree seedlings), and maintained with respraying it (4 L/ha) twice a year for weed control. Glyphosate (Atanor 48 Albaugh [Glifosato Atanor 48, Albaugh Agro Brasil LTDA., Resende, Brazil], composed by glyphosate isopropylamine salt at 48% (w/v) as active ingredient and 36% (w/v) of glyphosate equivalent) was sprayed mainly by the morning, with no rainy forecast and winds between 3 and 10 km/h, with a backpack sprayer equipped with a flat fan spray nozzle, and trained workers used personal protective equipment. In the low-frequency mowing treatment, we planted tree seedlings directly after tractor mowing, and further controlled weeds with a string trimmer twice a year. Weeding was performed at the same time as glyphosate spraying in the glyphosate treatment plots, to allow a direct comparison between weeding methods. In the mowing treatment plots, we planted tree seedlings directly after tractor mowing and used four mowing interventions per year, which is the frequency traditionally adopted by local restoration projects (see Fig. 1 for the timing of interventions). We removed weeds with a hoe in a 0.5 m radius around tree seedlings before glyphosate spraying and mowing in order to reduce the risks of herbicide drift and physical damages to seedlings' collar. On month six, we had to spray glyphosate twice due to operational flaws and both interventions were considered as a single maintenance.

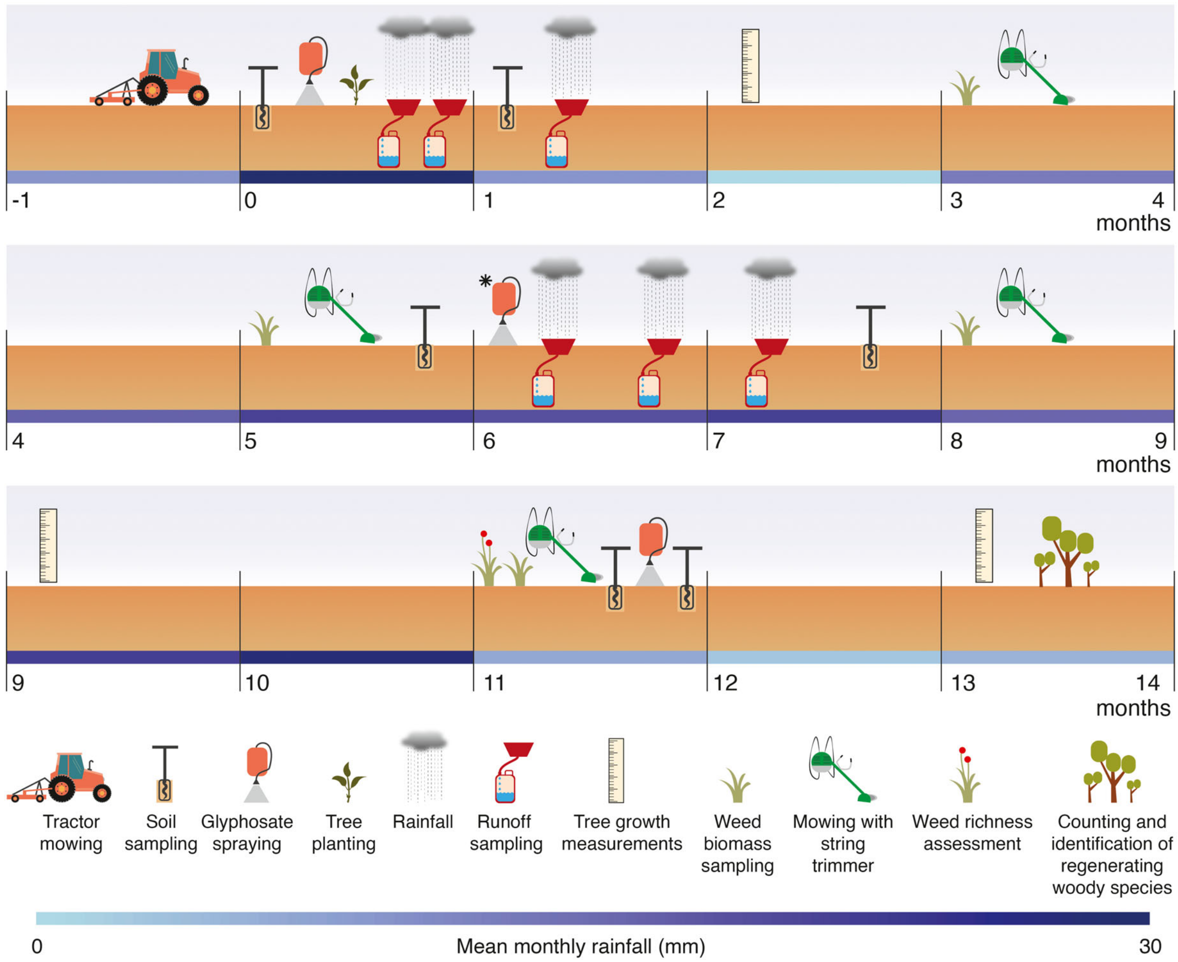

FIG. 1. Timing of weed control interventions and data collection along the experiment. The darkness of blue coloring the horizontal bar beneath each month represents the accumulated rainfall in the period, as expressed by the scale bar at the bottom of the figure. It was necessary to spray glyphosate twice as, due to operational problems on lack of homogeneity, there were still green weeds two weeks after spraying.

Experiment monitoring

Weed reinfestation.—Before each weeding intervention (Fig. 1), we randomly distributed four grids of 0.5 × 0.5 m in each plot, where we visually classified the proportion of invasive grass (i.e., *U. decumbens*) and other weeds (we included only herbaceous plants). We then collected the aboveground weed biomass, oven dried it at 60°C for 48 h, and weighed it. We summed the estimated dry biomass of each weed group (total weed biomass multiplied by the proportion of the weed group in the sample) collected during the experiment to estimate the total dry biomass per plot of each group of weeds.

Weeding costs.—The weeding costs were calculated based on labor and other inputs (Appendix S1: Table S3). Labor costs were calculated by multiplying the time required to control weeds (we timed the activities in each plot) and the per hour costs of rural labor in the region (Appendix S1: Table S3; IEA 2019). For glyphosate treatments, we considered as input costs the amount of glyphosate used (we weighed the backpack sprayer before and after glyphosate spraying in each plot to assess the volume of product used). For the low-frequency mowing and mowing treatments, we considered as input costs the amount of gasoline and oil to operate the string trimmer during the time required to control weeds in each plot (we considered the consumption of 1 L/h of the mixture 25:1 of gasoline and oil; Appendix S1: Table S3; Brasil 2017, IEA 2019). We also calculated equipment depreciation costs (backpack sprayer and string trimmer), but since they were negligible (0.39% of total costs per treatment), we did not consider them in the analyses. All evaluations were made at the plot level. The exchange rate used for conversion was US$1 = 4.02 BRL.

Performance of planted tree seedlings.—We assessed the performance of the planted tree seedlings by measuring: height (from seedling collar to the highest leaf), crown area (calculated by using two measurements of crown diameter and considering crown shape as circular), basal area (measured with a caliper at bole diameter), and seedling survival 2, 9, 13, and 28 months after planting (Fig. 1).

Glyphosate and AMPA residues in soil, water, and sediments.—We collected soil samples at 0–10 cm depth in both glyphosate and low-frequency mowing treatments at four time points: (1) before tree planting, (2) 25 d after the first glyphosate application, (3) right before the second glyphosate application, and 25 d after the second application (see Fig. 1 for sampling timing), in order to evaluate glyphosate and its metabolite (AMPA) percolation through soil macropores (Kjær et al. 2011). The interval of 25 d was defined based on the half-life value of 1.5 d for glyphosate and 26.4 for AMPA at warm and rainy regions (Bento et al. 2016). Runoff water was sampled in collectors installed perpendicularly to the direction of the runoff flow and connected to a water tank, which stored the first 5 L of runoff and discarded the rest. These collectors were installed at the lower portion of the plots at the end of V-shaped grooves created for concentrating runoff (Appendix S1: Fig. S2). We collected runoff samples directly after the three first rain events observed after the two glyphosate applications/mowing interventions (six collections; Fig. 1), and stored at 4°C before chemical analyses. We lost four samples collected after the second rain event of the first sampling period due to problems during transportation.

We assessed residues of glyphosate and AMPA in soil samples, and in water and sediments extracted from runoff samples (see methodological details in Appendix S1). Soil and sediment analyses were assessed by gas chromatograph (model CG Trace 1310, Thermo Scientific, Waltham, Massachusetts, USA), coupled to a simple quadrupole mass spectrometer (GC/MS; model ISQ, Thermo Scientific) with 1,250 μg/kg as the limit of quantitation; water analysis was performed in a liquid spectrometer (model Accela, Thermo Scientific), coupled to a triple quadrupole mass spectrometer (LC/MS/MS; model TAQ Quantum Access, Thermo Scientific) with 50 μg/L as limit of quantitation (detailed extracting procedures were described in Appendix S1).

Impacts on non-targeted organisms.—*1. Spontaneously regenerating plants.*—Eleven months after tree planting, we assessed the density of individuals and species of non-planted native woody species with height > 50 cm, and the species density per plot of spontaneously regenerating herbaceous plants (Fig. 1). All woody individuals and herbaceous species found within a plot were considered.

2. Soil bacteria.—We evaluated soil bacterial communities in the glyphosate and low-frequency mowing treatments, thus excluding the "mowing" treatment, as this analysis was focused on comparing a chemical and a non-chemical weeding approach. In each plot, we collected nine 0–10 cm soil subsamples with a soil probe, regularly distributed across the plot (Appendix S1: Fig. S3). We homogenized the subsamples by vigorously shaking them in a container and stored the composite sample at –80°C for further use. In the glyphosate treatment, we collected soil samples 5 months after the first maintenance spraying, immediately before the second spraying (5mo-gly), and another 24 h after the second spraying (5mo+24h-gly). On the low-frequency mowing treatment, we collected one sample (no-gly) at the same time as 5mo-gly (see Fig. 1 for sampling timing).

We then obtained a 0.50 g soil sample from the stored composite sample and extracted soil DNA using NucleoSpin Soil Kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany). DNA concentration was measured with Nanodrop (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA) and integrity was checked by agarose gel electrophoresis. The sequencing procedures were performed using the enterprise Helixxa (Paulinia-SP, Brazil), based on a paired-end 250 nt via Illumina MiSeq V2 Nano (Illumina Inc., San Diego, California, USA) of gene-specific primer for amplifying the V4-V5 region of 16 S rRNA (5′-CAACGCGAAGAACCTTACC-3′ and 5′-CGTCRTCCCCRCCTTCC-3′). PCR (Platinum Taq High Fidelity), and library building was based on Illumina protocols (Illumina 2019), with quantification and quality control done by Bioanalyzer 2100. Final normalization was standardized up to 5 ng/μL volume for one pool multiplex sequencing and quantification by qPCR (real time PCR).

Data analysis

Sequence analysis.—The files containing the raw reads were analyzed using Quantitative Insights Into Microbial Ecology (QIIME) software version 2 (Bolyen et al. 2019). Sequences were first grouped according to barcodes inserted in the forward primers and filtered by quality (qual.score = 25, maximum mismatch primer = 2, window of quality 50 and maximum number of homopolymers = 6). Sequences smaller than 180 base pairs (bp) were discarded. Similar sequences were assigned to operational taxonomic units (OTUs) using the Sortmerna method based on 97% similarity (Kopylova et al. 2012). Representative sequences of each OTU were subjected to taxonomic analysis through the PYNAST method against the Greengenes database (DeSantis et al. 2006), OTUs of low abundance (e.g., singletons and doubletons) were removed from the data set. Then, Sequence data sets were uploaded to the MG-RAST (Metagenomics Analysis Server) databank (job ID "MGP90305").

Bacterial communities were assessed using alpha- and beta-diversity methods. The alpha-diversity analysis determined the number of observed OTUs, diversity index (Shannon H'), Simpson $1 - D$, and Chao1 $- S'$). Statistical variation among samples were calculated using Tukey's Student Range Test ($P < 0.05$), in R software (R Development Core Team 2016).

We square-root-transformed the data to assess beta diversity and similarity matrices were generated based on the Bray-Curtis algorithm. Jaccard and Weighted and Unweighted Unifrac matrices supported the principal coordinate analysis (PCoA), which was drawn to exhibit the differences between the groups according to OTU percentage matrices, with Beta disperser and ADONIS as complementary nonparametric analyses. To observe the impact of glyphosate application on soil bacteria abundance, diversity, and composition, we analyzed the relative variation of identified families with $P < 0.05$ by Kruskal-Wallis. We compared the shifts on bacterial population at two distinct scenarios: between the same plots before and 24 h after glyphosate spraying, [(5mo+24h-gly/5mo-gly −1) 100] for positive and [−(5mo-gly/5mo+24h-gly −1) 100] for negative growth; and between distinct plots without and 24 h after glyphosate spraying, [(5mo+24h-gly/no-gly −1) 100] for positive and [−(no-gly/5mo+24h-gly −1) 100] for negative growth.

Univariate data analysis.—Homogeneity of variance and normality assumptions were tested by Bartlett and Shapiro-Wilk tests, respectively, before conducting one-way analysis of variance (GLM procedure) to compare independent effects in weed control costs, weed dry biomass, performance of planted tree seedlings, and richness of spontaneously regenerating plants. Pairwise Wilcoxon rank-sum test ($P < 0.05$) was used to compare woody species density as data did not show homogeneity of variance. Other variables were compared using Tukey's Student Range Test (HSD) ($P < 0.05$). All the analyses were made using R 3.0 environment (R Development Core Team 2016).

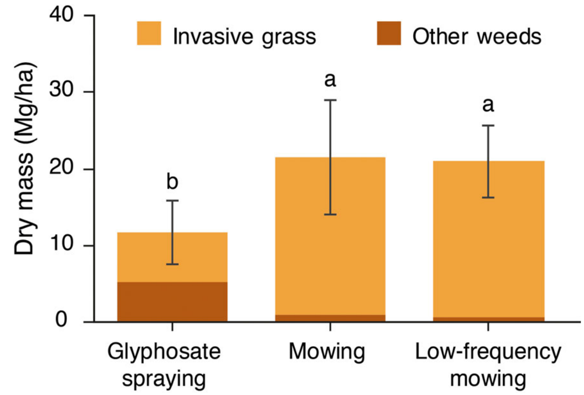

FIG. 2. Estimated aboveground dry biomass of *Urochloa decumbens* (invasive grass) and other weeds. Different letters above the bars indicate that total dry mass means were significantly different (Tukey tests, $P < 0.05$). Error bars represent the standard deviation.

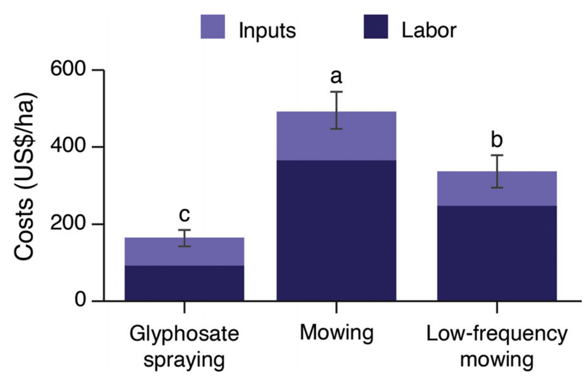

FIG. 3. Inputs and labor costs of weeding treatments. Different letters indicate that total costs means were significantly different (Tukey tests, $P < 0.05$). Error bars represent the standard deviation.

RESULTS

Compared to low-frequency mowing and mowing treatment, the glyphosate treatment reduced the accumulated aboveground biomass of weeds by one-half and of the invasive grass (*U. decumbens*) by one-third during the first plantation year (Fig. 2). However, the proportion of other weeds, different from *U. decumbens,* was much higher for the glyphosate (44.3% ± 22.5%; mean ± SE) than for the mowing (4.3% ± 2.6% for regular and 3.4% ± 2.7% for low frequency) treatments.

The costs of glyphosate weed control were at least half of that of mowing treatments, which differed from each other and were significantly lower, as expected, for the low-frequency mowing. Both labor and input costs were lower for glyphosate spraying, and the higher labor costs of mowing was the main determinant of the contrasting results observed between treatments (Fig. 3). The combination of reduced biomass of invasive grass and lower weeding costs suggests that glyphosate use was nearly six- and ninefold more cost effective than the low and the regular frequency mowing treatments, respectively.

The performance of planted tree seedlings was markedly favored by glyphosate spraying compared to mowing treatments by 28 months after planting, as expressed by improved seedling height (about two times higher), crown area (about five times higher), and basal area (about five times higher; Fig. 4). For crown and basal area, glyphosate spraying was nearly 15- and 10-fold more cost effective than the regular and the low-frequency mowing treatments, respectively (i.e., approximately one-third and one-half of the costs for getting fivefold growth). Seedling survival was higher for the glyphosate treatment compared to low-frequency mowing, whereas seedling survival in the regular frequency mowing did not differ from both treatments. Seedling growth and survival did not differ between mowing treatments, in spite of their contrasting frequency of interventions (Fig. 4).

Glyphosate spraying increased species density of both spontaneously regenerating woody and herbaceous

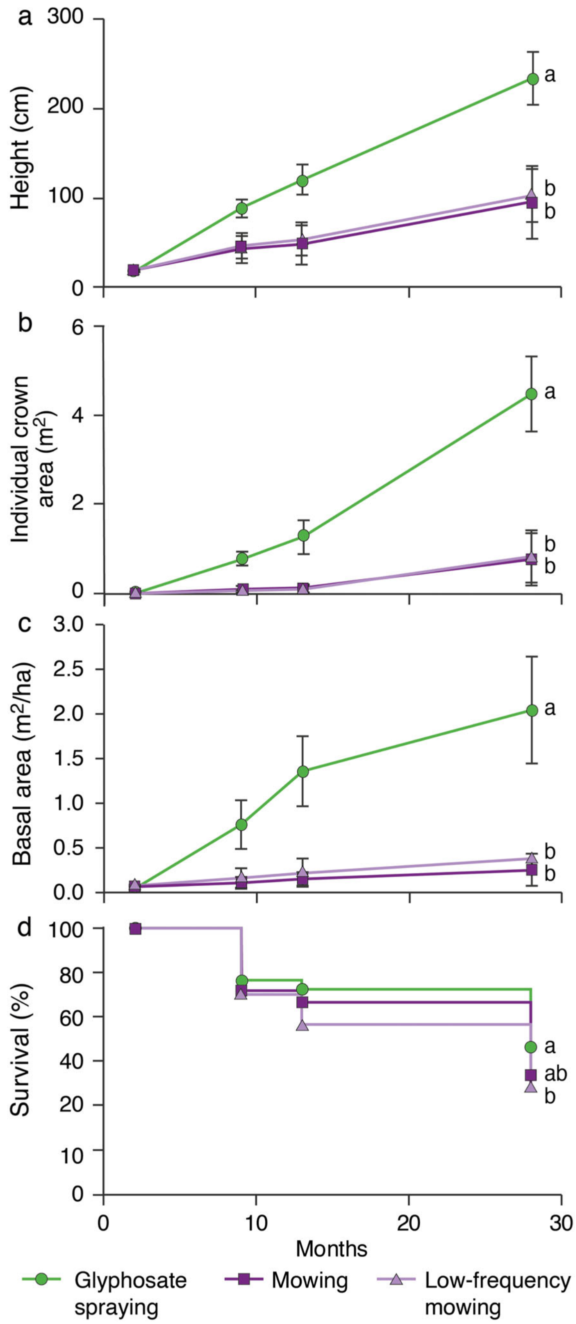

Fig. 4. (a) Seedling height, (b) crown area, (c) basal area, and (d) survival resulted from weeding treatments. Different letters indicate that means were significantly different (Tukey tests, $P < 0.05$). Error bars represent the standard deviation.

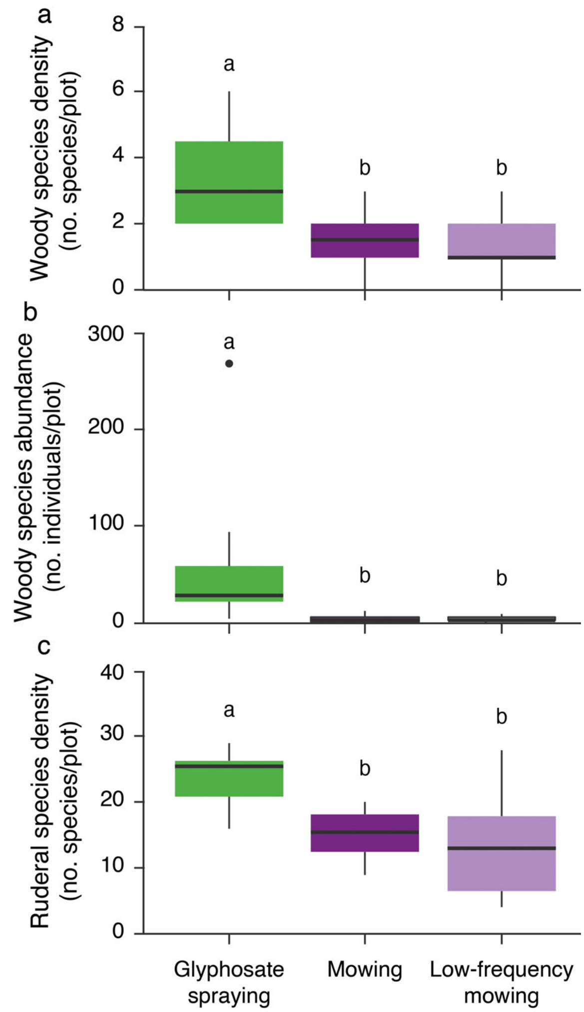

Fig. 5. Woody species (a) density and (b) abundance, and (c) ruderal species density resulting from weeding treatments. Different letters indicate that means were significantly different (species density: Tukey tests, $P < 0.05$; abundance: pairwise Wilcoxon rank-sum test, $P < 0.05$). Heavy lines represent the median, colored boxes represent the interquartile range, and error bars represent the standard deviation.

plants, as well the abundance of woody species, compared to mowing treatments, which did not differ from each other (Fig. 5). A total of 82 herbaceous species were found in the glyphosate treatment plots, while 59 and 66 species were found in the regular and the low-frequency mowing plots, respectively (Appendix S1: Table S4).

We obtained 610,810 high quality sequences from the rRNA 16S gene sequencing, which were rarified to 15,225 samples before assigning the OTU identities. Goods coverage index corresponded to 98% ± 0.03%, suggesting that most assessed bacterial groups were present in the databases. OTUs and Chao1 diversity index did not differ among plots but mowing plots without glyphosate spraying had lower Shannon and Simpson diversity indices regardless of the sampling time (Appendix S1: Table S5). The PCoA supported by Adonis analysis (Appendix S1: Table S6) showed dispersion homogeneity, displaying similar results among Bray Curtis, Jaccard, and weighted and unweighted UniFrac metrics. It indicates that glyphosate plots sampled either before or after spraying were statistically equal but differed from plots without glyphosate spraying (Fig. 6).

Proteobacteria was the most abundant phylum, followed by *Acidobacteria*, *Bacteroidetes*, and *Firmicutes*, but glyphosate spraying significantly affected *Proteobacteria* population ($P < 0.05$; Appendix S1: Table S7). Overall, the relative abundance of the 20 most abundant bacteria families (Appendix S1: Table S7) was higher after glyphosate spraying (Fig. 7a), except for the *Pseudomonadaceae* in the no-glyphosate spraying plots (Fig. 7b). Just 11 out of 88 assessed bacteria families had their abundance reduced in the glyphosate compared to the no-glyphosate plots (Fig. 7b). Seven families (Tsukamurellaceae, Holophagaceae, Syntrophaceae, Opitutaceae, Waddliaceae, Chlorobiaceae, and Haliangeaceae) had their abundance increased whereas only Saprospiaceae had it decreased considering both treatments (Fig. 7a and b). About 690 bacteria genera were found (Appendix S1: Table S8), and 142 presented significant variation between treatments. *Burkholderia*, *Janthinobacterium*, and *Pseudomonas* were the most abundant genera, mainly in the no-glyphosate spraying plots.

Glyphosate and AMPA residues were detected neither in water nor in the soil samples. However, they were found in 10 runoff sediment samples (out of 16 evaluated sampling points), at concentrations varying from 1.32 to 24.75 mg/kg for glyphosate and from 1.75 to 76.13 mg/kg for AMPA (Fig. 8). Unexpectedly, these molecules were also found in the sediments of three mowing plots, indicating that they were superficially transported by runoff throughout the plots.

Discussion

Compared to mowing, glyphosate spraying significantly reduced weed biomass, including for the aggressive pasture grass (*U. decumbens*), and it was also associated with improved performance of planted tree seedlings. Similar effects of glyphosate on grass growth and on planted seedlings were obtained in other experiments (e.g., Griscom et al. 2005, Craven et al. 2009, Brancalion et al. 2019), and reinforce the importance of effective weeding for reforestation success. *Urochloa decumbens* is an African grass grazed by large mammals in its natural habitat and has evolved a high resprouting capacity, which was further improved by breeding programs to use it as cattle fodder in the Neotropics (Williams and Baruch 2000). The ineffective control of *U. decumbens* by mowing demonstrates that more than four interventions are needed, which would inevitably increase maintenance costs and further reduce its cost-effectiveness compared to glyphosate spraying. A similar result was obtained for controlling the invasive grass *Saccharum spontaneum* in a restoration plantation in Panama (Craven et al. 2009), which reinforce the limitations of low-frequency mowing (two to four per year) to control invasive grasses.

Biomass accumulation is often described by a sigmoidal curve having an initial exponential phase followed by a stationary phase. It seems that mowing on higher frequency may have brought grasses back to the initial exponential phase, in which biomass accumulation and competition for resources are at their peaks, whereas

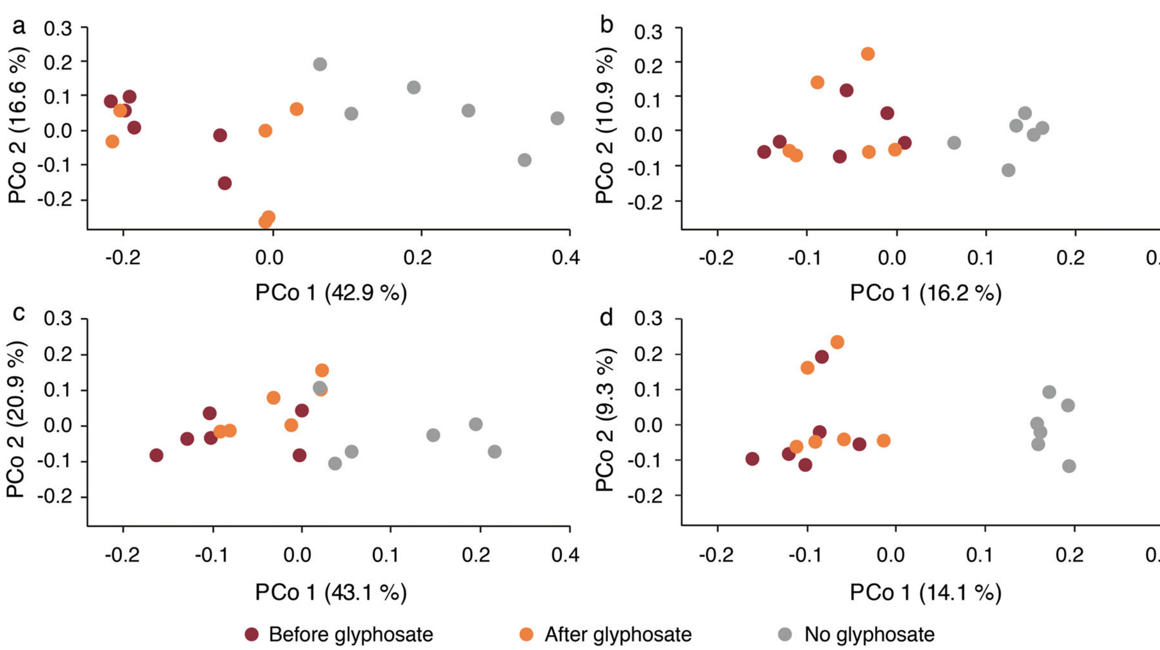

Fig. 6. Beta diversity analysis of soil bacterial communities before and after glyphosate spraying, and with no glyphosate spraying (number of plots per treatment = 6). Principal coordinate analysis (PCoA)-based (a) Bray-Curtis metrics, (b) Jaccard metrics, (c) weighted UniFrac metrics, and (d) unweighted UniFrac metrics.

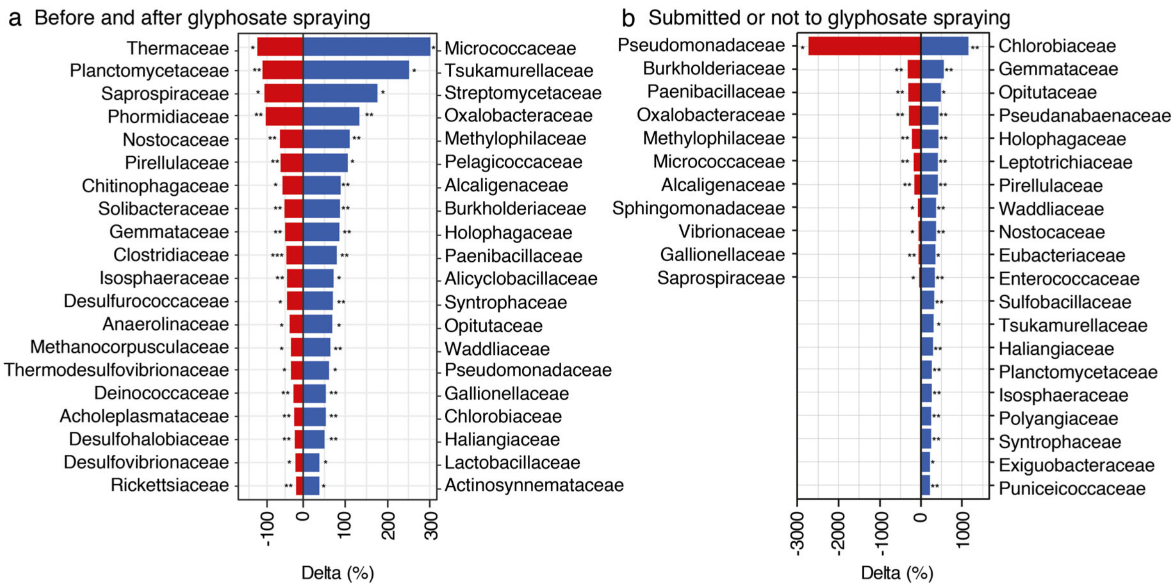

FIG. 7. Relative abundance variation in the 20 most abundant soil bacteria families: (a) history of glyphosate application ([(5mo+24h-gly/5mo-gly −1) 100] for positive (in blue) and −(5mo-gly/5mo+24h-gly −1) 100] for negative growth (in red)), and (b) no herbicide use ([(5mo+24h-gly/no-gly −1) 100] for positive (in blue) and [−(no-gly/5mo+24h-gly −1) 100] for negative growth (in red)). Sample names are explained in *Methods: Impacts on non-targeted organisms: Soil bacteria*. Only families with $P < 0.05$ were plotted and statistically significant differences using Kruskal-Wallis tests are indicated as $^{***}P < 0.001$, $^{**}0.01 < P \geq 0.001$, $^{*}0.05 < P \geq 0.01$. We considered (a) plots with glyphosate history and the same plots 24 h after spraying and (b) plots not submitted to glyphosate spraying with plots with glyphosate history 24 h after spraying.

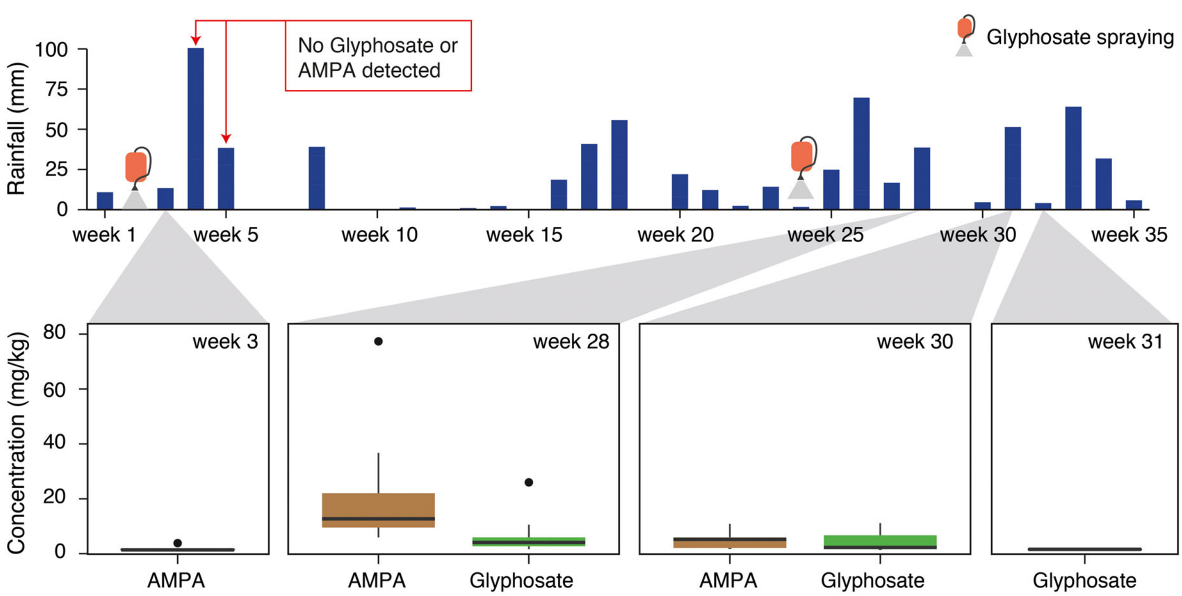

FIG. 8. Quantifiable residues of glyphosate and AMPA in sediments collected from runoff samples throughout time in weeding treatments. Vertical blue bars in the first panel represent the rainfall of each week, and sprayer icons represent the glyphosate application. The box plots show the concentration of these molecules in each runoff sampling day, counted from the planting day forward. Error bars in box plots represent the standard deviation.

mowing twice a year may have kept the invasive grass for a longer period at the stationary phase, thus with lower competition potential. The particular growth behavior of weeds may explain the contradictory result of no effect of mowing frequency on weed production biomass. Conversely, glyphosate is a systemic herbicide that kills invasive grasses and is not absorbed by tree seedling roots (Ratcliff et al. 2006). Therefore, glyphosate spraying is much more effective to reduce the competition between tree seedlings and invasive grasses, at least at regular mowing frequencies.

Weed control with glyphosate was 10–15-fold more cost effective than mowing in promoting seedling growth. Herbicide cost was lower than that of fuel and oil to operate the string trimmer, and herbicide spraying had an even lower labor cost, which may explain its wide use by forestry and large-scale restoration projects (Rolando et al. 2017, Weidlich et al. 2020). Another advantage of glyphosate use may be the reduction of the maintenance period, as the more effective control of weeds favors tree growth and accelerates the competitive exclusion of ruderal plants through shading (Craven et al. 2009, Campoe et al. 2010). For instance, Sweeney et al. (2002) found that restoration plantings maintained with mowing would take 20 yr more to reach to crown cover than those under glyphosate spraying. The financial impacts of glyphosate use in tree planting programs can be even greater if logistical costs are considered. For example, returning to the same site many times during the maintenance period, as needed in the case of mowing, may incur in additional transport, labor, accommodation, and meals costs.

The above results are rarely reported in the literature but are certainly not novel for practitioners accustomed to glyphosate use. However, when used in restoration, glyphosate impacts on non-target organisms and the environment are highly unknown. Glyphosate was efficient in controlling *U. decumbens* and other weeds in the first months after spraying, when all ruderal plants were killed. However, further elimination of *U. decumbens* stumps opened ecological niches for regeneration of other weeds and woody native plants. In fact, they were observed in higher biomasses, abundances, and richness in the glyphosate treated plots. Invasive grasses usually form a thick vegetation layer covering the ground, which can compromise seed bank expression (Zimmerman et al. 2000) and further arrest succession (Wheeler et al. 2016). The time interval between the two applications of glyphosate per year was enough to allow the effective regeneration of ruderal plants, which may have a lower competition potential than *U. decumbens*, a higher contribution to mutualistic interactions with pollinators and seed dispersers, and still protect the soil against erosion. Glyphosate spraying should then be focused on more aggressive weeds as *U. decumbens* and avoid affecting ruderal species of interest and regenerating native woody species. In addition to these more immediate benefits, *U. decumbens* control may favor restoration processes in the longer term. Another restoration experiment in the Atlantic Forest showed that the effective control of this weed by glyphosate enhanced biomass accumulation of planted seedlings, which was associated with an exponential increase in abundance and diversity of colonizing native tree species in the plantation understory (Brancalion et al. 2019).

However, glyphosate may negatively affect more sensitive organisms, such as soil bacteria due to the presence of the shikimate pathway in their metabolism for certain groups, which is the target metabolic route of glyphosate in plants (Yi et al. 2015). In vitro tests support this concern (Bonnet et al. 2007), but field tests are less conclusive since glyphosate molecules are strongly bound to soil colloids (Busse et al. 2001). In this study, 5mo-gly and 5mo+24h-gly bacteria community differed from no-gly but its total diversity was not significantly affected, as also observed by other authors (Schlatter et al. 2017, Bottrill et al. 2020, Kepler et al. 2020). The control of *U. decumbens* and its litter (Schlatter et al. 2017) and the regeneration of other ruderal species may have favored bacterial niche diversification (Kepler et al. 2020), but this effect was offset since the species density of ruderal plants only slightly changed before and after glyphosate application and sensitive species were already eliminated from plots having historical glyphosate application. *Pseudomonadaceae* family abundance decreased with glyphosate spraying, but the *Pseudomonas* genus is well known for having species capable of degrading glyphosate and other species sensitive to it (Sviridov et al. 2015), whereas *Saprospiaceae* are known to increase following glyphosate spraying (Guijarro et al. 2018). However, overall changes in the soil microbial communities caused by glyphosate tend to be transient and not last for more than a few days (Ratcliff et al. 2006). Still, microbiological studies of glyphosate impact in soil bacteria are often contradictory (Liu et al. 2018, Padilla and Selim 2020). We acknowledge that these impacts could have been magnified if we had used soil samples obtained more superficially, where glyphosate activity should be higher due to its lower mobility in the soil profile, instead of using a homogenized, 0–10 cm depth soil sample. But since we focused our study on ecosystem recovery processes and most of soil nutrients, organic matter, bacterial biomass, and fine roots are concentrated at 0–10 cm depth, we consider that the lack of evaluation of more shallow soil layers was not a critical methodological limitation of our work.

Glyphosate and AMPA residues were not detected in the water and soil samples. Other studies have found glyphosate residues in soils (Battaglin et al. 2014, Bento et al. 2019), but most of them have been performed in temperate ecosystems, characterized by long, biologically inactive winters and short growing seasons (Helander et al. 2012). In highly weathered tropical soils, the phosphorous portion of the glyphosate molecule is used as a source of nutrients by soil microbes or is strongly adsorbed by iron and aluminum oxides present in the clay fraction of the soils (Vereecken 2005), forming non-extractable residues (Gros et al. 2020). This bond chemically inactivates the herbicide, lowering its potential toxicity. Therefore, glyphosate is either immobilized or rapidly degraded from the soil solution in tropical soils (Maqueda et al. 2017). As a matter of comparison, glyphosate and AMPA half-lives correspond to 1.5 and 26 d under warm and humid weather, respectively (Bento et al. 2016) but increase to 197 and 240 d, respectively, under less favorable conditions like those found in temperate regions (Giesy et al. 2000). We acknowledge, however, that collecting soil samples from 0 to 10 cm depth may have diluted glyphosate and AMPA residues in the entire sample and pushed their concentration below detection levels, as such residues may be concentrated close to the soil's surface.

Nevertheless, 24.8 mg/kg of glyphosate and 76.1 mg/kg of AMPA were detected in the runoff sediments, higher compared to 10.9 mg/kg of glyphosate and 8.3 mg/kg of AMPA found by Bento et al. (2019) on eroded particles, and 0.58 and 0.48 mg/kg by Primost et al. (2017). Globally, there are no thresholds on soil for those molecules (Silva et al. 2019), but only predicted environmental concentrations, with values ranging between 6.62 mg/kg for glyphosate and 6.18 mg/kg for AMPA (EFSA 2015). Although with higher values, the concentrations found on our study must be associated with the total amount of sediments eroded in the catchment to infer potential impacts in water resources. High concentrations were found in two mowing plots, in which glyphosate was not sprayed, highlighting that glyphosate can be superficially transported by runoff or even by wind, but only attached to the sediments, thus reaching water bodies (Coupe et al. 2012). Glyphosate and AMPA are considered to have no acute toxicity and limited carcinogenicity to vertebrates due to the absence of the shikimate pathway in animals (WHO 2005, EFSA 2017, EPA 2020). However, glyphosate was classified as "probably carcinogenic to humans" (i.e., Group 2A), mainly because of genotoxicity and oxidative stress risks and potential long-term chronic effects like in non-Hodgkin lymphoma (Guyton et al. 2015). For instance, surfactants (i.e., compounds that lower water surface tension to enhance leaf absorption of herbicide droplets) are often more toxic than the herbicide itself (Perkins et al. 2000). Polyoxyethyleneamine (POEA), in particular, a major surfactant used in glyphosate formulations, is considered 10,000 times more toxic than glyphosate per se (Mesnage et al. 2013). Therefore, caution is needed when using this herbicide, especially in riparian buffers and temperate ecosystems.

Reducing glyphosate use and minimizing its risks in tree planting initiatives

Invasive grasses have a marked negative impact on tree planting performance and need effective control for increasing chances of successful reforestation projects. Glyphosate transport to water bodies through sediment runoff, potential negative impacts on soil bacteria, and health risks associated with its use may justify the preference for non-chemical control methods in certain cases (Weidlich et al. 2020). We highlight here two potential practical alternatives. The first one is intercropping green manure species with native tree seedlings, as green manure plants may occupy the ecological niche of invasive grasses and outcompete them (Reis et al. 2019). However, an initial control of invasive grasses with herbicides may be necessary to allow the effective establishment of green manure species (Cesar et al. 2013, de Souza et al. 2020), which could result in a marked reduction in glyphosate use, but not its elimination. The other alternative involves planting higher density of seedlings, which may speed up canopy cover and the exclusion of invasive grasses by shading. This strategy will considerably increase restoration costs, thus impeding its adoption in large scales. Direct seeding can be used as an alternative to enhance fast-growing native tree density at lower costs (Meli et al. 2018). Both approaches can be combined as already done in a large-scale restoration program in Brazil (Durigan et al. 2013).

Adoption of soil conservation practices and vegetative buffer strips can reduce glyphosate contamination of water courses (Reichenberger et al. 2007). For instance, glyphosate loads can be reduced by 39–78% just by leaving a 4–5 m strip of grasses at water course borders (Reichenberger et al. 2007, Lin et al. 2011, Lerch et al. 2017). In addition, avoiding spraying in windy and rainy days may also considerably reduce glyphosate runoff (Solomon and Thompson 2003). Practitioners can also choose more environmentally friendly glyphosate formulations, that vary according to their adjuvant composition and brands (Mesnage et al. 2015). For instance, surfactants (i.e., compounds that lower water surface tension to enhance leaf absorption of herbicide droplets) are often more toxic than the herbicide itself (Perkins et al. 2000). Polyoxyethyleneamine (POEA), in particular, a major surfactant used in glyphosate formulations, is considered 10,000 times more toxic than glyphosate per se (Mesnage et al. 2013). Therefore, there are potential alternatives to reduce or even avoid glyphosate use in tree planting, minimizing its environmental impacts. They should all be critically considered to reduce project failures while also preventing avoidable health and environmental risks.

Acknowledgments

We are indebted to J. Leighton Reid and Pedro Meirelles for their review of an early version of this manuscript. We thank José Zacarias, Joaquim Prates, Ebrahim X. de Souza, s Augusto, and Monique Alves for field work support, Italo Cegatta for statistical support, and Vanessa Sontag for improving the design of the figures. We are also thankful to SOS Mata Atlântica for providing the research site and financing plantation establishment and maintenance, and Agrosafety Monitoramento Agrícola for glyphosate and AMPA laboratory analyses. This research was funded by the São Paulo Research Foundation (FAPESP; grants #2012/11256-3 and #2012/19771-4). Author contributions: P. H. S. Brancalion led the project; F. G. Florido conducted the field experiment; P. A. M. Andrade and F. D. Andreote assisted the bacteria analysis and J. B. Regitano the environmental contamination evaluation; P. H. S. Brancalion and F. G. Florido led the writing and all authors contributed to revisions.

Literature Cited

Battaglin, W. A., M. T. Meyer, K. M. Kuivila, and J. E. Dietze. 2014. Glyphosate and its degradation product AMPA occur frequently and widely in U.S. soils, surface water, groundwater, and precipitation. JAWRA Journal of the American Water Resources Association 50:275–290.

Bento, C. P. M., S. van der Hoeven, X. Yang, M. M. J. P. M. Riksen, H. G. J. Mol, C. J. Ritsema, and V. Geissen. 2019. Dynamics of glyphosate and AMPA in the soil surface layer of glyphosate-resistant crop cultivations in the loess Pampas of Argentina. Environmental Pollution 244:323–331.

Bento, C. P. M., X. Yang, G. Gort, S. Xue, R. van Dam, P. Zomer, H. G. J. Mol, C. J. Ritsema, and V. Geissen. 2016. Persistence of glyphosate and aminomethylphosphonic acid in loess soil under different combinations of temperature, soil moisture and light/darkness. Science of the Total Environment 572:301–311.

Bolyen, E., et al. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nature Biotechnology 37:852–857.

Bonnet, J.-L., F. Bonnemoy, M. Dusser, and J. Bohatier. 2007. Assessment of the potential toxicity of herbicides and their degradation products to nontarget cells using two microorganisms, the bacteria Vibrio fischeri and the ciliate *Tetrahymena pyriformis*. Environmental Toxicology 22:78–91.

Bottrill, D., et al. 2020. Short-term application of mulch, roundup and organic herbicides did not affect soil microbial biomass or bacterial and fungal diversity. Chemosphere 244:125436.

Brancalion, P. H. S., O. Campoe, J. C. T. Mendes, C. Noel, G. G. Moreira, J. Melis, J. L. Stape, and J. Guillemot. 2019. Intensive silviculture enhances biomass accumulation and tree diversity recovery in tropical forest restoration. Ecological Applications 29:e01847.

Brancalion, P. H. S., and K. D. Holl. 2020. Guidance for successful tree planting initiatives. Journal of Applied Ecology 57:2349–2361.

Brasil. 2017. Instrução Normativa da Receita Federal do Brasil N° 1700, March 14th, 2017, Brasília, Brazil.

Busse, M. D., A. W. Ratcliff, C. J. Shestak, and R. F. Powers. 2001. Glyphosate toxicity and the effects of long-term vegetation control on soil microbial communities. Soil Biology and Biochemistry 33:1777–1789.

Campoe, O. C., J. L. Stape, and J. C. T. Mendes. 2010. Can intensive management accelerate the restoration of Brazil's Atlantic forests? Forest Ecology and Management 259:1808–1814.

Cesar, R. G., P. H. S. Brancalion, R. R. Rodrigues, A. M. S. Oliveira, and M. C. Alves. 2013. Does crotalaria (*Crotalaria breviflora*) or pumpkin (*Cucurbita moschata*) inter-row cultivation in restoration plantings control invasive grasses? Scientia Agricola 70:268–273.

Chazdon, R., and P. Brancalion. 2019. Restoring forests as a means to many ends. Science 365:24–25.

Coupe, R. H., S. J. Kalkhoff, P. D. Capel, and C. Gregoire. 2012. Fate and transport of glyphosate and aminomethylphosphonic acid in surface waters of agricultural basins. Pest Management Science 68:16–30.

Craven, D., J. Hall, and J.-M. Verjans. 2009. Impacts of Herbicide Application and mechanical cleanings on growth and mortality of two timber species in *Saccharum spontaneum* grasslands of the Panama Canal Watershed. Restoration Ecology 17:751–761.

de Souza, D. C., V. L. Engel, and E. C. de Mattos. 2020. Direct seeding to restore tropical seasonal forests: effects of green manure and hydrogel amendment on tree species performances and weed infestation. Restoration Ecology 29:e13277.

DeSantis, T. Z., P. Hugenholtz, N. Larsen, M. Rojas, E. L. Brodie, K. Keller, T. Huber, D. Dalevi, P. Hu, and G. L. Andersen. 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Applied and Environmental Microbiology 72:5069–5072.

Durigan, G., N. Guerin, and J. N. M. N. D. Costa. 2013. Ecological restoration of Xingu Basin headwaters: motivations, engagement, challenges and perspectives. Philosophical Transactions of the Royal Society B 368:20120165.

EFSA. 2015. Conclusion on the peer review of the pesticide risk assessment of the active substance glyphosate. EFSA Journal 13:107.

EFSA. 2017. Peer review of the pesticide risk assessment of the potential endocrine disrupting properties of glyphosate. EFSA Journal 15:e04979.

EPA. 2020. Glyphosate, Interim registration review decision case number 0178. Environmental Protection Agency, Washington, D.C., USA.

Giesy, J. P., S. Dobson, and K. R. Solomon. 2000. Ecotoxicological risk assessment for Roundup® herbicide. Pages 35–120 *in* G. W. Ware, editor. Reviews of environmental contamination and toxicology: continuation of residue reviews. Springer, New York, New York, USA.

Gregoire, C., S. Payraudeau, and N. Domange. 2010. Use and fate of 17 pesticides applied on a vineyard catchment. International Journal of Environmental Analytical Chemistry 90:406–420.

Griscom, H. P., P. M. S. Ashton, and G. P. Berlyn. 2005. Seedling survival and growth of native tree species in pastures: Implications for dry tropical forest rehabilitation in central Panama. Forest Ecology and Management 218:306–318.

Gros, P., R. Meissner, M. A. Wirth, M. Kanwischer, H. Rupp, D. E. Schulz-Bull, and P. Leinweber. 2020. Leaching and degradation of 13C2-15N-glyphosate in field lysimeters. Environmental Monitoring and Assessment 192:127.

Guijarro, K. H., V. Aparicio, E. De Gerónimo, M. Castellote, E. L. Figuerola, J. L. Costa, and L. Erijman. 2018. Soil microbial communities and glyphosate decay in soils with different herbicide application history. Science of the Total Environment 634:974–982.

Guyton, K. Z., D. Loomis, Y. Grosse, F. El Ghissassi, L. Benbrahim-Tallaa, N. Guha, C. Scoccianti, H. Mattock, and K. Straif. 2015. Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate. Lancet Oncology 16:490–491.

Helander, M., I. Saloniemi, and K. Saikkonen. 2012. Glyphosate in northern ecosystems. Trends in Plant Science 17:569–574.

Holl, K. D., and P. H. S. Brancalion. 2020. Tree planting is not a simple solution. Science 368:580–581.

IEA. 2019. Salários rurais. IEA, Paris, France.

Illumina. 2019. 16S metagenomic sequencing library preparation. Illumina, San Diego, California, USA.

Kepler, R. M., D. J. Epp Schmidt, S. A. Yarwood, M. A. Cavigelli, K. N. Reddy, S. O. Duke, C. A. Bradley, M. M. Williams, J. S. Buyer, and J. E. Maul. 2020. Soil microbial communities in diverse agroecosystems exposed to the herbicide glyphosate. Applied and Environmental Microbiology 86:e01744-19.

Kjær, J., V. Ernstsen, O. H. Jacobsen, N. Hansen, L. W. de Jonge, and P. Olsen. 2011. Transport modes and pathways of the strongly sorbing pesticides glyphosate and pendimethalin through structured drained soils. Chemosphere 84:471–479.

Kopylova, E., L. Noé, and H. Touzet. 2012. SortMeRNA: fast and accurate filtering of ribosomal RNAs in metatranscriptomic data. Bioinformatics 28:3211–3217.

Lerch, R. N., C. H. Lin, K. W. Goyne, R. J. Kremer, and S. H. Anderson. 2017. Vegetative buffer strips for reducing herbicide transport in runoff: effects of buffer width, vegetation, and season. JAWRA Journal of the American Water Resources Association 53:667–683.

Lin, C.-H., R. N. Lerch, K. W. Goyne, and H. E. Garrett. 2011. Reducing herbicides and veterinary antibiotics losses from agroecosystems using vegetative buffers. Journal of Environmental Quality 40:791–799.

Little, K. M., et al. 2006. Towards reduced herbicide use in forest vegetation management. Southern African Forestry Journal 207:63–79.

Liu, Y., et al. 2018. Glyphosate application increased catabolic activity of gram-negative bacteria but impaired soil fungal community. Environmental Science and Pollution Research 25:14762–14772.

Maggi, F., D. la Cecilia, F. H. M. Tang, and A. McBratney. 2020. The global environmental hazard of glyphosate use. Science of the Total Environment 717:137167.

Maqueda, C., T. Undabeytia, J. Villaverde, and E. Morillo. 2017. Behaviour of glyphosate in a reservoir and the surrounding agricultural soils. Science of the Total Environment 593–594:787–795.

Meli, P., I. Isernhagen, P. H. S. Brancalion, E. C. C. Isernhagen, M. Behling, and R. R. Rodrigues. 2018. Optimizing seeding density of fast-growing native trees for restoring the Brazilian Atlantic Forest. Restoration Ecology 26:212–219.

Mesnage, R., B. Bernay, and G. E. Séralini. 2013. Ethoxylated adjuvants of glyphosate-based herbicides are active principles of human cell toxicity. Toxicology 313:122–128.

Mesnage, R., N. Defarge, J. Spiroux de Vendômois, and G. E. Séralini. 2015. Potential toxic effects of glyphosate and its commercial formulations below regulatory limits. Food and Chemical Toxicology 84:133–153.

Padilla, J. T., and H. M. Selim. 2020. Environmental behavior of glyphosate in soils. Advances in Agronomy 159:1–34.

Perkins, P. J., H. J. Boermans, and G. R. Stephenson. 2000. Toxicity of glyphosate and triclopyr using the frog embryo teratogenesis assay—Xenopus. Environmental Toxicology and Chemistry 19:940–945.

Primost, J. E., D. J. G. Marino, V. C. Aparicio, J. L. Costa, and P. Carriquiriborde. 2017. Glyphosate and AMPA, “pseudo-persistent” pollutants under real-world agricultural management practices in the Mesopotamic Pampas agroecosystem, Argentina. Environmental Pollution 229:771–779.

R Development Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Ratcliff, A. W., M. D. Busse, and C. J. Shestak. 2006. Changes in microbial community structure following herbicide (glyphosate) additions to forest soils. Applied Soil Ecology 34:114–124.

Reichenberger, S., M. Bach, A. Skitschak, and H.-G. Frede. 2007. Mitigation strategies to reduce pesticide inputs into ground- and surface water and their effectiveness; A review. Science of the Total Environment 384:1–35.

Reis, L. K., A. Guerra, M. L. Z. Colado, F. L. G. Borges, M. D. R. Oliveira, E. X. Gondim, T. R. F. Sinani, N. Guerin, and L. C. Garcia. 2019. Which spatial arrangement of green manure is able to reduce herbivory and invasion of exotic grasses in native species? Ecological Applications 29:e02000.

Rodrigues, R. R., S. Gandolfi, A. G. Nave, J. Aronson, T. E. Barreto, C. Y. Vidal, and P. H. S. Brancalion. 2011. Large-scale ecological restoration of high-diversity tropical forests in SE Brazil. Forest Ecology and Management 261:1605–1613.

Rolando, C. A., B. R. Baillie, D. G. Thompson, and K. M. Little. 2017. The risks associated with glyphosate-based herbicide use in planted forests. Forests 8:208.

Schlatter, D. C., C. Yin, S. Hulbert, I. Burke, and T. Paulitz. 2017. Impacts of repeated glyphosate use on wheat-associated bacteria are small and depend on glyphosate use history. Applied and Environmental Microbiology 83:e01354-17.

Silva, V., H. G. J. Mol, P. Zomer, M. Tienstra, C. J. Ritsema, and V. Geissen. 2019. Pesticide residues in European agricultural soils—A hidden reality unfolded. Science of the Total Environment 653:1532–1545.

Solomon, K., and D. Thompson. 2003. Ecological risk assessment for aquatic organisms from over-water uses of glyphosate. Journal of Toxicology and Environmental Health, Part B 6:289–324.

Sviridov, A. V., T. V. Shushkova, I. T. Ermakova, E. V. Ivanova, D. O. Epiktetov, and A. A. Leontievsky. 2015. Microbial degradation of glyphosate herbicides (Review). Applied Biochemistry and Microbiology 51:188–195.

Sweeney, B. W., S. J. Czapka, and T. Yerkes. 2002. Riparian forest restoration: increasing success by reducing plant competition and herbivory. Restoration Ecology 10:392–400.

Vereecken, H. 2005. Mobility and leaching of glyphosate: a review. Pest Management Science 61:1139–1151.

Wagner, V., P. M. Antunes, M. Irvine, and C. R. Nelson. 2017. Herbicide usage for invasive non-native plant management in wildland areas of North America. Journal of Applied Ecology 54:198–204.

Weidlich, E. W. A., F. G. Flórido, T. B. Sorrini, and P. H. S. Brancalion. 2020. Controlling invasive plant species in ecological restoration: A global review. Journal of Applied Ecology 57:1806–1817.

Wheeler, C. E., P. A. Omeja, C. A. Chapman, M. Glipin, C. Tumwesigye, and S. L. Lewis. 2016. Carbon sequestration and biodiversity following 18years of active tropical forest restoration. Forest Ecology and Management 373:44–55.

WHO. 2005. Glyphosate and AMPA in drinking-water. Background document for preparation of WHO guidelines for drinking-water quality. World Health Organization, Geneva, Switzerland.

Williams, D. G., and Z. Baruch. 2000. African grass invasion in the Americas: ecosystem consequences and the role of ecophysiology. Biological Invasions 2:123–140.

Yi, S.-Y., G.-B. Wu, Y.-J. Lin, N. Hu, and Z.-D. Liu. 2015. Characterization of a new type of glyphosate-tolerant 5-enolpyruvyl shikimate-3-phosphate synthase from Isoptericola variabilis. Journal of Molecular Catalysis B: Enzymatic 111:1–8.

Zimmerman, J. K., J. B. Pascarella, and T. M. Aide. 2000. Barriers to forest regeneration in an abandoned pasture in Puerto Rico. Restoration Ecology 8:350–360.

Supporting Information

Additional supporting information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/eap.2472/full

Open Research

All OTU sequences are available at MG-RAST (https://www.mg-rast.org/), for download under job ID “MGP90305” for the 16Sr RNA gene sequences. Cost, regeneration, and plantation performance data are supplied at https://figshare.com/articles/dataset/A_comprehensive_experimental_assessment_of_glyphosate_ecological_impacts_in_riparian_forest_restoration/17029727/1.