

## Effects of temporary agroforestry systems and weeding techniques on the control of invasive species in Atlantic Forest restoration

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### Abstract

The restoration of tropical forests is greatly in demand but is limited by the lack of feasible technologies. Forest restoration can be associated with a temporary, productive herbaceous layer. Here, we compared the use of temporary agroforestry systems (tAFS) with conventional forest restoration through seedling planting. We quantified the growth estimators of native forest tree species and the effectiveness of the two methods for controlling invasive grasses. Seedlings of 20 native tree species were planted in 72 rows of 60 m each, totaling 1148 individuals. The restoration methods of planting tree seedlings only or trees coupled with herbaceous species (annual crops), along with weed control methods (mechanical and chemical), were systematically established in 12 plots (7920 m<sup>2</sup> for conventional techniques and 7920 m<sup>2</sup> for tAFS) per treatment (3960 m<sup>2</sup> each) and analyzed in a nested design using generalized mixed models. The chemical control of weeds was conducted using herbicides, and the mechanical control consisted of semimechanized mowing. The tAFS showed greater relative growth in both the height and diameter of native trees in comparison with the conventional method. Within the conventional restoration technique, chemical management led to an increased height of native trees and a decreased invasive biomass, but tAFS showed no differences between the invasive control methods. tAFS was efficient in ensuring the success of the tree seedlings and diminishing the invasive biomass, concomitant with the production of annual crops in tropical forest restoration.

**Keywords:** Ecological restoration; Herbaceous; Management; Native trees; Plantation.

**Abbreviations:** tAFS\_ temporary agroforestry systems.

### Introduction

The current loss of tropical forest areas and the ongoing reduction are serious global problems (Curtis et al., 2018). In Brazil, forests and associated phyto-physiognomies have been suppressed at an accelerated rate (Souza et al., 2020), and many of the remaining areas are located on private properties. In Brazil, the requirement for tropical forest restoration usually relies on land owners, who highlight the costs of the project itself, as well as the implementation and maintenance of ecological restoration actions as their main limitations. Therefore, economically viable alternatives must be forthcoming in the short term (Oliveira et al., 2021).

The most widely used restoration technique in Brazil is that of native tree seedling planting, focusing on fast-growing species, to create a structural canopy for the prevention of shade-intolerant invasive grasses at the initial stages (Viani et al., 2018). This method, referred to as conventional tree planting, is not easily applied to large scales, particularly because of the high number of invasive species that require invasive control methods by management, which results in increased costs (Oliveira et al., 2021). Ideally, alternatives for

tropical forest restoration should have reduced costs of implementation and maintenance, enabling economic returns (Schaeffer 2013; Tremblay et al., 2014; IPBES, 2019) or a certain level of food security (Vieira et al., 2009).

Agroforestry systems (AFS) are productive systems that combine woody and herbaceous species, covering a wide range of possible multistratified designs, with different diversities and successional groups. AFSs are commonly implemented by the addition of trees to land already being used for pasture or for growing annual crops (Elevitch et al., 2018). Restoration through AFS has economic, ecological, and social advantages in degraded areas, riparian forests, ecological corridors, and fragment edges (Boreux et al., 2016; Elevitch et al., 2018). Agroforestry provides cost-effective alternatives that can increase profits and meet environmental goals (Nair and Garrit 2012; Souza et al., 2016; Badari et al., 2020). In Brazil, environmental legislation obligates land owners to preserve a proportion of their land for the conservation of native ecosystems (the "Legal Reserve"). These areas represent nearly one-third of the

country's current native vegetation and are recognized for their role in protecting biodiversity and providing a wide range of ecosystem services (Metzger et al., 2019). In these areas, forest management, including the plantation of exotic species, has been allowed since the enactment of the Environmental Law of 2012 (Brasil, 2012) to encourage the conservation of these regions by reverting them to forests (Latawiec, 2015). Therefore, the intercropping of forest species and agricultural crops in the herbaceous layer, if only temporary, could be an attractive alternative for tropical forest restoration in its initial stages, especially for small and medium farms, where there is resistance to applying ecological restoration actions due to their high costs (Ehiagbonare, 2006; Bhagwat et al., 2008; Badari et al., 2020).

Invasion by weeds is considered one of the main obstacles to the success of restoration projects in tropical degraded areas (Brancalion et al., 2019). These invasive species reduce the growth of native tree seedlings and cause ecological imbalance by colonizing the remaining areas of native vegetation, which hinders or stagnates the regeneration processes in native ecosystems, leading to the extinction of endemic species (Vilá et al., 2011). Invasive grass control is one of the main challenges for restoration success, and its management represents a large share of the costs in the initial stage of restoration (Assis et al., 2021).

Mechanical and chemical methods are among the most commonly used to control invasive species. Therefore, temporary AFS emerges as an option for weed control through the cultivation of crops in the interrow space at the beginning of seedling development, thereby enabling native seedling survival while reducing restoration costs (Ikeda and Inoue, 2015; Singh et al., 2015).

Our objectives were as follows: (1) to test whether the restoration of tropical forests mixed with annual crops was more effective than the conventional method of planting seedling tree species and (2) to verify the responses of different weed control methods (mechanical vs. chemical) affecting both the growth of native tree species and the biomass of invasive grasses.

## Results

### **Weed management and tree species growth**

The analysis of traits (Table 1) showed that native tree species had higher relative growth both in height and diameter in the tAFS than in the conventional seedling plantation (Figure 1) in both the mechanized and chemical controls ( $p < 0.05$ ). The conventional seedling plantation showed less height than the tAFS, revealing further differences relative to the weed control method used. Chemical management led to a greater height of native trees compared to mechanized management. The diameter of native trees in the tAFS was also larger than that of conventional restoration, regardless of the invasive species control method (Figure 1).

Within tAFS, the chemical control showed a greater diameter (5.33 cm) than the mechanized control. The conventional seedling plantation displayed the least diameter growth, and the chemical control presented greater values compared to the mechanized (average of 3.7 vs. 2.81 cm). Summarizing traits with the PCA comprised most of the variation in the plots (~ 99%, Figure 2), and therefore, the use of the first axis of the PCA was a good proxy for the relative growth of native trees. Multivariate

analyses showed that the restoration strategy outweighed the differences found in the weed control methods, whereby tAFS led to greater growth than conventional seedling plantation (Figure 2).

### **Efficiency of different weed management methods**

Altogether, 19 weed species were observed in the experimental area over 18 months of monitoring. The weed species with the highest percentage of importance value (IVI) was *Eleusine indica* (L.) Gaertn. ("indian goosegrass"), 38.37%; *Eragrostis pilosa* (L.) P. Beauv. ("indian lovegrass"), 27.23%; *Ipomoea* sp. ("morning-glory"), 24.75%; *Bidens pilosa* L. ("hairy beggarticks"), 24.14%; and *Digitaria horizontalis* Willd. ("jamaican crabgrass"), 22.49%. Invasive species control (i.e., decrease in biomass) was 50% higher with chemical management than with mechanical control after 15 days ( $p < 0.05$ , Figure 2). The difference in invasive biomass between treatments exceeded 100% after 45 days. Invasive species biomass was related to the two factors and their interaction: the type of restoration (tAFS vs. conventional seedling plantation) and the type of weed management (mechanical or chemical).

## Discussion

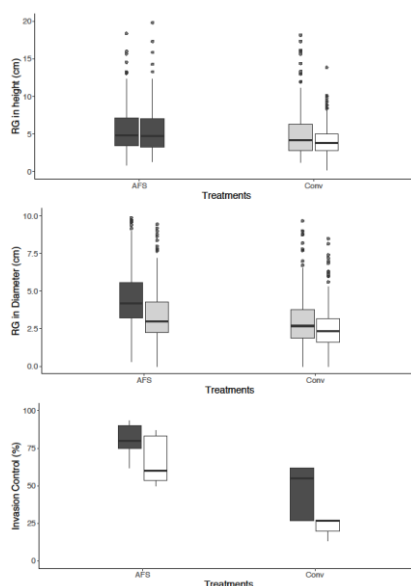
The combinations of herbaceous and tree species, intercropping forest, and agricultural components in rows and interrows showed no negative effects on tropical forest restoration success. In contrast, tAFS caused a greater relative growth of native trees and a reduced biomass of invasive species when compared to the conventional seedling plantation method, which has also been found in other circumstances for agroforestry systems with different arrangements and structures (Van der Werf et al., 2007; Elevitch et al., 2018; Souza and Piña-Rodrigues, 2013; Resende and Leles, 2017). Therefore, the deployment of different layers for one year, along with forest implementation, enhanced and accelerated tree seedling growth, likely because of the increased soil coverage and/or nutritional availability in the system.

Potential trade-offs between productivity and ecological benefits can occur when comparing agroforestry systems, high diversity plantations, and old-reference forests, as opposed to less rich or simplistic forest restoration (Guerin et al., 2020). Analysis of tree richness, abundance, regeneration, and biomass showed that the greater diversity of regenerating saplings in agroforests improved the success of the restoration, with higher canopy cover and richness of zoocoric species than restoration plantations (Badari et al., 2020). Therefore, the diversity promoted by tAFS should induce a greater variety of functional strategies from the complementary use of resources (Cardinale et al., 2006; Carmona et al., 2020) compared to silviculture forests.

The implementation of tree seedlings concomitantly with agricultural crops not only increased the restoration success but also led to earlier canopy formation, thereby decreasing the costs and management requirements. The use of tAFS as an ecological restoration option allows producers to generate financial income from intercrops, which can be used for restoration costs (Ferez et al., 2015). Moreover, tAFS design has simplified management because of the larger interrow spacing that allows for tractor operations. The implementation of restored tropical forests through tAFS is a feasible technique that could make the restoration alternative financially attractive for small and medium farms (Ehiagbonare, 2006).

**Table 1.** Results of the best generalized linear mixed models (GLMM; Bates et al. 2015), selected by ANOVAs. Both factors were determinants of our independent variables in all cases. Post hoc pairwise comparisons and significant differences between treatments are marked with an \* ( $p < 0.05$ )

Compared treatments	RG Height (cm)	RG Diameter (cm)	Inv. Control (%)
	<i>Treat*Control</i>	<i>Treat+Control</i>	<i>Treat*Control</i>
ConvMech - AFSMech	< 0.0001*	< 0.0001*	< 0.0001*
ConvMech - ConvChem	0.06	0.01*	0.002*
ConvMech - AFSCchem	0.05	< 0.0001*	0.001*
AFSMech – ConvChem	0.99	0.77	0.17
AFSMech - AFSCchem	0.99	0.002*	0.03*
ConvChem - AFSCchem	< 0.0001*	< 0.0001*	< 0.0001*

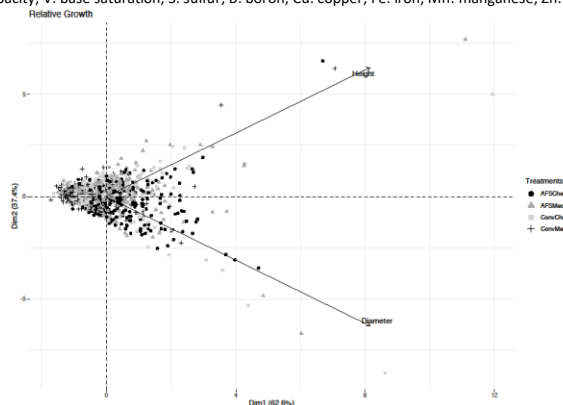


**Figure 1.** Relative growth in height (a; cm/year) and diameter (b; cm/year) of planted native species and the percentage of weed control (c; average of the percentage of invasive species biomass control, assessed at 15, 30, and 45 days), as a function of treatments (types of restoration tAFS – temporary agroforestry system and Conv – conventional system), divided into two types of weed management (mechanical and chemical control). The differences between treatments are represented by different letters, whereas differences in the control methods are shown by different colors (i.e., black and white;  $p > 0.05$ ).

**Table 2.** Chemical analysis of three soil samples - classified as NVdf (Yoshida and Stolf, 2016) - that make up the experimental area, collected according to the slope of the area.

SAMPLE	P mg/dm <sup>3</sup>	O.M g/dm <sup>3</sup>	pH CaCl <sub>2</sub>	K mmol/dm <sup>3</sup>	Ca mmol/dm <sup>3</sup>	Mg mmol/dm <sup>3</sup>	H+Al	Al	SB	CEC	V %	S mg/dm <sup>3</sup>	B	Cu	Fe	Mn	Zn
Sample 1	20	16	6.5	1.7	27	16	25	0.6	44.7	69.7	64	6	0.48	1.9	22	24.3	1.2
Sample 2	20	16	6.6	1.7	26	16	25	0.5	43.7	68.7	64	6	0.51	1.9	21	24.1	1.0
Sample 3	20.3	16	6.4	1.7	28	15	24	0.7	44.7	68.7	65	6	0.50	1.9	22	24.5	1.0

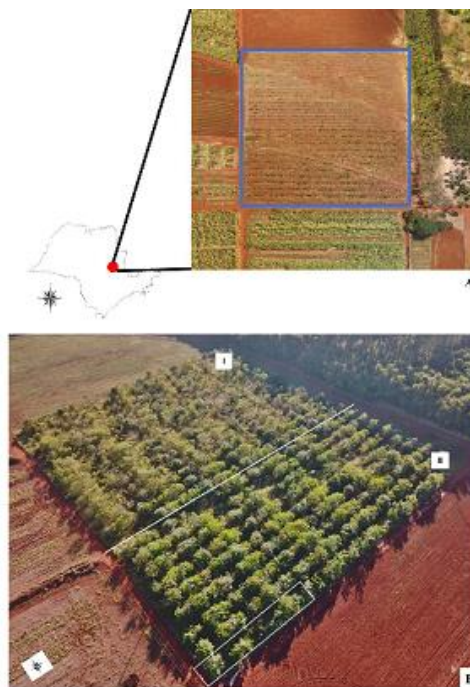
Source: Laboratory of Soil Fertility and Chemistry, UFSCar/CCA, Araras, SP. P: phosphorus; OM: organic matter; pH: soil acidity; K: potassium; Ca: calcium; Mg: magnesium; H+Al: potential acidity; Al: aluminum; SB: sum of bases; CEC: cation exchange capacity; V: base saturation; S: sulfur; B: boron; Cu: copper; Fe: iron; Mn: manganese; Zn: zinc.



**Figure 2.** Principal component analysis constructed from the morphological attributes of native seedlings (height and diameter) in both restoration methods in relation to the treatments analyzed. Variation in the attributes was greatly comprised by the PCA (99%). Treatments and control methods are represented by different colors: AFS for temporary agroforestry systems; Conv for conventional restoration; Chem for chemical control; and Mech for mechanized control of invasive species.

**Table 3.** Tree species from seasonal semideciduous forests planted in the restoration treatments. Species of the coverage group (C) and species of the diversity group (D). Species of the diversity group were not assessed in this study.

Family	Species	Group
Anacardiaceae	<i>Schinus terebinthifolius</i> Raddi	C
Bignoniaceae	<i>Handroanthus chrysotrichus</i> (Mart. ex DC.) Mattos	D
	<i>Handroanthus heptaphyllus</i> (Vell.) Mattos	D
	<i>Tabebuia roseoalba</i> (Ridl.) Sandwith	D
Boraginaceae	<i>Patagonula americana</i> L.	D
Euphorbiaceae	<i>Croton floribundus</i> Spreng.	C
	<i>Croton urucurana</i> Baill.	D
Fabaceae	<i>Anadenanthera colubrina</i> (Vell.) Brenan	D
	<i>Mimosa bimucronata</i> (DC.) Kuntze	D
	<i>Peltophorum dubium</i> (Spreng.) Taub.	C
	<i>Senna multijuga</i> (Rich.) H. S. Irwin & Barneby	D
Lamiaceae	<i>Aegiphila integrifolia</i> (Jacq.) Moldenke	C
Lythraceae	<i>Lafoensia pacari</i> A. St.-Hil.	D
Malvaceae	<i>Ceiba speciosa</i> (A. St.-Hil.) Ravenna	C
	<i>Guazuma ulmifolia</i> Lam.	C
	<i>Heliocarpus popayanensis</i> Kunth	C
Meliaceae	<i>Cedrela fissilis</i> Vell.	D
Rhamnaceae	<i>Colubrina glandulosa</i> Perkins	C
Urticaceae	<i>Cecropia pachystachya</i> Trécul	C
Verbenaceae	<i>Citharexylum myrianthum</i> Cham.	C



**Figure 3.** Aerial view of the experimental area, Center for Agricultural Sciences (CCA) of the Federal University of São Carlos (UFSCar), campus of Araras, São Paulo state. (A) General view; (B) more detailed image showing the two restoration methods adopted – (I) the agroforestry system and (II) the conventional seedling plantation. The white rectangle represents the experimental unit: 18 replications for each treatment, with 10 tree individuals in six planting rows with 60 m. Drone image credits: (A) Zenero et al. (2017); (B) Granus (2018).

Here, we increased the range of possibilities for tropical forest restoration, aiming to reverse the loss of natural areas and habitats for native species. In this regard, we have considered a few routes for implementing and managing crops under tAFS from an ecological restoration standpoint. tAFS implementation is expected to improve chemical, physical, and biological soil properties, as well as the functional attributes related to the biodiversity of the agroecosystem (Lemanceau et al., 2014; Duru et al., 2015;

Mancini et al. 2020). Although there were no significant differences found between the methods for managing invasive plants and the growth of native trees under tAFS, differences were noted in the conventional restoration treatment (mixed planting of native tree species); therefore, the choice of method (chemical or mechanical) would be a matter of assessing the costs relating to each strategy. For instance, when choosing an AFS design with chemically invasive plant management, the total size of the area for

restoration would be an important factor for cost determination and decision-making.

It is important to carefully select rapidly grown native species (of several plant life forms) for the interrows to expedite the shade element (Rodrigues et al., 2009; Brancalion et al., 2012). In addition, an adequate choice of agricultural crops for the interrows in the tAFS is needed, in which we highlight the use of native species, polyculture systems, and crop rotation; that is, the tAFS should be assembled, aiming to prevent invasion in the short term (Ramos et al., 2015; Colbach et al., 2019). The steady introduction of shade into the AFS requires diversification of crops and the range of ecosystem services, following the physiognomic, structural, and functional evolution of the tAFS (Kovács-Hostyánszki et al., 2017), and maintaining its potential for financial returns to the farmer in the medium or long term.

Since the growth of native trees was greater in the tAFS, the establishment of canopy cover in the interrows in the early establishment of the restoration might be related to the decrease in biomass of invasive species. Therefore, increasing fertility and preventing invasive species by filling this available niche increased the success of forest restoration in the tAFS. It is possible that interference and allelopathic effects of invasive species were precluded on native tree species, leading to increased overall growth (Monquero et al., 2015).

## Materials and methods

### *Description of the experimental site and soil*

The experimental area was located at the Center for Agricultural Sciences (CCA) of the Federal University of São Carlos (UFSCar), campus of Araras, São Paulo state (22°18'56" S and 47°23'20" W; 650 m altitude). The area was originally occupied by seasonal semideciduous forests (IBGE 2012) and has a history of sugarcane cultivation. Dystroferic Red Latosol (Oxisol) soil samples were retrieved from three systematically determined sites considering the slope of the area and sent for chemical analysis (Table 2) following the method of Yoshida and Stolf (2016). The experimental site is a fraction of the legal reserve area (a regimented conservation status in Brazil) and held no original vegetation at the time of the experiment implementation in 2016. The average annual temperature is 21.6 °C, with a minimum of 17.9 °C in July and a maximum of 24.3 °C in February. The annual precipitation is approximately 1400 mm, with a water deficit from April to October. The climate is Cwa (Köppen 1948): mesothermal, with hot and rainy summers and dry winters (from April to August).

### *Treatments, experimental design and study development*

The experiment consisted of 72 rows of 60 m in length each, covering 17,280 m<sup>2</sup>, planted with seedlings of seasonal semideciduous tree species (Figure 3). The tree species used can be seen in table 3. We defined a nested experimental design, first separating the total planting area into two restoration methods, which were further divided into mechanized and chemical control of weed species: tree seedlings only ("conventional seedling plantation") and a temporary agroforestry system (tAFS), which coupled the trees with herbaceous species (annual crops) in the interrows. These two restoration methods were further investigated. The two methods had different interrow widths; therefore, different numbers of planting rows and

total areas for each method were needed, which was accounted for in the models. The tAFS treatments had four rows of seedlings that were 6 m apart, whereas the conventional method used eight rows of seedlings 3 m apart. Only the species of the covering ecological group (present in both planting methodologies) were planted using the conventional method.

Within each of the two restoration techniques (tAFS and conventional seedling plantation), we randomly allocated two widely applied methods to control invasive species, chemical and nonchemical (mechanized) (Weidlich et al., 2020), summing up four different treatments in a nested design, as follows:

- 1 CONVmech, conventional restoration with mechanical control of weeds - is the standard used as control (Santana et al., 2020);
- 2 CONVchem, conventional restoration with chemical control of weeds;
- 3 AFSmech, temporary AFS restoration with mechanical control of weeds, and
- 4 AFSchem, temporary AFS restoration with chemical control of weeds.

The four treatments had 18 replications each, with 10 trees in six planting rows with 60 m considered as an experimental unit. The conventional seedling plantation used a spacing of 2 m between plants on the row and 3 m of interrow. Conventional restoration used 10 fast-growing tree species (the "covering group", Table 3), followed by 10 slow-growing tree species (the "diversity group") (Nave and Rodrigues, 2006). Eighteen plots, defined by 10 individuals from each species of the covering group, were established for each of the two restoration methods. All plants had their height and diameter measured monthly for 18 months to estimate the relative growth of each species. Tree seedlings were planted three weeks after harvesting the soybean crop.

### *Sowing of herbaceous crops in the temporary agroforestry system*

Three fast-cycle herbaceous crops were planted in the interrow of the tAFS plots during the first year of restoration: soybean, sorghum, and bean. Syngenta Intacta soybeans were sown at a density of 70 kg seeds ha<sup>-1</sup> in November 2016 before tree planting. Fertilization consisted of 400 kg ha<sup>-1</sup> of the formulation 06–30–20 (N–P–K) plus boron and zinc. Phytosanitary treatment consisted of the application of the fungicide Elatus 200 g/ha + oil 30 d after the crop was sown. Chemical weed control was performed with glyphosate at 1440 g a.i. ha<sup>-1</sup>. Planting fertilization was based on the soil chemical analysis (Table 1) and consisted of the application of 100 g of the formulation 08–28–16 (N–P–K) and 6 g of hydro retaining polymer diluted in 1 L of water in each planting pit, as determined by the manufacturer. Soybeans were harvested in March 2017, shortly after the native seedlings were planted, reaching a productivity of 50 bags ha<sup>-1</sup>.

Grain sorghum of the early variety BM 737 was then sown in the interrow of the tAFS area after soybean harvesting. We used an interrow spacing of 45 cm and 13 seeds per linear meter. The fertilizer used consisted of 150 kg ha<sup>-1</sup> of formulations 04–14–08 (N–P–K). Sorghum was harvested in July 2017, with a productivity of 45 bags ha<sup>-1</sup>. The bean of the variety "carioca" was then sown in September 2017 at a density of 50 kg seeds ha<sup>-1</sup> and fertilized with 400 kg ha<sup>-1</sup> of the formulation 04–14–08 (N–P–K) plus boron and zinc. The

harvest was conducted in January 2018, producing 40 bags  $\text{ha}^{-1}$ . No other crop was sown in the area after bean harvest, as the trees developed to the point of shading the interrows, reducing the crop cycle and rendering it impracticable.

### Weed management and assessments

Management practices were applied every 90 days to control invasive species and ensure seedling survival. Invasive species were sampled on both the rows and interrows with a  $0.5\text{-m}^2$  wooden frame placed at ten random locations in each plot. The invasive grasses inside the sample were cut close to the soil and identified by comparison with the literature and with the help of specialists. The occurrence of weed species was described by calculating the importance value (IVI) of the sampled species (Mueller-Dombois and Ellenberg, 1974).

The mechanical control consisted of mowing the weeds with hoes and rotary cutters. At the same time, chemical control was performed in the plots (six operations in 18 months). The choice of herbicides for chemical control was based on the floristic composition and characteristics of the weeds and crops identified. The application was performed using a 20-L knapsack sprayer with a single fan spray tip (11002VS). We used the following herbicides for weed control: glyphosate,  $1440\text{ g a.i. ha}^{-1}$  (six applications over 18 months); paraquat,  $200\text{ g a.i. ha}^{-1}$  (three applications over 18 months); glyphosate + metsulfuron-methyl,  $1440 + 2.4\text{ g a.i. ha}^{-1}$  (one application in 18 months); and clethodim,  $120\text{ g a.i. ha}^{-1}$  (one application in 18 months).

Systematic measurements were performed of the height and diameter of native tree species and the percentage of weed coverage to verify changes in native tree seedling growth and invasive species biomass among treatments. The measurements were implemented monthly for up to four months and, subsequently, every three months over a period of 18 months. Height was measured from the base of the stem to the last insertion of leaves using a measuring tape graduated in centimeters, and stem diameter was determined simultaneously at half the seedling height using a caliper.

The analysis of the height and diameter considered the relative growth rates after one year, according to the following formula:

$$(X_f - X_i)/X_i,$$

where  $X$  represents the morphological variable (height or diameter),  $X_f$  is the final reading at the end of the experiment, and  $X_i$  denotes the initial reading.

The invasive biomass was estimated visually at 15, 30, and 45 days after each management practice in each plot (ALAM, 1974).

### Statistical analysis

We performed a principal component analysis (PCA) on the trait matrix of the species using the *ade4* package (Dray and Dufour 2007) to assess correlations between them and with the treatments. We used generalized linear mixed-effects models (GLMMs) to detect the treatment effects on the relative growth of native tree species. Plots and species were treated as random factors, whereas weed control methods were nested within the restoration methods (tAFS and conventional seedling plantation), as their interactions were treated as fixed factors. The first axis of the PCA was the dependent variable in the mixed models to test whether linear mixed-effects models could be fitted using the *lme4* package (Bates et al., 2015). The significance of each

explanatory variable was tested using the *ANOVA* function in the *car* package (Fox and Weisberg, 2019). We then performed multiple comparisons based on GLMM to test whether the relative growth rates in height and diameter changed across treatments using the *emmeans* package (Lenth, 2020). All analyses were performed in R (R Core Team, 2020).

### Conclusion

We highlight the high efficiency of the tAFS as a restoration catalyzer that, considering the removal of chemical controls of invasive species, achieved the necessary growth of native trees. Fertilization of commercial crops, both in the planting rows and interrows, can explain the improved performance in the development of seedlings in the tAFS, as the volume of fertilizer available is higher than that in conventional cultivation. Despite chemical control having higher efficiency and decreasing the biomass of invasive species, it did not affect the growth of native tree species planted in the tAFS.

### Acknowledgments

We would like to express our sincere appreciation to Capes for scholarships granted to PPL (Processes no. 88887.583146/2020-00), Granus for providing the native tree seedlings used in this experiment, and to the Agrarian Science Study Group for their help with the field assessments.

### Declaration of Competing Interest

The authors have no conflicts of interest to declare.

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