

RUNNING HEAD: Soil amendments enhance assisted forest restoration

## **Soil amendments for cost-effective tropical forest restoration**

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### **Author contributions**

RJC conceived and designed the research, acquired funding, and led the writing; RJC and LKW analyzed the data; GQC led the field work and data collection; all authors contributed to writing and editing the manuscript.

## 1 ABSTRACT

2 **Introduction:** Low-cost soil amendments hold promise for improving assisted forest restoration  
3 outcomes and reducing dependence on emission-intensive inputs, yet rigorous field-based  
4 comparisons of novel and standard amendments, including cost-effectiveness, remain rare.

5 **Objectives:** We evaluated seedling performance and cost-effectiveness under three novel soil  
6 amendments: nutrient-rich agricultural biowaste (composted coffee pulp), native soil microbiome  
7 inoculation (live primary forest soil), and locally sourced effective microorganisms (LEM),  
8 against commercial fertilizer and an unamended control.

9 **Methods:** Treatments were tested on one early-successional (*Croton tenuicaudatus*) and one  
10 late-successional (*Persea caerulea*) seedling species. We assessed survival, relative growth rate,  
11 root mass fraction, soil and foliar nutrients, and cost-effectiveness (USD/kg plant carbon gain/ha)  
12 over the first year on degraded tropical pasture.

13 **Results:** Coffee pulp was the most effective amendment, significantly enhancing soil and foliar  
14 nutrients (particularly in *Croton*), matching fertilizer in *Croton* growth rate (0.182 vs. 0.160),  
15 improving survival by 13–19% over control and LEM treatments for both species, and reducing  
16 cost per unit carbon gain by up to 92% (*Croton*) and 68% (*Persea*) relative to controls. Native  
17 soil microbiome inoculation significantly enhanced *Croton* growth and cost-effectiveness  
18 relative to controls); LEM had no significant effect on growth or survival and significantly  
19 increased costs per unit plant carbon gain relative to other amendments for both species.

20 **Conclusions:** Composted coffee pulp and native soil microbiome inoculation improved first-year  
21 seedling performance and cost-effectiveness on degraded tropical pasture, with coffee pulp more  
22 cost-effective than commercial fertilizer; LEM provided no benefit.

23 **Implications for Practice:**

- Composted coffee pulp and other nutrient-rich agricultural biowastes significantly improve seedling performance and offer a scalable, low-cost alternative to emission-intensive synthetic fertilizers on degraded tropical land.
- Inoculation with live forest soil may also improve seedling growth, survival and restoration cost-effectiveness.
- We do not recommend LEM as a direct soil amendment as it may increase costs without ecological benefit.
- Soil amendments are a small share of total restoration costs but can greatly improve cost-effectiveness and should be evaluated with financial as well as ecological metrics.
- Future work should test more biowastes and soil microbiome inoculum sources and match amendments to species traits.

**Key words:** agricultural biowaste, coffee pulp, local effective microorganisms, microorganismos de montaña, soil microbiome inoculation, restoration costs, tropical forest restoration

## INTRODUCTION

There is an urgent need to scale up forest restoration to address the interconnected climate and biodiversity crises. Initiatives such as the Bonn Challenge (<https://www.bonnchallenge.org/>) have generated momentum for large-scale restoration, but many pledges remain unrealistic (Fagan et al. 2020), and progress toward meeting restoration goals is slow (Forest Declaration Assessment Partners 2024). A key barrier is the substantial landscape restoration finance gap,

estimated at \$300 billion annually (Ding et al. 2017). To maximize the impact of existing funding, it is critical to refine and field-test innovative restoration interventions that are cost-effective. Recent syntheses have identified promising strategies for upscaling assisted forest restoration but have underscored substantial gaps in testing these approaches in real-world settings (Brancalion & Holl 2024; Werden et al. 2024). Cost-effectiveness data are also scarce, with fewer than 5–6% of studies report financial metrics (Wortley et al. 2013; Iftekhhar et al. 2017; Mirzabaev & Wuepper 2023).

Business-as-usual practices to improve seedling survival and growth in tropical forest restoration typically rely on pasture grass and weed control until seedlings outcompete surrounding vegetation (Cole et al. 2024) and often include commercial fertilizer and chemical pest control applications (Dimson & Gillespie 2020). These interventions can contribute significantly to project costs (Toro et al. 2024). Fertilizer, commonly used in restoration projects that follow commercial forestry practices, may benefit fast-growing or non-native species, but can have neutral or negative effects on planted native species (Brancalion et al. 2019; Toro et al. 2024). Alternative soil amendments, such as microbial inoculants and repurposed agricultural biowaste, have shown benefits in agriculture, forestry, and greenhouse trials and may offer lower-cost options, but remain largely untested in tropical forest restoration.

The growth of many temperate and tropical tree species is strongly influenced by mycorrhizal fungi (Holste et al. 2016; Anthony et al. 2022), which often decline following agricultural land conversion (Aldrich-Wolfe 2007; Mueller et al. 2014; Tsiafouli et al. 2015). Accordingly, one emerging strategy involves soil microbiome transplants to rewild microbial communities in degraded ecosystems (Neuenkamp et al. 2019; Averill et al. 2022). Reintroducing native microbial communities can accelerate recovery and boost biomass

production (Wubs et al. 2018; Averill et al. 2022), however, the vast majority of research in this area has focused on temperate forests (Neuenkamp et al. 2019; Anthony et al. 2022) and grasslands (Kardol et al. 2006; Bauer et al. 2015), with few trials conducted in tropical ecosystems (Allen et al. 2003; Zahawi et al. 2015; Castillo-Bautista et al. 2024).

A complementary approach gaining traction in organic agriculture is the use of effective microorganisms, pioneered in Japan in the 1980s (Higa & Parr 1994). Effective microorganism treatments are produced by culturing beneficial, naturally occurring microbes such as lactobacilli, yeasts, actinomycetes, and photosynthetic bacteria (Higa 2000). These microbes are thought to enhance crop yield, nutrient availability, and facilitate decomposition while suppressing pathogens (Ney et al. 2018; Ney et al. 2020). A regional variation, locally sourced effective microorganisms (LEM), has been widely adopted in Central and South America, and is promoted by agricultural extension agencies (Suchini Ramírez 2012; Ney et al. 2018; Castro-Sowinski 2023). LEM is made by fermenting a carbohydrate-rich substrate inoculated with decomposing forest leaf litter. Despite widespread agricultural use, to our knowledge LEM has not been tested in a tropical forest restoration setting.

A third promising intervention involves the use of agricultural biowaste (Werden et al. 2024). Billions of tons of nutrient-rich waste, such as coffee pulp, citrus peels, and oil palm residues, are produced globally, often with limited alternative uses (Padmapriya et al. 2013; Millati et al. 2019). Studies have shown that orange peels and coffee pulp can significantly increase tree biomass and improve soil nutrients on degraded tropical soils (Janzen 2000; Treuer et al. 2018; Cole & Zahawi 2021). However, challenges like improper dumping, health risks and sanitation restrictions, as well as transport logistics limit scalability (Choi et al. 2018; Cole & Zahawi 2021). Composting biowaste offers a practical alternate solution, concentrating nutrients

and reducing health risks, and is widely practiced in sustainable agriculture (Trujillo-González et al. 2024). While composted biowastes, such as coffee pulp, are often available at little to no cost in agricultural producing regions (Echeverria & Nuti 2017; Cole & Zahawi 2021), their potential application in restoration settings remains largely unexplored.

Here, we contrasted three novel soil amendments (soil microbiome inoculation, LEM, and coffee pulp biowaste) against two business-as-usual treatments (chemical fertilizer plus nematicide, and an unamended control) for seedlings of one early- and one late-successional tree species planted in degraded pastures. Our objectives were to measure effects on (1) soil nutrients and (2) first-year seedling survival and above- and below-ground growth, and (3) to evaluate each treatment's cost-effectiveness for survival and plant carbon gain.

## METHODS

### *Study sites*

The study was conducted in 2023–2024 on degraded pastureland recently acquired for forest restoration by two organizations, the Tropical Landscape and Climate Program (TLCP) and Finca Cantaros Environmental Association (FCEA), in Coto Brus County, southern Costa Rica. Both sites were grazed for >30 years, causing erosion, soil compaction, and degradation from repeated burning and selective herbicide use. Soils in the region's former pastures are volcanic, mildly acidic, low in phosphorus, and relatively uniform in nutrient content (Holl et al. 2011; Cole et al. 2016). At the start of the study, sites were dominated by non-native pasture grasses (*Axonopus scoparius* (Flüggé) Kuhlman and *Urochloa brizantha* (Hochst. ex A.Rich.) R.D.Webster) and ruderal herbs (*Vernonanthura patens* (Kunth) H.Rob., *Vernonia arborescens*

(L.) Sw., and *Piper umbellatum* L.). The region is tropical premontane wet forest (Holdridge et al. 1971), with a mean annual temperature of ~21°C and ~3,500 mm annual rainfall, with a December–March dry season. Sites range from 1,230 to 1,480 m elevation, with steep slopes (12–30°).

### ***Seedling production***

Because our objective was to test native species being used in large-scale restoration efforts, we selected two species commonly produced in restoration-focused tree nurseries in the region. *Persea caerulea* (Ruiz & Pav.) Mez. (Lauraceae), is a later-successional species with intermediate growth rates, while *Croton tenuicaudatus* Lundell. (Euphorbiaceae), is a fast-growing, early successional species. During the year leading up to the field component of the study, TLCP nursery staff collected seeds from a minimum of 8 mother trees of each species for the experiment. Seedlings were grown under shade cloth in a standard, homogenized soil with no additional amendment or treatments until they reached sufficient size to be outplanted (7 months for *Persea* and 5 months for *Croton*). We removed the shade cloth 3 weeks prior to planting to harden seedlings.

### ***Amendments***

We tested three novel soil amendments (soil microbiome inoculation, LEM, and composted coffee pulp) against two business-as-usual treatments: chemical fertilizer plus a nematicide, and an unamended control. These treatments are hereafter referred to as soil microbiome, LEM, coffee pulp, fertilizer, and control.

For the soil microbiome treatment, we collected ~3kg of topsoil (0–10 cm depth) from each of 10 points along a 100-m transect in one of the region's largest and most intact old-growth forest remnants (~38 ha, at TLCP). Soil was thoroughly mixed and stored for  $\leq 24$  h before each field application. LEM was prepared following protocols from the Costa Rican Ministry of Agriculture and the Tropical Agricultural Research and Higher Education Center (CATIE) (Suchini Ramírez 2012; Tencio 2017). Specifically, we layered 50 kg of forest floor mulch, collected from multiple locations, with 46 kg of rice semolina and 3.7 L of molasses and fermented it anaerobically for one month. The resulting solid inoculum was mixed with water and molasses in a 150-L container to produce a liquid form. Coffee pulp was obtained free of charge from a local processing cooperative in March 2023 and composted for two months following industry-standard practices and Costa Rican health ministry guidelines (Hafner et al. 2018). The pulp was mounded into 1-m-high rows on plastic sheeting, turned twice monthly, and watered regularly; a light dusting of lye was applied weekly during the first month to reduce odor and suppress fly larvae. For the fertilizer treatment, we followed standard regional forestry practice (Holl et al. 2011) using a widely available slow-release fertilizer (NPK 10-30-10) plus the nematicide Mocap®, both purchased locally.

### ***Experimental design, seedling planting, and maintenance***

In July 2023, we cleared existing ruderal vegetation with machetes or string trimmers to prepare a 2-ha site at TLCP and a 1-ha site at FCEA. To account for site differences, we used a randomized block design (four blocks at TLCP, two at FCEA), with treatment as a fixed effect and block as a random factor. Each block contained five  $12 \times 12$  m plots spaced  $\geq 5$  m apart, each assigned one of the five treatments. Seedlings were planted in a grid at 1.5-m spacing. Because

of limited *Croton* germination in the nursery, each plot received 21 *Croton* and 27 *Persea* seedlings in alternating rows (48 per plot; 630 *Croton* and 810 *Persea* in total).

Seedlings were planted in uniform holes (35 cm deep  $\times$  30 cm diameter). For the coffee pulp and soil microbiome treatments, 1 L of composted coffee pulp or 150 g ( $\approx$ 1 cup) of fresh forest soil, respectively, was mixed into the planting hole in the seedling's root zone. For the fertilizer treatment, we followed the manufacturer's guidelines for forestry plantations, placing 30 g of fertilizer and 5 g of granular nematicide in a shallow circular trench 10 cm from the stem and lightly covering it with soil. For the LEM treatment, 100 mL of liquid was applied after planting with a backpack sprayer to the soil around each seedling's base. Control seedlings received no amendment.

LEM, coffee pulp, and forest soil were reapplied to the soil surface around each seedling every four months (for LEM and coffee pulp, following Costa Rican Ministry of Agriculture guidelines), whereas fertilizer was applied only at planting. As is standard local practice, pasture grasses and weeds within 0.5 m of each seedling were cleared every two months in all treatments using a machete or string trimmer.

### ***Plant measurements***

We measured and destructively harvested 12 seedlings of each species to determine initial dry above- and below-ground biomass. We measured the basal diameter and height of each planted seedling at the time of planting. In August 2024, one year after planting, we recorded mortality and remeasured basal diameter and height (stem base to the top of the apical meristem) of each surviving seedling. To determine final above- and below-ground biomass, we harvested three randomly selected surviving seedlings of each species per plot. Because leaf-cutter ant herbivory

caused high *Persea* mortality in two blocks, *Persea* was harvested in only four blocks, for a total of 90 *Croton* and 60 *Persea* seedlings. Roots were carefully excavated and washed free of soil, and roots, stems, and leaves were separated and dried to constant weight (~48 h at 65 °C).

### ***Soil and leaf nutrient measurements***

To evaluate amendment effects on soil nutrients, at harvest we collected and homogenized soil from the root zones of six seedlings in each of the 30 plots (30 samples; n = 6 per treatment). Samples were analyzed for total N (%), pH, organic matter, estimated nitrogen release, Bray-2 extractable P, base saturation (%) of Ca, Mg, K, and Na, and Mehlich-3 extractable macro- and micronutrients (P, K, Ca, Mg, Mn, Zn, B, Cu, Fe, Al, S), following standard procedures at Brookside Laboratories, New Bremen, OH (<https://www.blinc.com/soil-testing-methods> and (Gavlak et al. 2003).

For foliar nutrients, we collected three fully expanded leaves from each of three non-harvested seedlings per species in each plot (*Croton*, n = 6 per treatment; *Persea*, n = 4). Leaves were pooled by species and plot, air-dried, and analyzed for N, P, K, Ca, Mg, Na, S, Mn, Cu, Zn, Fe, and Al, also at Brookside Laboratories (<https://www.blinc.com/plant-testing-methods> and Gavlak et al., 2003).

### ***Cost Effectiveness***

To compare costs among treatments, we recorded the labor time and the labor and material costs needed to establish and maintain each treatment during the first year. To assess cost-effectiveness for survival, we divided the total cost of establishing 1,000 seedlings/ha under each treatment by the number of surviving seedlings, yielding a cost per surviving seedling (USD).

We used 1,000 seedlings/ha as a reference density because it is widely reported in the restoration literature (Cole et al. 2024), and scales easily to other densities.

We also estimated the cost per unit of total (above- and below-ground) plant carbon (C) gained during the first year, calculated as final minus initial plant C. Biomass was converted to C using a standard factor for tropical trees (47% of dry biomass; Martin & Thomas 2011; Chave et al. 2014). Costs are expressed in USD per kg of total plant C gained, with both scaled to 1 ha. Finally, for each amendment we calculated the average cost per surviving seedling (as above) and the average cost per additional surviving seedling relative to the control. Calculations are detailed below.

### ***Statistical analysis***

Unfortunately, *Persea* seedlings in two blocks, both at the FCEA site, were killed through complete defoliation by leafcutter ants during the height of the dry season; thus, only the four blocks at TLCP were used in all analyses for this species ( $n = 4$  blocks). There was no leafcutter herbivory of *Croton* seedlings, thus all blocks ( $n = 6$  blocks) were used in all analyses.

In order to non-destructively estimate total biomass for the non-harvested seedlings, we developed allometric models for each species based on harvested seedlings. Preliminary analysis showed that root mass fraction (RMF: root biomass / total biomass) did not differ significantly among treatments for either *Croton* ( $\chi^2 = 7.10$ ,  $df = 4$ ,  $p = 0.131$ ) or *Persea* ( $\chi^2 = 3.49$ ,  $df = 4$ ,  $p = 0.479$ ), so we included seedlings harvested in all treatments when developing the final allometric model for each species (see below for analysis details).

Furthermore, to provide a more comprehensive assessment of seedling growth we used combined below- and above-ground plant biomass to develop these relationships (total biomass).

228 We used two predictor variables, diameter (D) (cm) and height (H) (cm), with total dry biomass  
229 (B) (g) as the response variable based on measurements of the 90 *Croton* and 60 *Persea*  
230 seedlings harvested. We applied logarithmic transformations as this is commonly used in the  
231 construction of allometric equations to account for heteroscedasticity of the data on the original  
232 scale and satisfy requirements for normality of residuals and homogeneity of variances. We then  
233 tested the fit of various linear and non-linear models commonly used for allometry development  
234 (e.g., (Chave et al. 2014). The best-fit model for each species was selected based on the lowest  
235 AIC values in combination with highest  $r^2$  value. This process yielded the following two linear  
236 models, which we then used to estimate total initial and final seedling biomass for each species:  
237

238 *Croton*:  $\ln(B) = 0.4140 + 0.7640 \cdot \ln(D^2 \times H)$  ( $r^2 = 0.877$ ,  $\text{adj } r^2 = 0.875$ ,  $\text{AIC} = 128$ )

239 *Persea*:  $\ln(B) = -0.1484 + 0.7546 \cdot \ln(D^2 \times H)$  ( $r^2 = 0.850$ ,  $\text{adj } r^2 = 0.845$ ,  $\text{AIC} = 107$ )

240

241 Back-transforming log-scale predictions introduces a systematic downward bias (Sprugel, 1983).  
242 Because the corresponding correction factor is constant for a given model, it cancels when RGR  
243 is calculated from initial and final biomass estimated with the same equation; we therefore did  
244 not apply it to biomass estimates used for RGR.

245 We then calculated relative growth rate ( $\text{RGR}_{\text{total biomass}}$ ) using initial and final estimated  
246 total dry biomass, applying the classic RGR equation ( $\ln[\text{final biomass}] - \ln[\text{initial biomass}]$ ) /  
247 (final day – initial day) following (Hoffmann & Poorter 2002; Paine et al. 2012). We also  
248 calculated  $\text{RGR}_{\text{height}}$  using direct height measurements of seedlings. Because preliminary  
249 analysis showed the same trends in all cases for both  $\text{RGR}_{\text{height}}$  and  $\text{RGR}_{\text{total biomass}}$ , we present  
250 only the results using  $\text{RGR}_{\text{total biomass}}$  as the more robust overall measure of growth.

All statistical models were fit in R version 4.4.0 (R Core Team 2024) with treatment as a fixed effect and block as a random effect throughout. Seedling survival was analyzed as a binomial response using a generalized linear mixed model (GLMM) with a logit link, fit using the lme4 package (Bates et al. 2015). Root mass fraction was analyzed using a GLMM with a beta distribution and logit link, fit using the glmmTMB package (Brooks et al. 2017). RGR and carbon cost (kg C / ha) were analyzed using linear mixed models (LMMs); carbon cost data were log-transformed prior to analysis while  $RGR_{\text{total biomass}}$  required no transformation. Soil and foliar nutrient concentrations were each analyzed separately using LMMs (lme4, lmerTest; (Bates et al. 2015; Kuznetsova et al. 2017), with normality of residuals assessed using the Shapiro-Wilk test; non-normal variables were log-transformed. Where residuals remained non-normal after log transformation (soil Zn and *Croton* foliar Al), data were analyzed using a Gamma GLMM with a log link (glmmTMB; (Brooks et al. 2017)), with model fit verified using simulated residuals in DHARMA (Hartig & Lohse 2022). For all response variables, the overall treatment effect was assessed using a likelihood ratio test comparing the full model to a null model containing only the block random effect, and treatment means were compared using Tukey-adjusted pairwise contrasts via the emmeans package (Lenth 2023).

## RESULTS

### *Soil and foliar nutrients*

Treatment significantly affected 11 of 16 soil properties (Fig 1. A-K, Table S1). Soil nitrogen was significantly higher under coffee pulp and fertilizer treatments than under control, LEM, and Soil treatments ( $\chi^2 = 42.35$ ,  $df = 4$ ,  $p < 0.001$ ). Similarly, available phosphorus (P) and Bray II P

were strongly affected by treatment ( $\chi^2 = 51.65$  and  $70.35$ ,  $df = 4$ , both  $p < 0.001$ ), with fertilizer having the highest values, followed by coffee pulp, while control, LEM, and soil microbiome did not differ significantly from each other. Soil pH was significantly higher under coffee pulp than all other treatments ( $\chi^2 = 56.58$ ,  $df = 4$ ,  $p < 0.001$ ), as was organic matter ( $\chi^2 = 19.36$ ,  $df = 4$ ,  $p < 0.001$ ), potassium ( $\chi^2 = 60.77$ ,  $df = 4$ ,  $p < 0.001$ ), and magnesium ( $\chi^2 = 11.94$ ,  $df = 4$ ,  $p = 0.018$ ), with coffee pulp consistently showing the highest values. Aluminum was significantly lower under coffee pulp than all other treatments ( $\chi^2 = 26.70$ ,  $df = 4$ ,  $p < 0.001$ ). Total cation exchange capacity, calcium, sodium, sulfur, iron, manganese, and copper did not differ significantly among treatments ( $p > 0.05$ ; Table S1).

Treatment significantly affected 11 of 12 foliar nutrients in *Croton*, with calcium showing marginal differences ( $p = 0.050$ ) and only zinc showing no significant effect ( $\chi^2 = 5.74$ ,  $df = 4$ ,  $p = 0.219$ ; Figure 2, Table S2). Coffee pulp consistently elevated foliar concentrations, with significantly higher nitrogen, potassium, boron, sulfur, and phosphorus than most or all other treatments; phosphorus was also lowest in LEM (all  $p < 0.001$ ). Foliar manganese was significantly lower in coffee pulp and control than in fertilizer, soil microbiome, and LEM ( $\chi^2 = 39.55$ ,  $df = 4$ ,  $p < 0.001$ ), while iron and aluminum were both significantly higher in LEM than in fertilizer ( $p = 0.005$  and  $p = 0.002$ , respectively). Magnesium was significantly higher in control than in LEM ( $\chi^2 = 14.27$ ,  $df = 4$ ,  $p = 0.007$ ), and copper was significantly higher in control and coffee pulp than in LEM ( $\chi^2 = 17.77$ ,  $df = 4$ ,  $p = 0.001$ ). Treatment effects were more limited in *Persea*: only foliar nitrogen was significantly higher in coffee pulp than all other treatments ( $\chi^2 = 10.49$ ,  $df = 4$ ,  $p = 0.033$ ; Figure 2L), with no significant effects for remaining foliar nutrients (all  $p > 0.051$ ; Table S2).

## 297 *Seedling performance*

298 Seedling survival differed significantly among treatments for both *Croton* ( $\chi^2 = 11.53$ ,  $df = 4$ ,  $p =$   
299  $0.021$ ) and *Persea* ( $\chi^2 = 19.56$ ,  $df = 4$ ,  $p < 0.001$ ). For both, survival was lowest in control and  
300 LEM and highest in coffee pulp and fertilizer (Figure 3A, B). For *Croton*, coffee pulp showed  
301 the highest survival ( $0.929$ ), significantly exceeding control ( $0.819$ ;  $p = 0.010$ ) and LEM ( $0.838$ ;  
302  $p = 0.039$ ), while fertilizer ( $0.911$ ) and soil microbiome ( $0.882$ ) were intermediate and did not  
303 differ significantly from any treatment (Figure 3A). For *Persea*, coffee pulp ( $0.924$ ) and fertilizer  
304 ( $0.905$ ) had the highest survival and did not differ from each other, but both significantly  
305 exceeded control ( $0.770$ ) and LEM ( $0.768$ ; all  $p \leq 0.004$ ). Soil microbiome was again  
306 intermediate ( $0.872$ ) with no significant differences from any treatment (Figure 3B).

307 The two species had different growth responses to amendments. *Croton*  $RGR_{total\ biomass}$   
308 differed significantly among treatments ( $\chi^2 = 62.22$ ,  $df = 4$ ,  $p < 0.001$ ), with the lowest growth in  
309 control ( $0.084$ ) and LEM ( $0.094$ ), and highest in fertilizer ( $0.160$ ) and coffee pulp ( $0.182$ );  
310 neither pair differed significantly from each other. Interestingly, the soil microbiome treatment  
311 showed intermediate  $RGR$  ( $0.117$ ), significantly higher than control and LEM ( $p = 0.002$  and  $p =$   
312  $0.044$ , respectively) but lower than coffee pulp and fertilizer (both  $p < 0.001$ ; Figure 3C). In  
313 contrast, *Persea*  $RGR_{total\ biomass}$  did not differ across treatments ( $\chi^2 = 7.73$ ,  $df = 4$ ,  $p = 0.102$ ),  
314 with estimated means ranging from  $0.001$  in LEM to  $0.050$  in coffee pulp and no significant  
315 pairwise differences (Figure 3D). Root mass fraction (RMF) was also unaffected by amendments  
316 in either species (*Croton*:  $\chi^2 = 7.10$ ,  $df = 4$ ,  $p = 0.131$ ; *Persea*:  $\chi^2 = 3.49$ ,  $df = 4$ ,  $p = 0.479$ ), with  
317 estimated means ranging from  $0.286$ – $0.352$  in *Croton* and  $0.352$ – $0.397$  in *Persea* and no  
318 significant pairwise differences detected (Figure S1).

319

## Cost-effectiveness

The costs of producing, preparing the site, and planting seedlings, alongside the first year of necessary maintenance summed \$2,700 per ha planted. This was almost all of the total cost of implementation (94 – 100%; Table 1), while the cost of purchasing, preparing and applying amendments was comparatively low (1.2 – 6.1% of total costs). The LEM amendment was the least expensive amendment to apply (\$49/ha) while fertilizer was the most expensive (\$164/ha), despite being applied only once compared to three times for all other amendments (Table 1).

When we estimated USD / kg plant carbon gained per ha, however, costs differed significantly among treatments for both *Croton* ( $\chi^2 = 43.05$ ,  $df = 4$ ,  $p < 0.001$ ) and *Persea* ( $\chi^2 = 29.10$ ,  $df = 4$ ,  $p < 0.001$ ) (Fig. 3E, F; Table 2). For both species, coffee pulp and fertilizer treatments had the lowest carbon costs while LEM and control had the highest. In *Croton*, estimated costs ranged from \$107 / kg plant carbon / ha in coffee pulp to \$1,282 / kg plant carbon / ha in control, with LEM and control not differing significantly from each other but both being significantly more costly than coffee pulp and fertilizer (all  $p \leq 0.046$ ). In *Persea*, costs ranged from \$483 / kg plant carbon / ha in coffee pulp to \$2,355 / kg plant carbon / ha in LEM with control, coffee pulp, fertilizer, and soil microbiome not differing significantly from each other (Figure 3F, Table 2). We note that the cost for LEM appears to be very high because of the very small  $RGR_{total\ biomass}$  value and this metric becomes unreliable as growth approaches zero (i.e., costs are divided by a near zero value). Thus, *Persea* cost results across amendments should be interpreted as being driven primarily by small variations in growth rather than large variation in actual amendment costs. We also show average costs per surviving seedling under each treatment and average costs per each additional surviving seedling relative to the control treatment (Table 2).

## DISCUSSION

Although tropical forests can regenerate naturally in less degraded areas, unsustainable land use has severely degraded vast regions, impeding recovery and necessitating assisted restoration (Chazdon et al. 2021). Achieving global restoration targets would benefit from low-cost strategies to accelerate these efforts (Werden et al. 2024). We compared three novel soil amendments with commercial fertilizer and an unamended control for a fast-growing, early-successional species (*Croton tenuicaudatus*) and a slow-growing, late-successional species (*Persea caerulea*) planted on degraded former pasture and quantified first-year costs per surviving seedling and per kg of plant carbon gained. Several amendments improved early survival, total plant carbon accumulation, and cost-effectiveness, although responses varied by species. Composted coffee pulp, an agricultural biowaste, yielded the most consistent gains in soil and foliar nutrients and seedling performance. It exceeded fertilizer's effect on growth in the early-successional species (RGR: 0.182 vs. 0.160) and improved survival by up to 15–16% over the control and locally sourced effective microorganisms (LEM) in both species. Fertilizer also improved growth and survival in both species but was less cost-effective than coffee pulp. Microbial treatments produced mixed results: native soil microbiome inoculation increased growth in the early-successional species and improved cost-effectiveness by 53–69% relative to controls in both species, whereas LEM, despite being widely recommended in agriculture, had no significant effect on growth or survival and substantially increased costs. These findings highlight the value of directly comparing amendments in field trials, the potential of agricultural

biowaste and soil microbiome inoculation for restoration, and the importance of matching amendments to species' growth strategies to make the most of limited restoration funds.

### ***Performance of Biowaste Amendment***

Composted coffee pulp was the most effective amendment tested. It significantly enhanced soil nutrient levels, increasing concentrations of N, P, K, Mg, and organic matter (OM) compared to the control and microbial-based treatments, and matched or exceeded the effects of fertilizer in all but phosphorus. These results are consistent with other studies showing that composted agricultural biowaste increases soil nutrient concentrations (Haynes & Zhou 2016) and offers a viable alternative to commercial synthetic fertilizers (Chojnacka et al. 2020; Lawrence & Melgar 2023). Coffee pulp also significantly reduced soil aluminum, which may have further benefited seedlings on these degraded soils (Ofoe et al. 2023). Soil nutrient gains were reflected in foliar nutrients, especially in the early-successional *Croton*, which had elevated leaf N, P, K, and B. The late-successional *Persea* had a more limited response, however, showing only elevated foliar N. Nonetheless, the coffee pulp amendment increased *Persea* survival relative to the control and LEM treatments and raised *Croton* growth to levels matching or exceeding fertilizer. Notably, it was also more cost-effective than fertilizer, at approximately 57% and 76% of fertilizer's cost per kg of plant carbon gained per hectare for *Croton* and *Persea*, respectively.

The outcomes of coffee pulp compost align with previous research on nutrient-rich agricultural biowaste, such as orange-peel waste (Janzen 2000; Treuer et al. 2018) and raw coffee pulp (Cole & Zahawi 2021), which greatly accelerate forest recovery on degraded land. Compost also has notable advantages over the mass applications of raw biowaste used in those trials. Raw applications can cause nutrient leaching, water contamination, odor, and insect

problems that conflict with health and waste management regulations in many countries (Chew et al. 2019; Trujillo-González et al. 2024). Importantly, the compost's smaller volume and low application rates make large-scale use feasible, even on remote, steep sites that are difficult to access. Our results reinforce calls to integrate biowaste into forest and landscape restoration (Werden et al. 2024). Coffee is grown in more than 70 countries, and processing of the ~10 million tons of beans produced annually generates an estimated 12–19 million tons of pulp and husk each year (Murthy & Madhava Naidu 2012; Tsigkou et al. 2025), while global agricultural residues, including oil palm and citrus processing wastes, total roughly 2 billion tons annually (Millati et al. 2019). Together, these figures highlight the global potential of composted biowaste as a scalable, low-cost restoration input.

### ***Performance of microbiome amendments***

The two microbiome amendments had contrasting effects on growth and cost-effectiveness. Soil microbiome inoculation (live primary forest soil) significantly enhanced *Croton* growth and reduced cost by 69% relative to the control (\$401 vs. \$1,282/kg plant carbon gain/ha). It also had the lowest cost per additional surviving seedling of any amendment (\$0.33 vs. \$1.38 for fertilizer and \$0.53 for coffee pulp). Effects on *Persea* were modestly positive but not statistically significant and merit further testing; even so, its small survival gains translated into the lowest cost per additional surviving seedling (\$0.19 vs. \$1.11 for fertilizer and \$0.48 for coffee pulp). These cost-effective outcomes are notable, especially at scale and given the mixed species responses reported in other tropical restoration studies. For instance, AMF inoculation (a subset of the soil microbiome) boosted growth of late-successional species but had neutral effects on early-successional trees in Mexican dry forests (Huante et al. 2012). Inoculum source is also an

important factor: For example, Allen et al. (2003) showed that early-successional AMF improved growth across successional stages, while Fischer et al. (1994) found pasture-derived inoculum to be the least beneficial. More broadly, inoculation with soil from reference ecosystems increases plant biomass by an average of 64% across multiple ecosystems (Averill et al. 2022). Our findings reinforce the idea of live soil microbiome inoculation as a cost-effective restoration strategy and underscore the need for further research to optimize species–inoculum pairings across diverse ecological contexts.

LEM, though widely recommended for sustainable agriculture across Central and South America, did not improve soil or foliar nutrient concentrations, seedling growth, or survival in our study. Unlike soil microbiome inoculation, LEM provided no significant cost benefits relative to the control and actually increased costs for *Persea* by 56% with no significant growth benefits. It also had the highest cost per additional surviving seedling of any amendment for both species. The only other study we know of that tested microbial applications on trees also found no growth benefits for two European forest species (Bzdyk et al. 2018). Recent agricultural research suggests LEM is more effective at accelerating decomposition of organic waste or compost than enhancing plant growth. For instance, combining LEM with swine effluent (Ney et al. 2018) and broiler litter (Ney et al. 2020) increased the rate and amount of soil N mineralization but not crop productivity, and pairing effective microorganisms with biowaste improved decomposition rates and soil organic matter quality (Żukowska et al. 2024). The anaerobic conditions used to prepare LEM likely reduce populations of mycorrhizal symbionts and other plant-beneficial elements, while favoring microbes that support organic matter breakdown (Gao et al. 2012; Jusoh et al. 2013). Based on our findings, we do not recommend LEM as a direct soil amendment for tree establishment on degraded tropical lands.

Cost effectiveness assessments are rare in restoration practice (Cole et al. 2024) and research (Bodin et al. 2022), yet they are essential for identifying scalable strategies (Brancalion et al. 2019; Brancalion & Holl 2020). Our analysis shows that soil amendments can reduce costs per unit carbon gain (USD/kg plant carbon gain/ha) by up to 92% for *Croton* and 68% for *Persea* relative to controls. Coffee pulp compost was the most cost-effective treatment, ranging from \$107/kg plant carbon gain/ha for *Croton* to \$483 for *Persea*. Fertilizer and soil microbiome inoculation also improved cost-effectiveness relative to the control for both species. In contrast, LEM was among the costliest treatments, reaching \$2,355/kg plant carbon gain/ha for *Persea*, significantly higher than all treatments except the control. That said, amendment costs accounted for just 1.2–6.1% of total restoration expenses (~\$2,700/ha). As in other Latin American tropical forest projects (Cole et al. 2024; Toro et al. 2024), site preparation, planting, and maintenance made up the remainder. Although commercial fertilizer improved early-successional seedling growth and was the second most cost-effective treatment for both species, its high carbon footprint and reliance on subsidies pose challenges for climate mitigation goals (Chew et al. 2019; Houlton et al. 2019). Previous studies also report neutral or negative effects of fertilizer on native tree performance alongside higher project costs (Shannon et al. 2023; Toro et al. 2024). These findings highlight a critical gap in restoration science: the need to incorporate economic as well as ecological metrics in assessments (Brancalion & Holl 2024; Werden et al. 2024). Our study provides field-based evidence that evaluating both biological and financial performance is not only feasible but essential to designing cost-effective restoration at scale.

While our results are promising, several caveats remain. First, this study reflects only one year of monitoring, and amendment effects may shift over time. In dry tropical forests, for example, amendment benefits did not persist beyond two years (Werden et al. 2018), and Toro et

al. (2024) reported negative fertilizer effects over the same period. Longer-term trials are needed to assess persistence, reapplication needs, and delayed impacts, but the strong responses to specific treatments suggest robust trends worth exploring at larger scales. Second, the soil microbiome inoculum came from transects in a single remnant forest patch; other remnants may harbor different microbial communities and yield different results. Third, our species-specific responses underscore the need to test amendments across diverse tree functional types. Linking amendments to species traits, such as nutrient uptake strategy, growth rate, or mycorrhizal association, could guide restoration approaches (e.g., Werden et al. 2018). Finally, other novel amendments, such as biochar, merit testing (Thomas & Gale 2015; Werden et al. 2024).

Our results demonstrate that locally available soil amendments—particularly agricultural biowaste and native soil microbiome inoculation—can significantly improve early seedling performance and cost-effectiveness in tropical forest restoration, even over short timeframes, while ineffective amendments such as LEM can increase costs without corresponding ecological benefit. Further, testing and adapting locally available, low-cost biowastes represents a promising direction for expanding scalable assisted restoration approaches in degraded tropical landscapes. Such novel amendments offer scalable, low-cost strategies with the potential to enhance restoration outcomes while minimizing reliance on more traditional high-emission, subsidy-dependent inputs like synthetic fertilizers. By linking amendment effects to species traits such as growth strategy, and incorporating financial as well as ecological metrics, future research can further refine restoration strategies that are both impactful and economically viable at scale.

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679 **Tables and Figures**

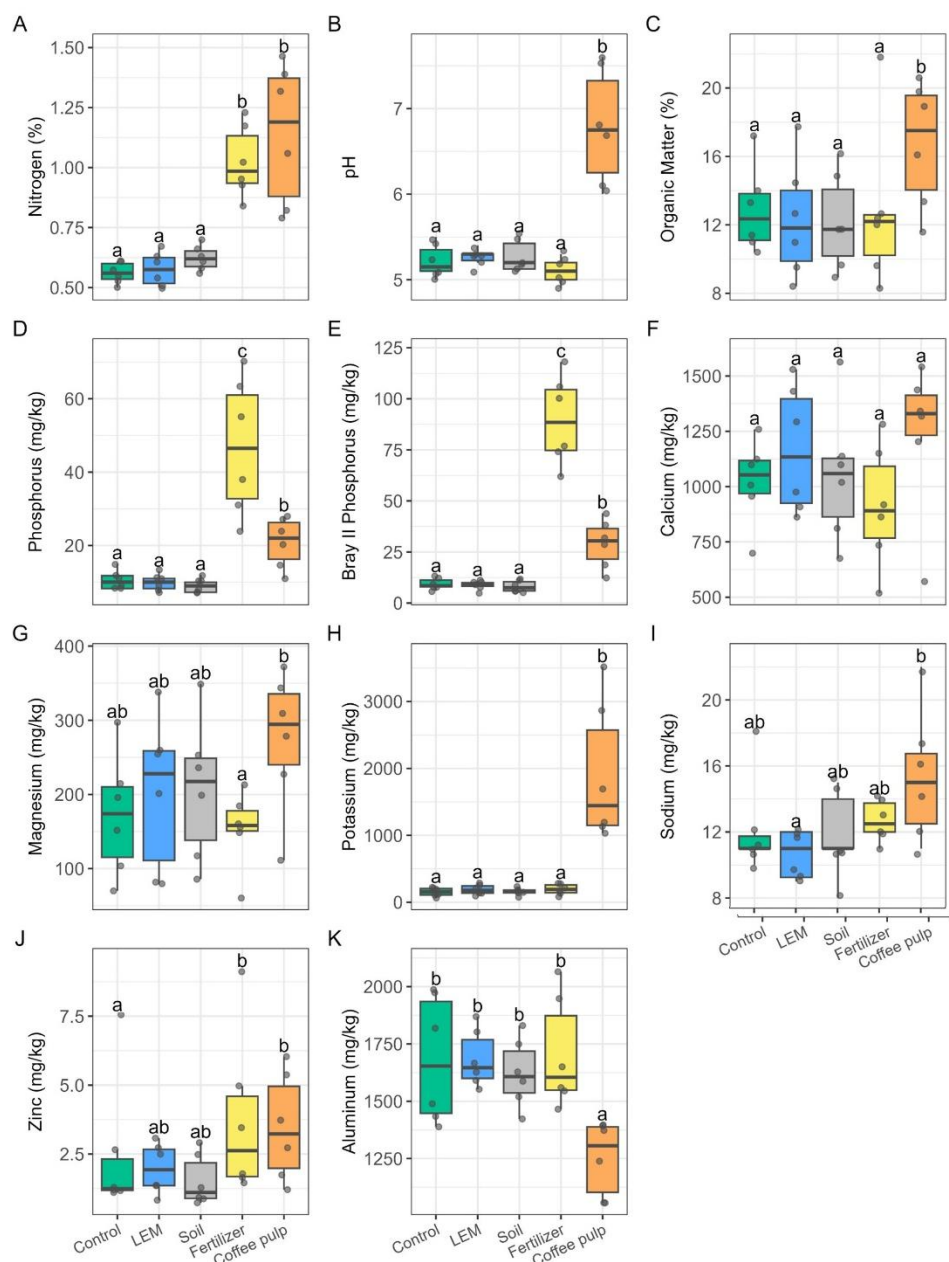
680 **Table 1.** Cost breakdown for each step of restoration project installation, and addition of each of the soil amendments tested. Costs  
681 are in units of \$USD/ ha (planting density is 1000 seedlings/ha).

| Costs of planting and maintenance                                 |         | Costs of amendment addition      | Control (No amendments) | LEM     | Soil microbiome | Fertilizer | Coffee pulp |
|-------------------------------------------------------------------|---------|----------------------------------|-------------------------|---------|-----------------|------------|-------------|
| Seedling production                                               | \$1,250 | Supplies                         | \$0                     | \$30    | \$0             | \$158      | \$0         |
| Site preparation                                                  | \$500   | Preparation                      | \$0                     | \$15    | \$15            | \$0        | \$68        |
| Planting                                                          | \$500   | Application time (person/hours)  | \$0                     | \$4     | \$6             | \$6        | \$7         |
| Post-planting maintenance                                         | \$450   |                                  |                         |         |                 |            |             |
| Total cost of planting and maintenance                            | \$2,700 | Total cost of amendment addition | \$0                     | \$49    | \$21            | \$164      | \$75        |
| Total cost (USD/1000 seedlings planted under amendment treatment) |         |                                  | \$2,700                 | \$2,749 | \$2,721         | \$2,864    | \$2,775     |

**Table 2.** Comparison of mean costs per surviving seedling, mean cost of each additional surviving seedling relative to the Control treatment, and mean cost per kg plant carbon gain / ha

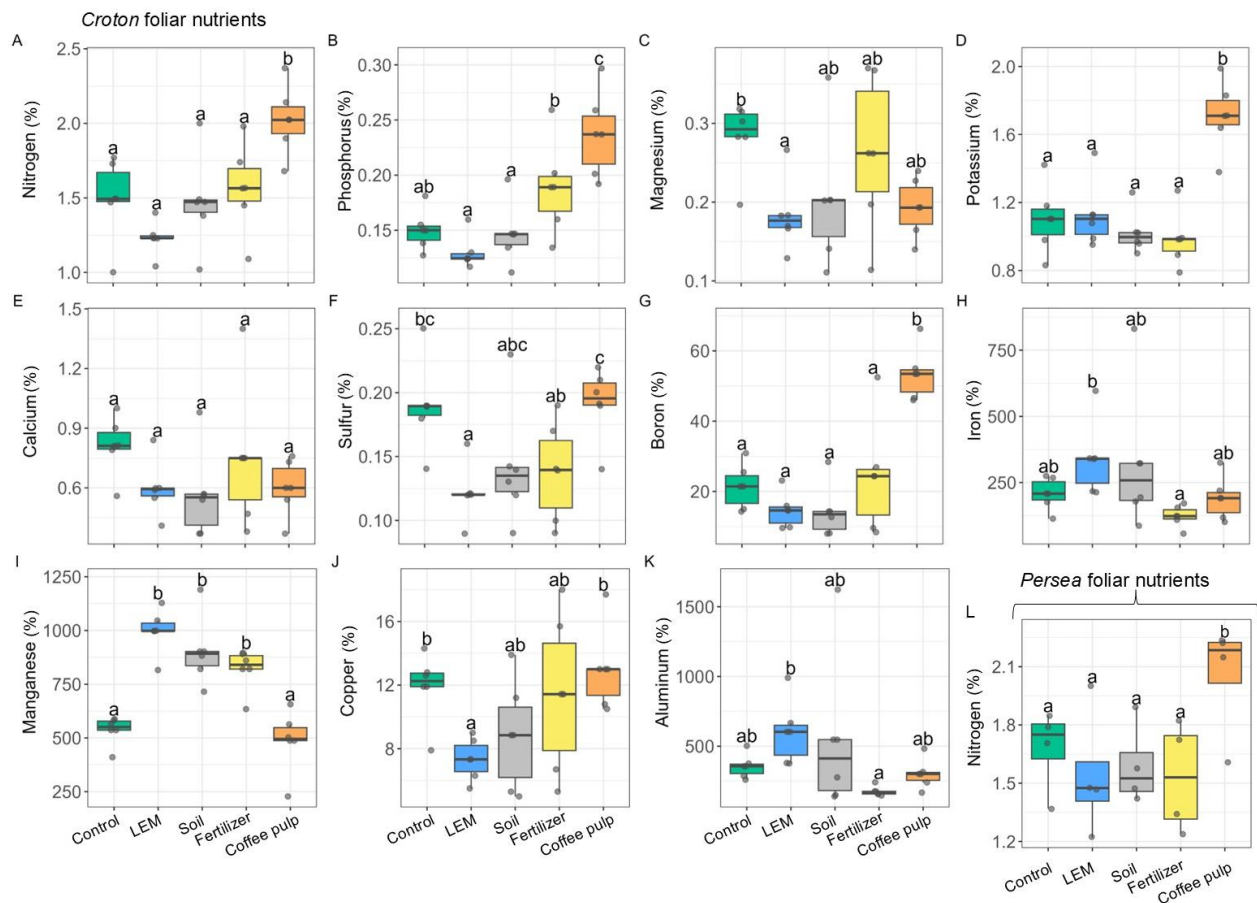
| Treatment       | Croton                     | Persea | Croton                                                   | Persea   | Croton                            | Persea      |
|-----------------|----------------------------|--------|----------------------------------------------------------|----------|-----------------------------------|-------------|
|                 | \$USD / surviving seedling |        | \$USD / surviving seedling relative to control treatment |          | \$USD / kg plant carbon gain / ha |             |
| Control         | \$3.43                     | \$3.50 | na                                                       | na       | \$1,281.50                        | \$1,281.50  |
| LEM             | \$3.33                     | \$3.56 | \$2.06                                                   | *\$24.50 | \$968.40                          | *\$2,109.61 |
| Soil microbiome | \$3.10                     | \$3.07 | \$0.33                                                   | \$0.19   | \$400.70                          | \$710.50    |
| Fertilizer      | \$3.05                     | \$3.10 | \$1.38                                                   | \$1.11   | \$188.50                          | \$638.30    |
| Coffee pulp     | \$2.87                     | \$2.97 | \$0.53                                                   | \$0.48   | \$107.20                          | \$483.20    |

\* Cost for *Persea* in the LEM treatment appear inflated as gains relative to the control treatment are small (i.e., costs are divided by a number close to zero) for both survival and plant carbon gain.



**Figure 1.** Soil nutrients in rooting zone of seedlings following 1 year of amendment applications.

Only soil nutrients with significant differences are shown here; the results of all soil nutrients across all treatments plus comparison with composted coffee pulp and reference forest soil are shown in Table S1. Different letters indicate significant differences among treatments based on Tukey-adjusted pairwise contrasts ( $\alpha = 0.05$ ;  $n = 6$ ).



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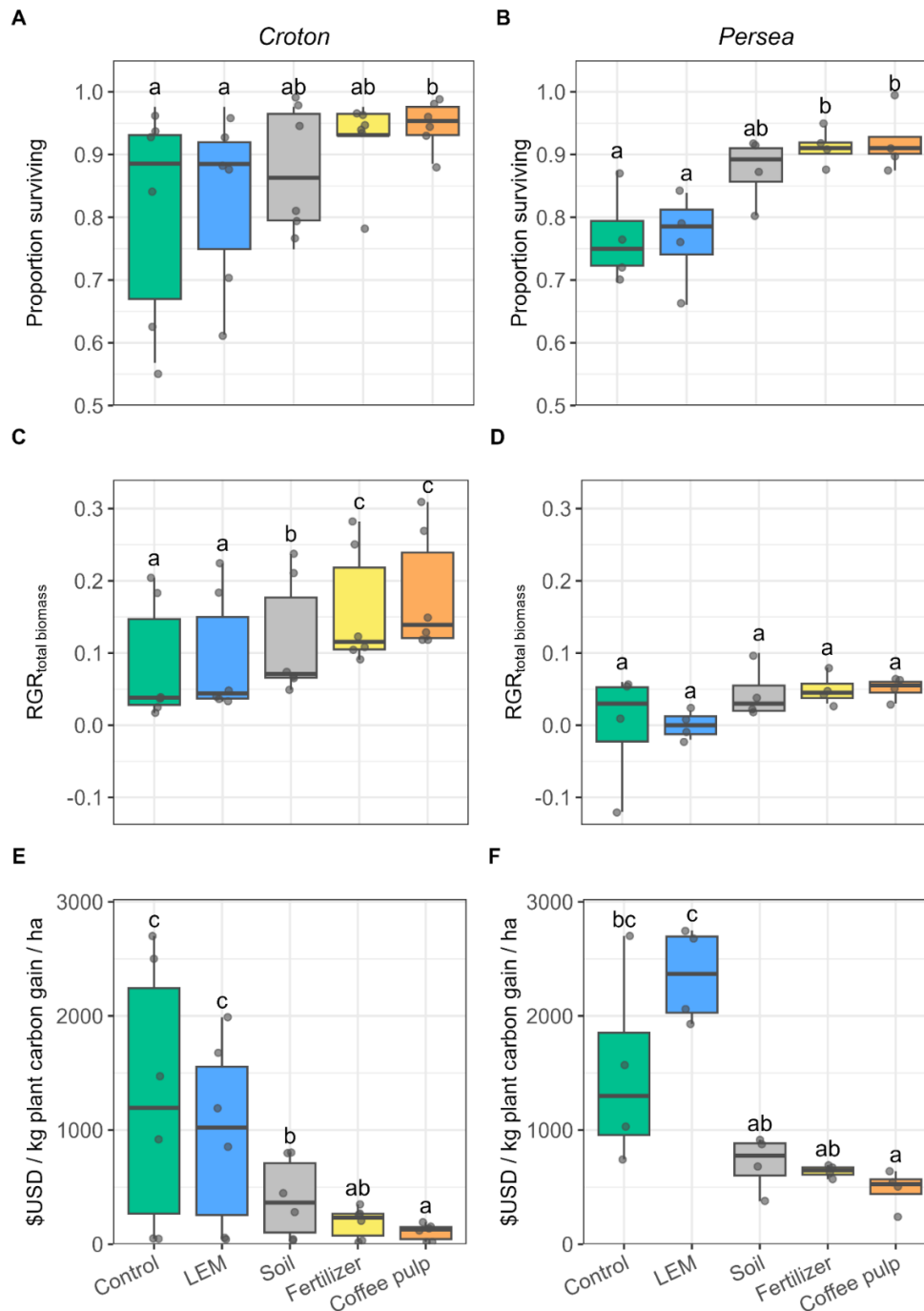
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**Figure 2.** Foliar nutrients in *Croton* (A - K) (n = 6) and *Persea* (L) (n = 4) following 1 year of amendment applications. Foliar nutrients with significant differences ( $p < 0.050$ ) and one marginally significant difference (Calcium  $p = 0.050$ ) are shown. The results of all foliar nutrients across all treatments are in Tables S2 (*Croton*) and S3 (*Persea*). Different letters indicate significant differences among treatments based on Tukey-adjusted pairwise contrasts ( $\alpha = 0.05$ ).



**Figure 3.** Percent survival for *Croton* (A) and *Persea* (B). Relative growth rate (RGR) of dry total biomass (g/month) for *Croton* (C) and *Persea* (D). Cost effectiveness in USD per kg of carbon gained in the first year of growth /ha (1000 seedlings) planted for *Croton* (E) and *Persea* (F). This estimate considers the average percent mortality under each treatment for each species.

719 Cost for *Persea* in the LEM treatment (F) appear inflated as growth was close to zero (i.e., costs  
720 are divided by a number close to zero). In all cases,  $n = 6$  for *Croton* and  $n = 4$  for *Persea*;  
721 different letters indicate significant differences among treatments based on Tukey-adjusted  
722 pairwise contrasts ( $\alpha = 0.05$ ).