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Research article

Biochar effects on NTFP-enriched secondary forest growth and soil properties in Amazonian Ecuador

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ABSTRACT

Deforestation in the Amazon has resulted in large areas of depleted soils on abandoned pastures and agricultural sites that present a restoration challenge central to protecting biodiversity and ecosystem function in the region. Biochar – charcoal made from waste materials – can improve soil physical, chemical, and biological properties, but the few tropical field trials to date do not give consistent results regarding tree growth. This study presents three years of soil performance and tree growth of a secondary forest shading nontimber forest product (NTFP) plantations of *Ocotea quixos* (Lauraceae), *Myroxylon balsamum* (Fabaceae), and their mixture. Open kiln and traditional mound biochars were added at 10 t ha^{-1} at two sites with contrasting soil types. Biochar additions resulted in pronounced effects on soil properties that varied over time and with depth in the soil profile. Biochar additions generally increased soil organic matter, electrical conductivity, and plant nutrients (in particular K, Ca, and N), but there were interactive effects of NTFP treatments, and stronger responses on the poorer soil type. Biochar amendments resulted in increased tree growth, with a 29 ± 12% increase in aboveground biomass (AGB) on plots amended with kiln biochar and a 23 ± 9% increase in plots with mound biochar compared to controls. Tree species also varied in growth responses to biochar additions, with the largest increases observed in *Jaccaranda copaia* and *Piptocoma discolor*. Significant interactions between biochar and NTFP treatments were also seen for tree growth responses, such as *Cecropia* spp., which only showed increased biomass on mound biochar plots planted with *Ocotea quixos*. Overall, our results demonstrate a stronger effect of biochar in less favorable soil conditions, and an overriding effect of the legume NTFP in richer soils, and suggest that additions of biochar and legumes are important options to increase productivity and ecological resilience in tropical forest restoration.

1. Introduction

The Amazon rainforest, accounting for about 10% of global biodiversity, is considered one of the most diverse (Maretti et al., 2014), yet one of the most threatened ecosystems on Earth (Sakschewski et al., 2016; Winemiller et al., 2016). Deforestation driven by agricultural expansion and land-clearing for pasture, among other human activities, is decreasing forest cover across the landscape (Achard et al., 2008; Tritsch and Le Tourneau, 2016). Amazonia is also jeopardized by climate change. In 2005, a severe drought reduced biomass growth in northwestern Amazonia increasing tree mortality (Phillips et al., 2009). In 2010, southwestern and southern Amazonia were also affected, reducing aboveground biomass due to increased tree mortality and reduced tree recruitment (Feldpausch et al., 2016). There is evidence for a reduction in ecological resilience in the region, due to extended dry periods, with more pronounced effects closer to human-modified environments (Boulton et al., 2022). Ecosystem models predict forest dieback, especially in northern parts of the Amazon (Parry et al., 2022).

Threats to Amazonian forests are exacerbated because soils depend on natural forest cover. Highly weathered Oxisols and Ultisols account for 40% (Laurance et al., 1999) and 24% (Mendonça-Santos et al., 2006) of the Amazon basin, respectively; Inceptisols are the most abundant in the Ecuadorian Amazon (Sánchez et al., 2018) and throughout much of the foothills of the Andes. Generally, availability of the most limiting

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elements – phosphorus (P) in old forests (Davidson et al., 2007) and nitrogen (N) in secondary tropical forests (Davidson et al., 2007; Laurance et al., 1999) – are directly linked to aboveground biomass production. Once forest biomass is removed and transformed into pasture or agriculture, plant nutrients persist for 5–7 years; after which pH declines, and nutrient leaching is pronounced (Herpin et al., 2002). Rapid soil depletion is a major driver of regional deforestation: progressive conversion of forest to agriculture is driven by the subsistence needs of Amazonian rural families (Lavelle et al., 2016), with increasing consequences for soil loss and environmental impoverishment.

Biochar, the name coined for charcoal intended for use as a soil amendment, may present a viable option for forest restoration and management with long-term benefits (Thomas and Gale, 2015; Bruckman and Pumpanen, 2019). In general, potential mechanisms for the positive effects of biochar on tree growth include increased nutrient and water retention, beneficial effects on physical soil properties, and liming effects on acid soils (Joseph et al., 2021). In the Amazon, slow-pyrolysis biochars show promise in helping retain water (Santos et al., 2022), especially important under drought scenarios (Batista et al., 2018; Sun and Lu, 2014). Biochar in tropical regions may also improve other soil properties through nutrient provisioning and liming effects in acid soils (Jeffery et al., 2017). Improvements to soil physical properties include increased porosity that enhances root growth, increased water retention, and enhanced soil aggregate formation biologically mediated after biochar application (Vijay et al., 2021). Biochar's high pH may improve the conditions of tropical soils that generally have high Al^{+3} concentrations. Unlike most forms of organic matter, biochar is recalcitrant and remains in soils for very long periods, as evidenced by *terra preta* soils found throughout the Amazonian region (Guo, 2015). The benefits of biochar may even increase with time due to biochar oxidation by aging, which enhances soil cation exchange capacity and may increasingly retain plant nutrients in available forms (Basak et al., 2022; Blanco-Canqui, 2021).

To date, most results on biochar addition come from agricultural experiments (Aller, 2016b; Basak et al., 2022; Blanco-Canqui, 2021; Jeffery et al., 2017; Schmidt et al., 2021). Field studies of biochar's effects on soil properties in forest environments in the tropics remain few. Biochar additions to sandy soils in the Indo-Malayan region resulted in increased soil P and K concentrations and increased stable soil aggregates after 3 years of biochar addition at 10 t ha^{-1} (Prakongkep et al., 2021). Increased soil organic matter and pH were found following biochar additions at 7.5 t ha^{-1} to a tea plantation in Bangladesh (Karim et al., 2020). Biochar resulted in reduced Cd concentrations in soil and leaves in cocoa plantation trials (Ramtahal et al., 2019). In a recent study in the Ecuadorian Amazon, additions of biochar at 6 t ha^{-1} increased soil Ca, Zn, and total N concentrations, as well as Ca and Zn retention, but did not significantly enhance tree growth when compared with unamended soils (Gonzalez Sarango et al., 2022).

Plant growth responses to biochar addition vary among species (Gale et al., 2017) and are also dose- and substrate-dependent (Gale and Thomas, 2019). Studies suggest that biochar generally increases tree growth with a larger impact on tropical than temperate trees (Thomas and Gale, 2015); however, most data are from short-term pot trials. A recent forest restoration study in Mauritius found higher soil pH, and large increases in seedling growth of six native tree species in response to 25 t ha^{-1} and 50 t ha^{-1} to biochar additions (Sujeeun and Thomas, 2022). Another recent study in the Peruvian Amazon found that slow-pyrolysis biochar can improve tree survival in nursery plantations that use low organic matter soil from mine tailings as substrate (Lefebvre et al., 2019), and a related field experiment suggested that species with lower wood density species show better responses to biochar addition (Román-Dañobeytia et al., 2021). However, other tropical field trials have not detected positive tree growth responses. Biochar did not result in detectable growth increases in a 4-year study in the Brazilian Amazon involving a *Eucalyptus* hybrid and *Tachigali vulgaris* (de Farias et al., 2016). Similar null results were found with *Schizolobium parahyba* and *Gmelina arborea* in the Ecuadorian Amazon in a 4-year field trial. In this case, soil type and nutrient status had an overriding effect on tree growth (Gonzalez Sarango et al., 2021, 2022).

Biochar may be particularly viable as a management option for valuable nontimber forest products (NTFPs), which in South America are predominantly produced in the Amazon region (Herrero-Jáuregui et al., 2013; Silva et al., 2020). The experiences from tea plantations in subtropical Asia show that biochar increased tea yields and soil N retention with a potential for net economic benefits if carbon credits are strong (Lin et al., 2023). Other results suggest that the large benefits of biochar are mainly seen in degraded soils of tea monocultures (Karim et al., 2020). Biochar also holds a potential for reducing aluminum toxicity, which is a major factor in tropical soils, yet field experiments are needed to corroborate laboratory and pot results (Shetty et al., 2021). There is a need for more field studies addressing the specific conditions under which biochar can result in positive effects on tree growth and performance in the tropics.

In the present study, we examine soil and tree responses to biochar additions in the context of a 3-year field trial conducted in Amazonian Ecuador at two sites with different soil conditions. The wood-feedstock biochars used included both a traditional mound charcoal and a biochar produced using an open conical kiln system. The following hypotheses were tested: (1) biochar additions will enhance the availability of soil organic matter and mineral nutrients; (2) biochar additions will enhance tree growth, particularly in the lower nutrient status soil; (3) biochar generated using the open conical kiln method will show superior effects on soil nutrients and tree growth compared to traditional mound charcoal; (4) tree species will vary in their responses to biochar additions.

2. Methods

2.1. Preparation and characterization of biochars

Biochar types were obtained from *Piptocoma discolor* (Kunth) Pruski (Family: Asteraceae) (local common name pigue), a widespread pioneer species native to neotropical wet forests, which is used regionally for pallet and fruit-box construction (Erazo et al., 2013). Biochar was produced from pigue off-cuts that were pyrolyzed in a slow pyrolysis open conical kiln (B1) with 2.7 h mean residence time and a peak temperature of ~550 °C. The kiln was similar in design to the flame-curtain "Kon-Tiki" kiln design intended for use in tropical rural settings (Cornelissen et al., 2016; Karim et al., 2020). Traditional mound charcoal (B2) was purchased from local charcoal producers who also used pigue as a feedstock material; pyrolysis conditions are unknown, but typical traditional mound charcoal has a residence time that goes from 12 to 24 h to several days (FAO, 1983), and typical peak temperatures of 350–400 °C (UNDP, 2009). Biochar pH and electrical conductivity were measured electrometrically at the Soil Laboratory of Universidad Estatal Amazónica using a 1:20 biochar:distilled water ratio with a Sartorius PP-20 multiparameter meter. Total Calcium (Ca), Magnesium (Mg), Potassium (K), and Phosphorus (P) were measured at the Analyst lab of the University of Toronto; samples were digested in nitric acid and analyzed by ICP-OES elemental analysis using a Perkin Elmer Optima 7300DV instrument. Biochar physiochemical property values are presented in Table 1.

2.2. Experimental design

The study was carried out in the facilities of the Amazonian Experimental Center for Production and Research (CEIPA; formerly CIPCA) (Fig. S1). CEIPA is located in the border area of Napo and Pastaza provinces in Amazonian Ecuador. The local forest formation is an Amazonian evergreen forest, with thermotropic pluvial humid bioclimatic conditions, with a local topography dominated by medium to high ridges and rounded hills (MAE, 2013). The precipitation can exceed 4000 mm a year, with April as the rainiest month and September as the

Table 1
Physiochemical properties of biochar produced from *Piptocoma discolor* feedstock used in experimental trials.

Biochar	pH	EC (μS.cm^{-1})	Max Temp (°C)	Moisture content (%)	Carbon content (%)	Ca (mg·L^{-1})	Mg (mg·L^{-1})	K (mg·L^{-1})	P (mg·L^{-1})
B1 (kiln)	10.17	1579	~550	54	83.4	0.146	0.189	0.439	21.25
B2 (mound)	8.8	191	–	48	73.3	0.384	0.100	1.449	79.19

driest (INAMHI, 2019).

Nontimber forest product (NTFP) plantations were established as a potential green economy alternative for Amazonian populations (Ríos Guayasamín, 2013). *Myroxylon balsamum* (L.) Harms (Family: Fabaceae) produces exudates that are used in the cosmetic and perfume industries (Herrero-Jáuregui et al., 2013), with potential antimicrobial and insecticide properties (Riesmeier et al., 2021). *Ocotea quixos* (Lam.) Kosterm. (Family: Lauraceae) also constitutes a species whose leaves have essential oil content that is locally used in soap and cosmetics (Noriega, 2016), with potential insecticide uses (Arteaga-Crespo et al., 2021). Four hundred fifty trees were originally planted in 2014, from which 288 were inside the 72 plots constituting the biochar experiment. The sites under study were weeded of understory vegetation every three to six months; however, natural regrowth of secondary forest trees was allowed for shading purposes, such that the NTFP species were rapidly over-topped and constituted a small part of total forest biomass.

In 2014, two sites with a Latin square design of three replicates and three treatments were established in a poor alluvial (sandy) soil (−1.242954, −77.899227) and a colluvial soil (−1.241924, −77.891903) with higher nutrient conditions. Three NTFP treatments were established: monocultures of *Myroxylon balsamum* (L.) Harms (Family: Fabaceae) (T1), *Ocotea quixos* (Lam.) Kosterm. (Family: Lauraceae) (T2), and a mixture of 50% *O. quixos* and 50% *M. balsamum* (T3), planted at 5 m × 5 m spacing with 25 trees in an area of 625 m^2 (5 × 5 arrays of trees); the total research area was 1.125 ha with 18 plots at each site (Ríos Guayasamín, 2013). Three years later, a factorial design (3 biochar treatments × 3 NTFP treatments at each site) was established within the four-year-old plantations. By this time a mixture of native pioneer trees had established within the NTFP treatments. Each site had nine amended plots of kiln-made slow pyrolysis biochar (B1), nine traditional mound-made biochar plots (B2), and eighteen control plots where no biochar addition was made (C). Each plot was 10 m × 10 m, with 36 plots per site and a total of 72 experimental units (Fig. S2). Biochars were added at 10 t ha^{-1} to plots corresponding to the B1 and B2 treatments. Biochar was uniformly spread on top of the mineral soil.

2.3. Soil measurements

The soils of the study were characterized as Inceptisols (Maldonado, 2006). They are in the Sub-Andean Amazonian soil region, derived from quaternary sediments of Andean origin, derived from material originating in the mountainous and sub-mountainous reliefs that range from 500 to 1000 m.a.s.l. in the Napo Mountain range (Moreno et al., 2018). The soil close to the river (alluvial) is part of the alluvial plains near the foothills, which are developed from sand and coarse sediments, having low fertility with higher water tables, identified as fluvial landscapes that may be seasonally flooded. The inland soil (colluvial) is developed from geologically recent volcanic ash deposition that has been weathered by high precipitation and consists of a mixture of ash and clayey colluviums over limestone of sedimentary origin (Sánchez et al., 2018).

Soil samples from the uppermost 5 cm were taken in each of the 72 plots annually, right after biochar addition for two consecutive years (year 0, year 1, year 2), and pH, electrical conductivity (EC), bulk density (BD), phosphorus (P), calcium (Ca), magnesium (Mg), potassium (K), aluminum (Al), and organic matter (OM) were analyzed at the Soil Laboratory of UEA. In the second year, each plot was also sampled at various soil depths (0–5 cm, 5–10 cm, and 10–30 cm) and the soil parameters indicated above were also quantified for each depth. BD was obtained after drying a known soil volume to constant weight in a drying oven at 105 °C (Blake and Hartge, 1986). pH and electrical conductivity were measured using an Orion Star A215 multiparameter meter (Rhoades, 1982). Available quantities of Ca, Mg, K, and P were determined following modified Olsen extraction (Díaz-Romeu and Hunter, 1978; Olsen and Sommers, 1982). Ca, K, and Mg were measured by atomic absorption using a Perkin Elmer AA 800 instrument, and P using a Thermo GENESYS™ 10UV UV–Vis Spectrophotometer. Exchangeable Al was determined by basic titration (Barnhisel and Bertsch, 1982). Finally, OM was obtained by loss on ignition as described by Eyherabide et al. (2014). In the last year of the study total nitrogen (TN) Ammonia (NH_4^+) and Nitrate (NO_3^-) availability were also evaluated using ion exchange resins (plant Root Simulators (PRS) probes: Hangs et al., 2002), with a 12-day burial length.

The Soil Evaluation Factor (SEF), developed for tropical soils, was used as an integrated measure of soil fertility. The SEF index was developed to evaluate soil fertility patterns in tropical forest succession; values lower than 5 indicate poor soil fertility (Lu et al., 2002). SEF was calculated as:

$$SEF = [Ca + Mg + K - \log(1 + Al)] \times OM + 5 \tag{1}$$

where Ca, Mg, K, and Al are concentrations expressed in Meq 100 g^{-1}, and OM is organic matter in percent (Lu et al., 2002).

2.4. Tree measurements

All naturally recruiting secondary forest trees, within the 72 10-m × 10-m experimental units, were measured for three consecutive years starting one year after biochar addition (year 1, year 2, year 3). The diameter at breast height (DBH) at 1.3 m of trees >5 cm DBH was measured in all tree species of the secondary forest regrowth. In the alluvial soil (Alu) 167 trees were initially measured, while 130 of them were measured in the colluvial soil (Col), but only trees that lived during all three years were considered for statistical analysis (Alu = 150, Col = 120). Experts at the ECUAMZ herbarium of UEA identified trees to the species level. For *Cecropia* species, local manuals and identification keys were also used (Barriga et al., 2004; Berg, 2002). Measurements of tree height and survivorship of planted NTFP trees were made in 2019; these trees made up a small part (<2%) of total biomass and were not considered in stand biomass calculations.

Aboveground biomass (AGB) growth was estimated using a pantropical allometric equation (Chave et al., 2014, equation 7), for which the climatic conditions of the closest climatic station (Tena) were considered, following the average parameters presented in the meteorological bulletins available for 2006 to 2015 (INAMHI, 2019). Wood density and biomass estimations were made using the BIOMASS R package (Réjou-Méchain et al., 2017).

2.5. Statistical analysis

An analysis of deviance on each of the nine soil parameters measured was performed using generalized linear models (GLMs) to evaluate soil parameters as a function of soil type (site), year, non-timber forest planting treatment, and biochar treatment; the effect of soil sampling depth was also considered in the final year. Linear mixed-effect models (LMMs) were then performed for each site under study to evaluate the responses of soil parameters to NTFP treatments, biochar addition, and the interaction of these factors across all three years, considering year as a random variable. GLMs were performed within each site for each depth to examine the variation of specific elements responding to biochar addition, NTFP planting treatment, and their interaction. Linear mixed models were also used to assess the effects of biochar addition, NTFP treatment, and their interaction on the variation of the log-transformed AGB growth at the stand level and for individual species (where possible considering sample size) using year as a random variable. Interaction terms were dropped from models if not statistically significant. The lmer4 package (Bates et al., 2015), in R studio (R core Team, 2013) was used for all statistical analyses. Post-hoc Tukey pairwise comparisons were performed for all values that showed statistically significant results ($p < 0.05$), but only parameters that present mean differences with both the *t*-test and z-test were considered when interactions were not significant, using the multcomp (Hothorn et al., 2008), and emmeans (Lenth, 2023) packages. The ggpubr (Kassambara, 2020) and ggplot2 (Wickham, 2016) packages were used to make all figures.

3. Results

3.1. Soil changes after biochar addition

All soil parameters showed pronounced and highly significant differences between sites; hence the two sites were analyzed separately (Fig. S3). In the alluvial site, EC ($F_{(4,97)} = 3.22$, $p < 0.05$) and OM ($F_{(2,97)} = 4.86$, $p < 0.05$) showed significant interactive effects of biochar and NTFP treatments (Fig. 1; corresponding ANOVA tables in Table S1). When compared with control plots, on average, EC increased by 30%, and OM by 16% over the two years of study in plots planted with *M. balsamum* that were amended with B2. The pattern differed in plots planted with *O. quixos*, where OM increased by 8% on soils amended with B1, but decreased by 12.5% in plots amended with B2, with a similar pattern for EC. Available Al was lower on plots amended with B2, on average, and pH was marginally higher in the tree mixture NTFP treatment. In the colluvial soil, K showed a significant interactive effect of biochar and NTFP treatments ($F_{(4,\ 97)} = 3.64$, $p < 0.05$). K had an average increase of 58% in plots planted with *O. quixos* that were amended with B1, compared to a 29% increase in plots planted with *M. balsamum,* and amended with B2. The time effect was highly significant for all parameters, with the exception of organic matter.

All soil parameters showed significant variation with sampling depth and clear distinctions between the alluvial and colluvial soils (Fig. S4 corresponding ANOVA tables in Table S2). GLMs, in this case, were based on Gamma residual models due to strongly right-skewed residual distributions. There was a significant biochar x NTFP interaction in the alluvial soil for OM content at the uppermost 5 cm ($F_{(4,27)} = 2.32$, $p < 0.05$), with OM 18% higher in plots with *O. quixos* amended with B1, but declining by 26% in plots with B2 addition when compared with controls (Fig. 2c; corresponding ANOVA tables in Table S2). *M. balsamum* and mixed plantations had a higher OM content when amended with B2, with a lower increase in plots amended with B1 (Fig. 2c). Ammonium-N, at 5–15 cm depth, was the only soil parameter that showed significant effects: plots amended with B1 (Fig. 2g) were on average 208% higher than the control ($F_{(2,27)} = 4.15$, $p < 0.05$). NTFP treatments also influenced soil properties on the alluvial soil. Nitrate-N from 5 to 15 cm, EC from 0 to 5 cm and from 10 to 30 cm, and K from 10 to 30 cm had higher values in plots planted with *O. quixos* compared to mixed plantations; however, available Ca was higher in the latter (Fig. 2). The colluvial soil did not show any increase in OM; nonetheless, nitrate-N showed a significant interactive effect ($F_{(4,27)} = 3.01$, $p < 0.05$), increasing in the *O. quixos* plantations amended with B1 (83.7%) and B2 (43.6%). The opposite trend was seen in plots with the tree mixture (Fig. 2f). Plots planted with *M. balsamum* had a clearer trend, with 191% more nitrate-N in plots amended with B2 than in control or B1 addition plots. At both sites, the ratio of NO_3^-:NH_4^+ showed significant variation in NTFP, but no mean difference was detected in post-hoc tests (Fig. S5).

The SEF value (equation (1)), which gives an integrated picture of soil fertility, varied from 22 (±1.11-SE) in the alluvial site to 71 (±5.56-SE) in the colluvial site (Fig. S6, and Fig. S7). The alluvial soil presented higher Al content with respect to the other cations (Ca, K, Mg, lower Ca:Al ratio) used in the SEF calculations. SEF showed increasing values over time (Fig. 3, corresponding ANOVA tables in Table S3) and decreasing values with depth (Fig. 4, corresponding ANOVA tables in Table S4). The alluvial site showed a steady increase in SEF over time. An analysis of repeated measures with time as a random factor and biochar and NTFP treatments as fixed factors (LMM analysis) showed a marginal main effect of biochar treatments ($F_{(2,101)} = 2.4$, $p = 0.1$) for the alluvial site. SEF values showed small increases from year 1–2 in the colluvial site when compared with year 0, and a marginal effect of NTFP treatments ($F_{(2,101)} = 2.91$, $p = 0.06$), with time showing significant variation in both soil types. For the final year data, GLM analysis showed a marginal increase in SEF on the alluvial soil in response to biochar addition in the top 5 cm soil layer ($F_{(2,31)} = 2.9$, $p = 0.06$). The SEF values for the colluvial soil showed a significant effect of NTFP treatments at the top 5 cm ($F_{(2,27)} = 4.35$, $p < 0.05$), and at 10–30 cm depth ($F_{(2,31)} = 4.35$, $p < 0.05$), but with no distinguishable mean difference in post-hoc tests, and no significant effects of biochar treatments.

3.2. Tree responses to biochar addition

There were 123 individual NTFP trees within treated plots in the alluvial site (mean height of 1.75 m ± 1.07-SE) and 131 (mean height of 3.17 m ± 1.95-SE) in the colluvial site (Fig. 5; corresponding ANOVA tables in Table S5). The height of NTFP trees was significantly different between sites, with no significant biochar effect. GLMs based on Gamma regression between biochar treatments and NTFP showed no significant interactions. Biochar addition showed a marginal effect on NTFP tree height ($F_{(2,118)} = 2.87$, $p = 0.06$) in the alluvial soil, while a significant effect of NTFP treatments ($F_{(2,126)} = 14.76$, $p < 0.05$) was seen in the colluvial soil, with the *M. balsamum* monoculture 85% and 45% higher than *O. quixos* and the tree mixture respectively.

There were 270 natural regrowth secondary forests trees followed through three years, including 20 species, of which 13 were identified to the species level, and 5 to the genus level. The most abundant genus was *Cecropia*, with 6 species represented by 89 individuals. The most abundant species was *Piptocoma discolor* (70 individuals), followed by *Jacaranda copaia* (60), *Cecropia ficifolia* (29), *Cecropia angustifolia* (25), *Cecropia marginalis* (19), *Vismia baccifera* (16) and *Cecropia sciadophylla* (11); other species had less than 10 individuals (Table S6). The two sites differed in tree dominance, with *Piptocoma discolor* the most abundant on the alluvial soil, and *Cecropia* spp. dominant on the colluvial soil.

Estimated AGB was significantly different at the stand level between the sites (Fig. 6 corresponding ANOVA tables in Table S7). LMMs indicated a significant biochar effect on AGB at the stand level in the alluvial soil site ($F_{(2,439)} = 6.27$, $p < 0.05$), with B1 and B2 treatments showing 29% and 23% increases in AGB, respectively, compared to control plots (Figs. 6c and 7e). Among common species for which species-specific analyses were possible, *Jacaranda copaia* showed, on average, a significant increase in AGB of 39.7% in B2 compared to control plots (Fig. 7a), while B1 was only 7.7% higher ($F_{(2,145)} = 3.87$, $p < 0.05$). No significant effects were found for *P. discolor* AGB (Fig. 7c), but a marginal biochar x NTFP type interaction term was present ($F_{(4,169)} = 2.29$, $p = 0.06$). On the colluvial soil, the NTFP treatments had a significant influence on AGB, but not the biochar treatments. On average, the AGB of the cover trees had an increase of 33.7% in plots planted with *M. balsamum* (Fig. 7d) compared to plots with *O. quixos*, or the tree mixture planting ($F_{(2,349)} = 5.64$, $p < 0.05$). *Cecropia* trees (all species pooled) showed a significant interaction effect of biochar and NTFP treatments ($F_{(2,229)} = 3$, $p < 0.05$) with a 26% AGB increase in plots planted with *O. quixos* amended with B1. *Cecropia* spp. showed higher growth in monocultures of *O. quixos and M. balsamum* plantations than in the tree mixture (Fig. 7b), with about 33% higher AGB found in the monoculture plots ($F_{(2,229)} = 4.22$, $p < 0.05$). The time effect of repeated measures was

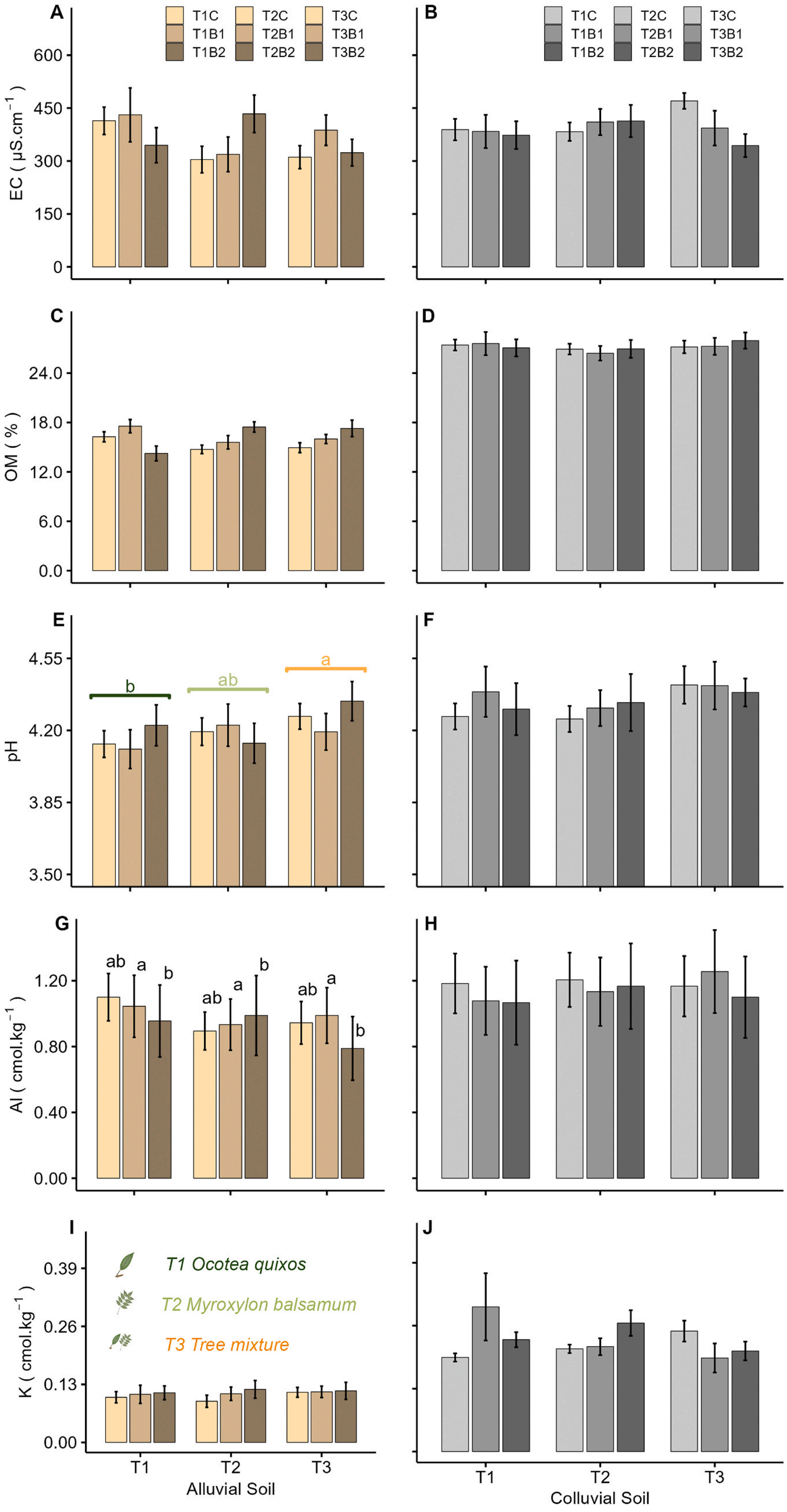

Fig. 1. Soil parameter responses to biochar addition from the uppermost 5 cm in soils amended with kiln (B1) or mound (B2) biochars in non-timber forest plantations of *Ocotea quixos* (T1), *Myroxylon balsamum* (T2) and a mixture of those trees (T3) in the Ecuadorian Amazon. Letters indicate significant differences in treatment means (±SE) at $p < 0.05$ (by Tukey HSD comparisons).

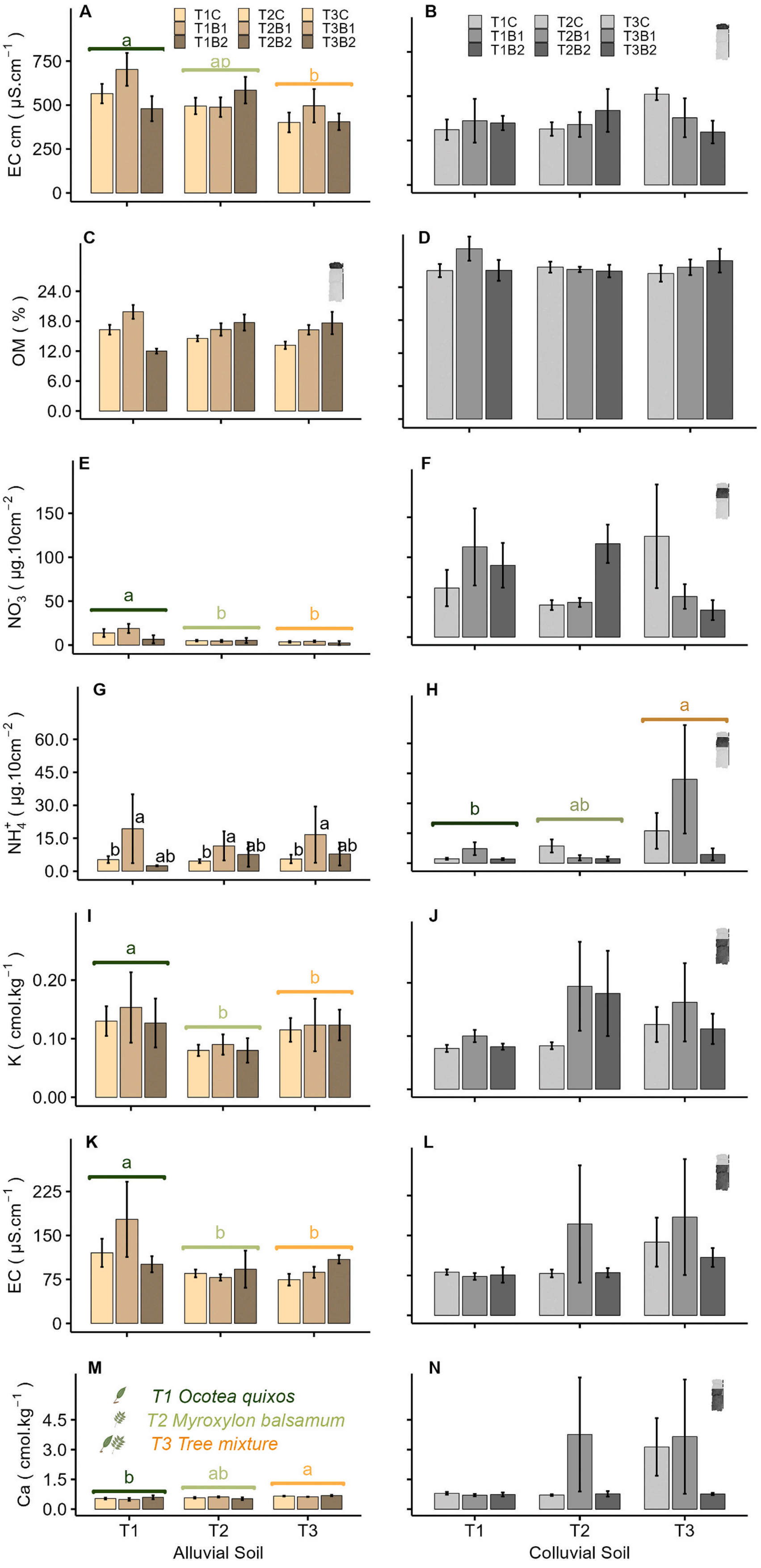

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Fig. 2. Second-year soil parameter responses to the addition of biochar in the 0–30 cm soil layer, amended with either kiln (B1) or mound (B2) biochars on non-timber forest plantations of *Ocotea quixos* (T1), *Myroxylon balsamum* (T2) and a mixture of these species (T3) in the Ecuadorian Amazon. Letters indicate significant differences in treatment means (±SE) at $p < 0.05$ (by Tukey HSD comparisons). NO_3^- and NH_4^+ nutrient concentrations were measured per 10 cm^2 of sampler membrane area during 12 days of burial period.

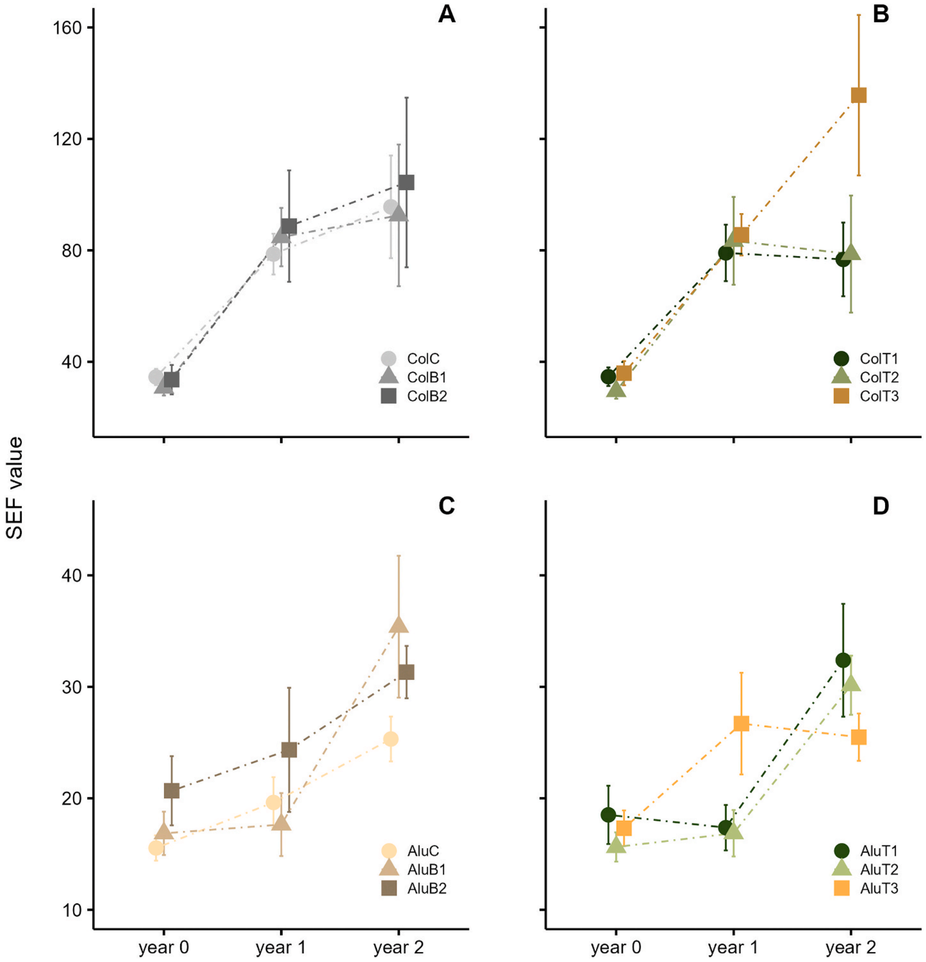

Fig. 3. Soil Evaluation factor (SEF) values in Colluvial (A,B) and Alluvial (C,D) soils from the uppermost 5 cm in soils amended with kiln (B1) and mound (B2) biochars in non-timber forest plantations of *Ocotea quixos* (T1), *Myroxylon balsamum* (T2) and a mixture of those trees (T3) in the Ecuadorian Amazon. Symbols and lines represent treatment means (±SE).

highly significant for all parameters.

The mean effect size metric for tree biomass response to biochar additions, pooled across both soil types and biochar types was 22% (157 ± 14.8 kg·tree^{-1}, $p = 0.06$) in year 1, 20% (200 ± 17.9 kg·tree^{-1}, $p = 0.07$) in year 2, and 17% (236 ± 21.9 kg·tree^{-1}, $p = 0.1$) in year 3 higher than control. The mean effect size pooled across the three years was 19% (198 ± 10.7 kg·tree^{-1}, $p < 0.05$) higher than unamended plots.

4. Discussion

4.1. Soil responses

An understanding of baseline soil nutrient status is important in predicting potential vegetation responses to biochar additions. Based on SEF values (Lu et al., 2002), the soils under study have a higher nutrient status than those found at lower altitudes in the Amazon, consistent with the generally higher nutrient status of Inceptisols from the northwestern Amazon, albeit with great variation depending on pedogenesis, topography, and vegetation (Quesada et al., 2010; Sánchez et al., 2018). The studied soils are comparable to the average values of Alfisols in Brazil (with higher values in the colluvial site) but with superior values to Ultisols and Oxisols (compared to values presented by Lu et al., 2002). The higher SEF value in the colluvial soil from 10 to 30 cm may indicate lower nutrient leaching (Lu et al., 2002) and a higher influence of the above -and below-ground biomass in the soil nutrient cycle of the forest (Nagy et al., 2017; Zieger et al., 2018). This likely corresponds to higher microbial abundance and diversity as well (Soong et al., 2020). The alluvial site shows an increasing SEF value over time, but its rapid reduction with depth, coupled with a high Al content, indicates its low soil development (Quesada et al., 2010), and P availability (Nagy et al., 2017). These conditions are expected to produce slower nutrient cycling and a less developed microbial community (Soong et al., 2020).

The poorer alluvial soil was hypothesized to show more pronounced effects of biochar additions. Consistent with this prediction, we observed increased OM and NH_4^+ in response to biochar additions on the alluvial soil, but not the colluvial. Moreover, there was a reduction of Al through time in response to biochar addition that largely accounts for the increasing SEF values in biochar-amended alluvial soils (Fig. 4c). More than two-thirds of the Amazonian soils have a dominant Al fraction (Quesada et al., 2010); based on our results, biochar application may generally be useful in soils that have this condition. Liming effects are also expected to increase the pool of available cations (Qian and Chen,

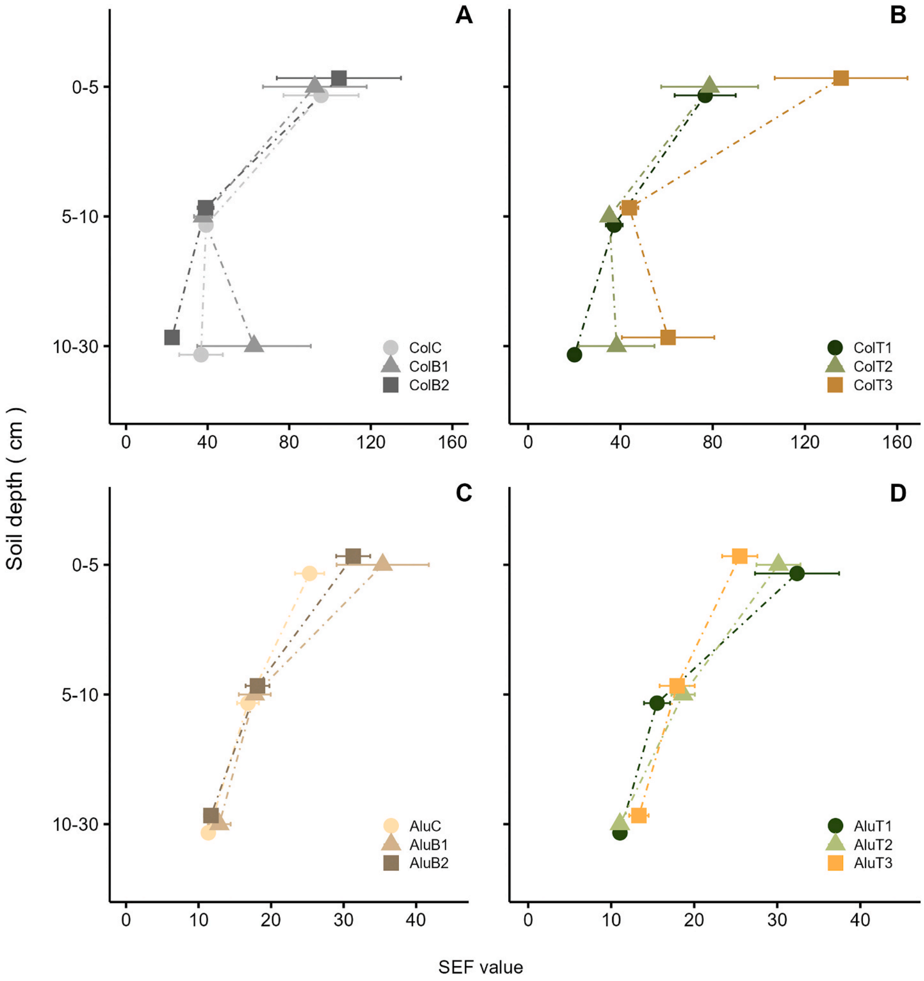

Fig. 4. Soil Evaluation factor (SEF) variation from 0 to 30 cm in the second year of biochar addition in soils amended with kiln (B1) and mound (B2) biochars on non-timber forest plantations of *Ocotea quixos* (T1), *Myroxylon balsamum* (T2) and a mixture of those trees (T3) in the Ecuadorian Amazon. Symbols and lines represent treatment means (±SE).

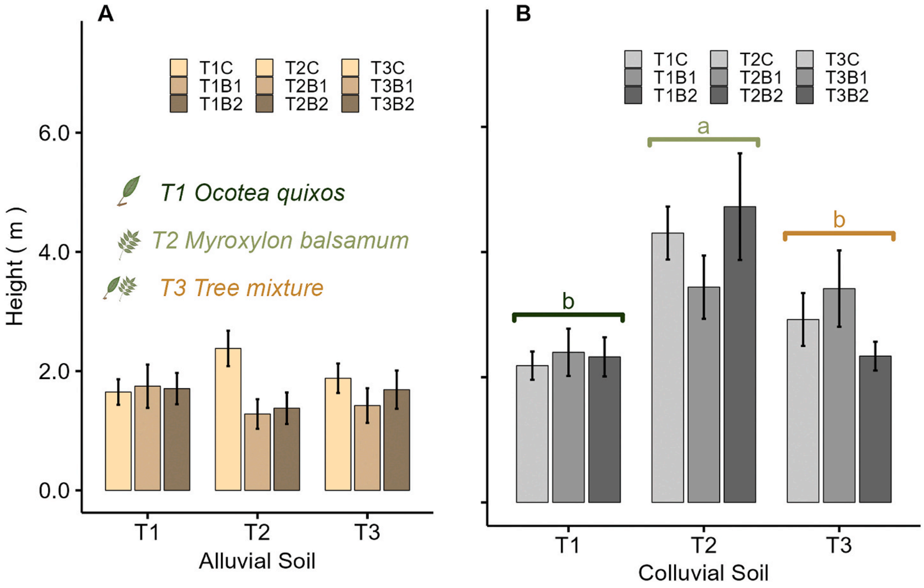

Fig. 5. Height response of non-timber forest product plantations of *Ocotea quixos* (T1), *Myroxylon balsamum* (T2), and a mixture of those trees (T3), in which kiln (B1) and mound (B2) biochars were added after three years of study on Alluvial, and colluvial soils in the Amazon rainforest of Ecuador. Letters indicate significant differences in treatment means (±SE) at $p < 0.05$ (by Tukey HSD comparisons).

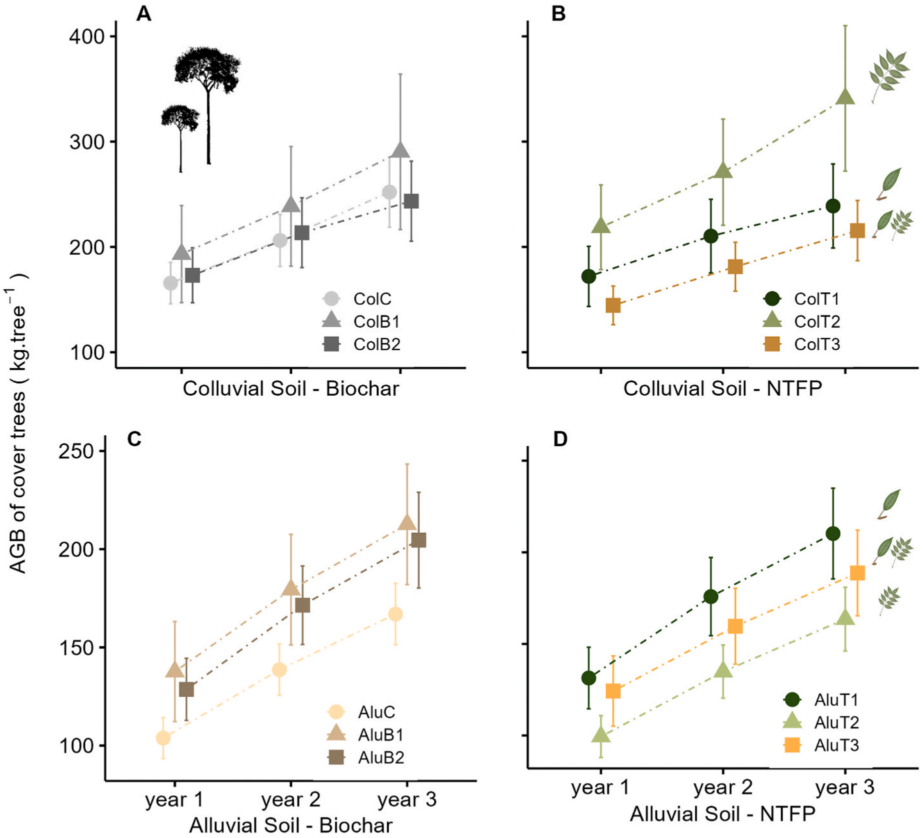

Fig. 6. Above-ground biomass responses of cover trees protecting non-timber forest plantations of *Ocotea quixos* (T1), *Myroxylon balsamum* (T2), and a mixture of those trees (T3), in which kiln (B1) and mound (B2) biochars were added after three years of study on alluvial (Alu), and colluvial (Col) soil types in the Amazon rainforest of Ecuador. Symbols indicate general means (±SE).

2014; Shetty et al., 2021); however, significant biochar effects on soil pH were not detected. This is consistent with some prior field trials on degraded tropical soils (e.g., Raboin et al., 2016), though other studies have found substantial liming effects (e.g., Karim et al., 2020). In many cases, higher dosages would likely be required to overcome soil buffering capacity (Aller, 2016; Basak et al., 2022; Jeffery et al., 2017; Joseph et al., 2021). The colluvial site, an Inceptisol with Ca as a dominant cation (Quesada et al., 2011), was expected to show less response to biochar additions. The colluvial soil did not show statistical changes in OM or NH_4^+, but increases in K were seen, consistent with the large amount of K directly provided by biochar (e.g., Karim et al., 2020). The site effect in the soils under study was large, confirming similar findings in soils with contrasting fertility levels in southern Amazonian Ecuador (Gonzalez Sarango et al., 2021, 2022).

Theoretical models have suggested that the early stages of tropical forest regrowth (up to 40 years) are limited mainly by N (Nagy et al., 2017), especially on young soils with higher sand content (Nardoto et al., 2014). Biochar generally does not directly provide available N and can act to immobilize NH_4^+ in the short term (Clough et al., 2013). However, biochar can enhance NH_4^+ retention and N-fixation (Yin et al., 2021). Here, consistent with our hypothesis, we detected increased NH_4^+ availability in plots amended with biochar in plots amended with kiln biochar at the alluvial site (Fig. 3g). This contrasts with null effects found in Ultisols in the region (Gonzalez Sarango et al., 2022), which may be related to very low Ca:Al ratios present in this soil type (Quesada et al., 2010). In the alluvial soil, plant-root-simulator measurements of NH_4^+ flux were, on average, higher than those for NO_3^-, consistent with prior studies in early successional stages elsewhere in Amazonia (Figueiredo et al., 2019). Nitrate-to-ammonium (NO_3^-:NH_4^+) ratio concentrations were lower, suggesting a stronger influence of biochar in N retention. In the colluvial site, NO_3^-:NH_4^+ were much higher; in this case, NO_3^- increments are associated with traditional mound biochar applications in *M. balsamum* treatments. Legumes may increase NO_3^- concentrations and change the microbial community in forest plantations (Rachid et al., 2013), indicating the influence of the NTFP on the effects of the more nutritious soil.

4.2. *Tree responses*

Biochar additions resulted in increased growth of secondary forest trees on the alluvial site, with the highest tree growth seen in the open kiln biochar (T1); the colluvial site also showed the highest AGB with open kiln biochar, but this pattern did not reach significance (Fig. 6). Other recent field trials in Amazonia have obtained variable results. Biochar alone improved tree survivorship on Amazonian gold mine tailings, while tree growth was enhanced with a biochar plus fertilizer combination (Román-Dañobeytia et al., 2021). In nursery plantations using a similar mixed tailings substrate, increased seedling performance was enhanced at low but not high biochar dosages (Lefebvre et al., 2019). Other Amazonian studies have found null results after biochar addition (de Farias et al., 2016), instead finding significant variance among soil conditions (Gonzalez Sarango et al., 2021). In contrast, substantial positive effects were found in a 30-month field trial of biochar effects on native trees on the island of Mauritius, though in this case, the main mechanism was thought to be biochar sorption of allelochemicals (Sujeeun and Thomas, 2022). The pooled mean biomass response to biochar in the present study was an increase of 19%, somewhat lower than that reported for a prior meta-analysis of tree responses heavily weighted toward pot trials (+41%: Thomas and Gale, 2015). However, as hypothesized, species varied substantially in their responses (Fig. 7). For example, the AGB of the second most dominant tree species in the alluvial site, *Jacaranda copaia*, was ~40% higher in

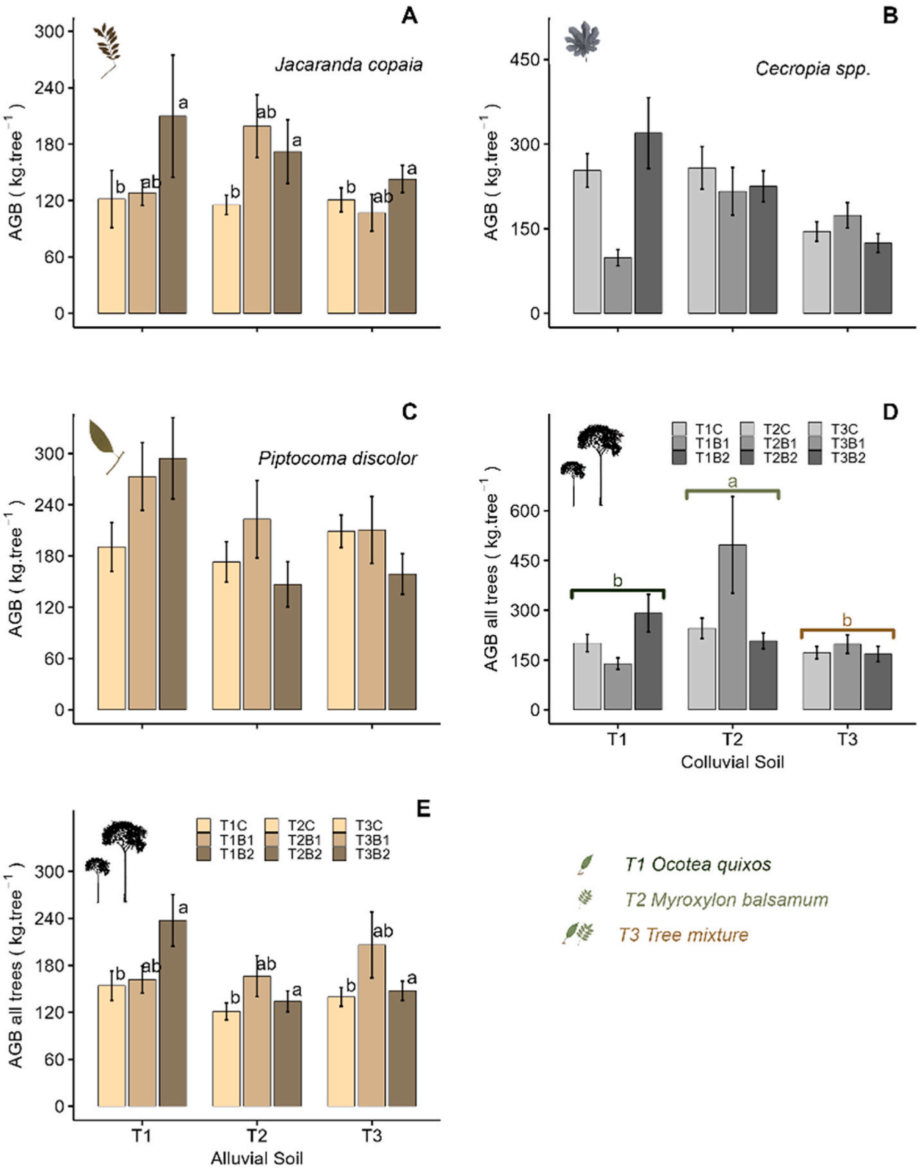

Fig. 7. Aboveground biomass (AGB) variation in soils amended with kiln (B1) and mound (B2) biochars on non-timber forest plantations of *Ocotea quixos* (T1), *Myroxylon balsamum* (T2) and a mixture of those trees (T3) in the Ecuadorian amazon rainforest. Letters indicate significant differences in treatment means (±SE) at $p < 0.05$ (by Tukey HSD comparisons).

plots amended with one biochar types investigated over the other.

Secondary forest regrowth also showed large responses to NTFP treatments. In the colluvial site, the effect of biochar on AGB was overridden by the effect of *Myroxylon balsamum* (Fig. 7b), which was significantly higher than the other NTFP treatments. The combination of biochar and N-fixing species is a potentially important strategy to increase N availability and enhance productivity (Thomas et al., 2019). Legumes are generally less abundant in wet forests (Gei et al., 2018); therefore, their addition may be more important in wet forest systems to enhance N during early succession. In addition, species-specific responses to the combination of biochar and NTFP treatments were also important, as in the case of the dominant *Cecropia* spp., which had a higher AGB in the plots planted with *M. balsamum* and amended with traditional mound biochar (B2). Pyrolysis process effects also varied among tree species. Although the open kiln biochar generally produced larger growth responses, *Jacaranda copaia* showed significant growth increases only with the traditional mound biochar.

Non-timber forest products that may improve people's livelihood in the Amazon region are heavily dependent on the type of forest management (Hajjar et al., 2011), where specific legislation (Silva et al., 2020) and multipurpose species (Herrero-Jáuregui et al., 2013) may enhance the options for farmers living in remote areas and nutrient-poor soils conditions. Our results show NTFP plantations of *M. balsamum* and *O. quixos* may enhance forest services with improved soil conditions after biochar additions to improve local livelihoods. Biochar has been used in agroforestry systems elsewhere in the amazon, improving crops and fruit trees productivity that supplements subsistence means where soil productivity has decreased (Miltner and Coomes, 2015). A comparable phenomenon could also be seen in tea plantations of tropical Asia, with stronger carbon markets (Lin et al., 2023).

5. Conclusions

Tropical soils vary greatly in their physical properties and nutrient status. Here, the poorer alluvial site, with lower SEF values than a colluvial site, showed stronger responses to biochar addition both in terms of soil parameters and forest growth. SEF values may be of broad use as a predictor of potential forest responses to biochar additions in tropical soils. However, due to cost and logistical constraints, biochar additions seem unlikely to find general use in forest restoration in Amazonia other than in the context of crops that co-exist with regenerating forests, such as NTFPs. The large variation in responses among species, and the interactive effects of NTFP crop species, soils, and biochar types on secondary forest growth suggest both considerable scope for optimization of systems, and a substantial need for additional experimentation.

Author contributions

P.R.G.: Conceptualization, Writing – original draft, research permit responsible, field data collection, S.T.: funding, data collection, Conceptualization, Writing – review & editing, Supervision, S.S.: funding, Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2023.119068.

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