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Research Article

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Posted Date: June 8th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9794144/v1

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Abstract

Substituting synthetic agrochemicals with biologically based inputs is increasingly promoted to improve the sustainability of tropical cropping systems, yet empirical evidence from commercial cacao plantations remains limited. This study evaluated the agronomic and economic consequences of partially replacing synthetic foliar fertilizers and fungicides with an on-farm fermented biol in a CCN-51 cacao monoculture on the Ecuadorian coast. Methods: Six management regimes were evaluated over two consecutive production cycles. Pod production, dry bean yield, disease incidence (Frosty Pod Rot and Witches' Broom), climatic variables, and production costs were analyzed using mixed-effects models and partial budget analysis. Despite substantial differences in foliar input regimes, cumulative pod production and dry bean yield remained statistically comparable among treatments, indicating that partial substitution did not compromise productivity when soil fertilization was maintained. Disease incidence was not significantly affected by treatment but was associated with rainfall patterns occurring one to two months prior to symptom expression, confirming the dominant role of climate. Economic performance varied markedly with application frequency: biol applications every 15 days generated negative returns (net margin −884 USD ha^{-1}; benefit–cost ratio 0.9), whereas monthly applications (50% biol) restored profitability comparable to conventional management (BCR 2.2) with production costs of approximately 1.10 USD kg^{-1}. Strategic monthly substitution of foliar synthetic inputs with a 50% biol offers a viable entry point for reducing chemical dependency in intensive cacao systems, but only when embedded in an integrated management framework that includes rational application schedules, climate-informed sanitation, and labor efficiency.

Keywords: *Theobroma cacao*, biofertilizers, economic viability, sustainable intensification, integrated nutrient management.

1. INTRODUCTION

Global cacao (*Theobroma cacao* L.) production is at a crossroads, balancing growing industrial demand with increasing pressures for environmental sustainability and resilient crop management. Although West Africa dominates global supply, Latin America—particularly Ecuador—plays a strategic role in international cocoa markets through both fine-flavor cacao and high-yielding commercial cultivars. Maintaining stable production under increasing climatic variability, disease pressure, and rising input costs has therefore become a central challenge for cacao producers and the global chocolate industry (Läderach et al. 2013; ICCO). Within this context, the CCN-51 clone has emerged as one of the most widely adopted high-yielding cacao cultivars across Latin America, including Ecuador, Peru, Colombia, Brazil, and parts of Central America (Jaimez et al. 2022). Developed in the 1960s by Homero Castro Zurita in Ecuador's Guayas Province, CCN-51 (Colección Castro Naranjal 51) was selected for exceptional productivity, environmental adaptability, and relative tolerance to several endemic diseases. Its rapid expansion over the past three decades has made it a dominant cultivar in commercial plantations in several producing countries. Although research on CCN-51's performance in African and Asian cacao belts remains sparse or largely anecdotal outside the peer-reviewed literature, its increasing recognition among producers and researchers underscores its relevance beyond its country of origin (Xocolat 2026).

CCN-51 combines vigorous vegetative growth with high reproductive capacity, traits that support exceptional productivity when adequate agronomic management is provided. Physiological characteristics such as strong osmotic adjustment and extended pollen viability contribute to yield stability across contrasting edaphoclimatic conditions. However, achieving this productive potential in practice typically requires intensive nutrient and phytosanitary management (energy inputs) (Pérez Neira 2016), as high-yielding genotypes impose substantial metabolic and nutritional demands on production systems (Boza et al. 2014; Benjamin et al. 2025). Recent physiological evidence suggests that high-yielding genotypes like CCN-51 have elevated metabolic demands that can outpace native soil fertility, reinforcing the need for precise input strategies (Benjamin et al. 2025).

Across the Neotropics, the major constraints to sustained cacao yield remain endemic fungal diseases, particularly Frosty Pod Rot (FPR) caused by *Moniliophthora roreri* and Witches' Broom (WB) caused by *M. perniciosa*. These hemibiotrophic pathogens have sophisticated life cycles that manipulate host physiology and can cause severe yield losses in unmanaged or inadequately managed systems (Evans 2016a, b). Historically, control has relied on copper-based fungicides and the gradual introgression of partial disease tolerance into breeding

populations, but breeding for durable resistance remains a long-term challenge. Even relatively tolerant cultivars such as CCN-51 therefore require integrated management combining sanitation, canopy regulation, and chemical or Biological interventions to limit disease impact (Gutiérrez et al. 2016).

Concerns about the long-term environmental impacts and economic costs of synthetic agrochemical inputs have stimulated increasing interest in biologically based alternatives capable of supporting crop nutrition and plant health. Among these approaches, microbial biological control agents (BCAs) and fermented organic biofertilizers such as “Biols” have gained attention because they can suppress pathogens, stimulate plant growth, enhance nutrient availability, and potentially strengthen plant defence responses (Ten Hoopen and Krauss 2016; van Lenteren et al. 2025). These agronomic pressures have stimulated interest in strategies that improve input efficiency or substitute synthetic inputs with biologically based alternatives, representing early stages of agroecological transition (Gliessman 2014). At the production-system level, such transitions are also linked to efforts to improve the energetic and environmental sustainability of cacao supply chains (Pérez-Neira et al. 2020).

Despite growing interest in biological inputs, empirical evidence evaluating their agronomic and economic performance under commercial cacao production conditions remains limited. Most studies have focused on pathogen suppression or small-scale trials rather than whole-system productivity. In particular, few studies have assessed whether biological inputs can partially substitute synthetic agrochemicals in intensive monoculture systems dominated by high-yielding cultivars such as CCN-51. Moreover, integrated analyses combining agronomic performance, disease dynamics, climatic variability, and economic outcomes remain scarce, despite their importance for determining the practical viability of alternative management strategies. Importantly, most previous evaluations of biological inputs in cacao have been conducted under experimental or short-term conditions, leaving limited evidence on their performance in commercial plantations facing endemic disease pressure and real farm management constraints.

The objective of this study was to evaluate the agronomic and economic consequences of partially substituting synthetic foliar fertilizers and fungicides with a standardized on-farm fermented Biol in an intensive CCN-51 cacao monoculture under commercial farm conditions. Specifically, the study examined how different Biol application frequencies affected yield, disease incidence, and production costs across two consecutive production cycles while accounting for climatic variability and phenological sensitivity.

2. MATERIALS AND METHODS

2.1. Study site and experimental conditions

The study was conducted at *Hacienda San Rafael* located in Bucay, Guayas Province, Ecuador (Fig. 1), a commercial cacao plantation previously analyzed in sustainability assessments of Ecuadorian cacao production systems (Pérez Neira 2016), providing an opportunity to interpret the present agronomic results within a broader production-system context. *Hacienda San Rafael* is a full-sun monoculture of grafted CCN-51 cacao (*Theobroma cacao* L.). The plantation represents an intensive cacao production system under tropical lowland conditions, characterized by endemic pressure of Frosty Pod Rot (FPR, *M. roreri*) and Witches' Broom (VWB, *M. perniciosa*). The plantation was established seven years prior to the experiment on sandy loam alluvial soils, with a planting density of 1,086 trees ha^{-1}. The upper soil horizon exhibits variable depth (20–60 cm, locally up to 90 cm), overlying stony alluvial layers typical of the Guayas river basin. These soil conditions are representative of commercial cacao plantations in the region.

Before the initiation of the trial, the field was managed under a high-input conventional system, including intensive synthetic fertilization, technified irrigation, and calendar-based pesticide applications. Nutrient management relied primarily on soil-applied N, P, K, and Mg fertilizers, while pruning was performed twice per year (maintenance pruning mid-year and formative pruning at the end of the year), following standard commercial practices. The experimental area was located within a production block of the San Rafael farm (Fig. 1A). The entire block has a documented yield history typical of intensively managed CCN-51 systems in the region of 531.7 kg ha^{-1} (11.7 qq ha^{-1}) in 2012, 834.6 kg ha^{-1} (18.4 qq ha^{-1}) in 2013, 848.2 kg ha^{-1} (18.7 qq ha^{-1}) in 2014, and 797.0 kg ha^{-1} (17.58 qq ha^{-1}) in 2015, using a conversion factor of 45.36 kg qq^{-1} (Sánchez-Mora et al. 2015). FPR was the most prevalent disease at the site, and routine sanitary harvests were conducted monthly to reduce inoculum pressure. A previous outbreak of *M. perniciosa* affecting floral cushions and vegetative shoots had been recorded several years before the present study, confirming the endemic nature of both diseases at the site.

The experimental site is located in the Guayas River Basin, within the tropical humid zone of coastal Ecuador. The climate is characterized by a stark seasonal contrast: a rainy winter season (December–May) averaging 1,800 mm of precipitation, and a dry summer season (June–November) with less than 100 mm monthly. Mean annual temperature is 25.3°C, with minimal thermal variation. This seasonality defines the agricultural calendar, concentrating both flowering peaks and *Moniliophthora* spp. disease pressure during the wet months.

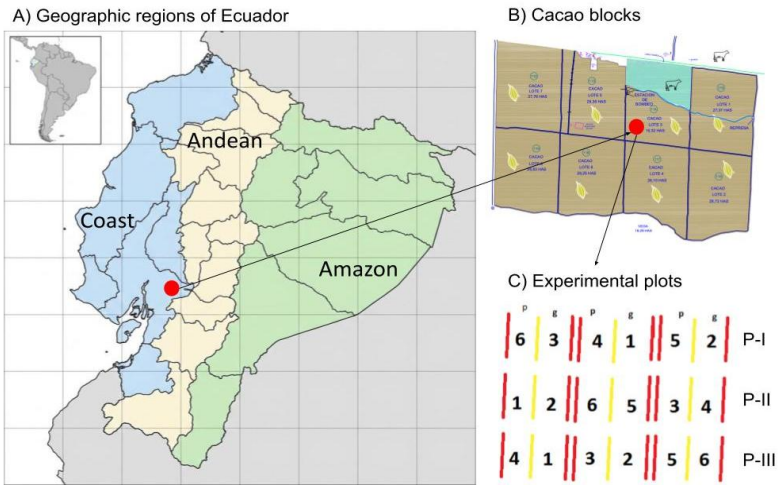


Figure 1. Experimental site and design. (A) Geographic location of the study site in the Coastal Region of Ecuador (2.1835° S, 79.2333° W; 143 m a.s.l.).. (B) View of the experimental block. (C) Diagram of the experimental layout, detailing the distribution of the six treatments (T1–T6) randomized within the three plots (PI–PIII).

2.2. Experimental design and treatments

The experiment was arranged in a randomized complete block design with three blocks used as replicates (Fig. 1B and C). Each block constituted a macro-plot with six treatments, randomly assigned to subplots, resulting in a total of 18 experimental units. Each experimental unit consisted of 10 central trees, representing a net plot area of approximately 92.1 m². In total, 180 trees were monitored over the two-year study period (January 2015 – November 2016). The six treatments are described in detail in Table 1.

Table 1. Description of treatments based on product type, quantity, and number of applications per year. All treatments received identical cultural practices (pruning, weeding).

Treatment-ID	**Description**	**Nº products / Nº applications**			**Concentration / Nº Applications**
		Soil fertilization [1]	**Foliar fertilization [2]**	**Fungicides [3]**	**BIOL [4]**
T1	Conventional. Farm's standard high-input	7 / 14	8 / 22	7 / 8	--
T2	Optimized for fertilization	5 / 4	--	7 / 8	100% / 2
T3	Biol at 100%	7 / 14	--	--	100% / 30
T4	Biol at 100%	7 / 14	--	--	100% / 15
T5	Biol at 50%	7 / 14	--	--	50% / 30
T6	Biol at 50%	7 / 14	--	--	50% / 15

Notes: Detailed descriptions of soil fertilization (¹), foliar fertilization (²), fungicides (³), and Biol application rates (⁴) are provided in the Supplementary Material (Supplementary Methods 1).

2.3. Biol production and characterization

A standardized liquid Biol was used across all Biological treatments. The formulation and production protocol followed a previously developed and validated methodology at ESPOL (León 2018), based on controlled anaerobic fermentation of organic substrates. This protocol has been applied consistently in prior agronomic studies and ensures batch-to-batch reproducibility (Vera-Morales et al. 2020). Biol fermentation was initiated using a mother solution and allowed to mature for four months prior to field application (Fig. 2). The fermentation process was conducted on-farm under ambient tropical conditions. Physicochemical properties of the Biol were monitored monthly as part of quality control. Electrical conductivity (EC) and pH were measured in the mother solution after one month of fermentation and subsequently in two independent Biol batches at one and four months of maturation.

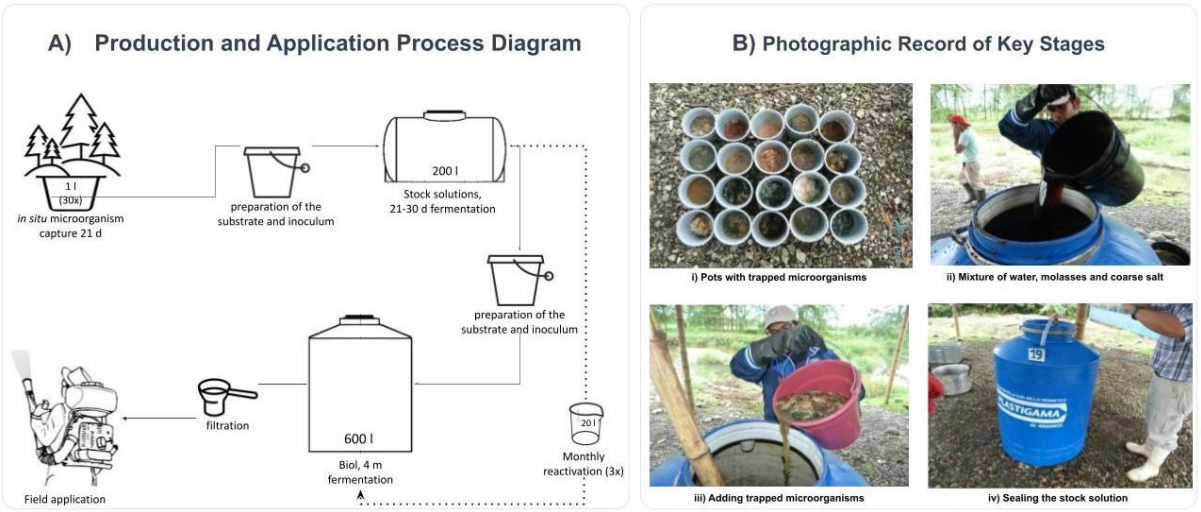


Figure 2. A) Standardized protocol for on-farm Biol production and B) microbial capture and in situ stock solution preparation. The process includes microorganism capture, fermentation in 540-L tanks, filtration, and electrostatic spraying. See Supplementary material for details.

In addition to physicochemical monitoring, microbiological quality of the mature Biol was assessed prior to field application. Analyses included detection of *Salmonella* spp. and *Escherichia coli*, enumeration of total coliforms, and total aerobic microbial counts. None of both pathogens were not detected, and total coliform counts were low (30 CFU mL^{-1}). Total aerobic microbial populations were on the order of 10^5 CFU mL^{-1}, consistent with stabilized fermented organic bio-inputs. These analyses were conducted to verify sanitary quality and to confirm the absence of pathogenic contamination. Comparable Biols produced using the same methodology under similar tropical conditions have previously shown total dissolved solids in the range of 8–9 g L^{-1}, salinity of approximately 10

ppt, and fermentation temperatures of 27–28 °C, providing additional context for the production environment, although these parameters were not measured in the present study (Vera-Morales et al. 2020).

2.4. Crop management and input application

Synthetic fertilizers and fungicides were applied following standard commercial recommendations for intensive CCN-51 production in the region (Table 1). The application of the biological treatment (Biol) was standardized across four alternative experimental variants to evaluate the interaction between concentration and total volume. Applications were performed using calibrated backpack sprayers to ensure uniform coverage. All plots received identical irrigation schedules, sanitary pruning, weed control, and pest management practices, except for the defined input substitution strategies.

2.5. Yield assessment and disease monitoring

Yield and plant health assessments were conducted biweekly and monthly, respectively, over a two-year evaluation period. Yield was quantified on a biweekly basis by recording the number of healthy harvested pods per plant and the corresponding fresh bean weight (g). Fresh bean weight was converted to cumulative dry bean yield per plant using a standard conversion factor of 0.4, commonly applied for CCN-51 cacao under commercial conditions (Sánchez-Mora et al. 2015). Production efficiency was additionally evaluated through the Pod Index, calculated as the number of pods required to produce 1 kg of dry cacao beans.

Plant health was monitored monthly by recording the incidence of FPR, expressed as the number of pods showing visible symptoms, the number of vegetative witches' brooms (VWB) associated with M. perniciosa, and the number of dry cherelle. Dry cherelle were defined as fruits smaller than 8–10 cm in length and were recorded without distinguishing between physiological abortion or disease-related causes, following the criteria proposed by Krauss and Soberanis (2002). Based on these observations, pod potential was calculated as the sum of healthy pods and FPR-affected pods. FPR incidence (%) was computed using the following expression:

FPR incidence (%) = (Number of symptomatic pods / Total potential pods) × 100

All diseased pods, VWB, and dry cherelle recorded during each evaluation were subsequently removed from the plants and covered with leaf litter within the crop row, following standard sanitary management practices aimed at reducing inoculum pressure while maintaining organic matter recycling within the system.

2.6. Statistical analysis of agronomic responses

To evaluate treatment effects on cumulative and temporal production responses, linear mixed-effects models were applied. Accumulated dry bean yield per plant (DW_kg) over the two-year period was analysed using a linear mixed-effects model including *Year*, *Treatment*, *Block*, and their interactions as fixed effects. To account for repeated measurements on individual trees, a random intercept and a random slope for *Year* were specified for each plant (*ID*), with plants nested within blocks. This structure allowed separation of treatment effects from plant-level temporal variability.

The same modelling framework was applied to cumulative response variables, including total number of pods, pod index, number of FPR pods, number of VWB, and number of cherelle. All analyses were conducted in R v. 4.5.1 using the *nlme* package. Model assumptions of residual normality and homoscedasticity were assessed using diagnostic plots (residual histograms and Q–Q plots). When significant main effects or interactions were detected, post-hoc pairwise comparisons among treatments were performed using estimated marginal means (*emmeans* package). To control for multiple testing, *p*-values were adjusted using the False Discovery Rate (FDR) procedure. Statistical significance was determined at $\alpha = 0.05$.

2.7. Analysis of environmental influence on yield and disease

To assess the influence of environmental variability on yield performance and disease expression, climatic and agronomic datasets were jointly analysed using exploratory and inferential statistical approaches. Meteorological data, including air temperature and precipitation, were obtained from a weather station located approximately 1 km from the experimental field. Monthly climatic records were synchronised with production and phytosanitary observations. Given the phenological cycle of *T. cacao*, which spans approximately 140–150 days from anthesis to harvest, time-lagged climatic variables were generated to capture delayed environmental effects on flowering, fruit set, pod development, and disease progression. Climatic predictors were evaluated at time lags ranging from one to five months prior to harvest (t−1 to t−5), allowing identification of critical windows of climatic influence across successive production cycles.

Initial associations between climatic variables and agronomic responses were explored using Spearman's rank correlation matrices. This non-parametric approach was used to detect monotonic relationships and to identify candidate time lags for subsequent modelling, without assuming linearity or normality of the data. The magnitude

and significance of environmental drivers were then formally evaluated using mixed-effects modelling frameworks. Dry bean yield was analysed using Linear Mixed Models (LMM), while disease-related response variables were analysed using Generalized Linear Mixed Models (GLMM). Experimental block and individual plant (nested within block) were treated as random effects in all models to account for repeated measurements over time and spatial heterogeneity within the plantation.

Disease incidence variables, including the number of FPR–affected pods and VWB, were modelled using GLMMs with a Poisson error distribution and a log-link function, appropriate for count data and the presence of zero-inflated observations. Both concurrent climatic variables and lagged precipitation accumulations were included in the models to distinguish immediate environmental effects from delayed responses associated with pathogen incubation and host susceptibility dynamics. For the analysis of dry cherelle incidence (N_Cherelle), representing early fruit abortion, the concurrent number of developing pods per plant (N_Pods) was included as a covariate. This adjustment allowed differentiation between environmentally induced abortion and density-dependent physiological thinning resulting from internal competition among sinks.

All climatic predictors were standardised (z-scores) prior to modelling to facilitate direct comparison of regression coefficients (β) across variables and time lags. The objective of these analyses was not to establish direct climatic causation, but rather to contextualise yield variability and disease expression within prevailing environmental conditions and to identify dominant climatic signals potentially amplifying or buffering agronomic responses under intensive cacao monoculture.

2.8. Economic analysis

A descriptive economic assessment was conducted to evaluate the financial efficiency of partially or totally substituting synthetic agrochemical inputs with Biol under intensive cacao monoculture. The analysis was explicitly framed around the concept of input efficiency, defined here as the capacity of each management strategy to retain yield while reducing the cost per unit of production. The assessment focused exclusively on variable production costs directly affected by the treatments, including the acquisition of soil and foliar fertilizers, fungicides, and on-farm Biol production, as well as the labour required for their application. Fixed costs, such as land tenure, infrastructure depreciation, and administrative overhead, were deliberately excluded to isolate the marginal economic effects attributable to input substitution rather than whole-farm profitability.

Financial performance was evaluated using three complementary indicators:

- Net margin (USD ha^{-1}): calculated as gross income—defined as total annual dry bean yield multiplied by the average farmgate price—minus total variable costs. This indicator reflects the absolute economic return of each strategy per unit area.
- Unit production cost (USD kg^{-1}): calculated as the ratio of total variable costs to total dry bean yield. This metric represents the cost required to produce one kilogram of cacao and serves as a direct proxy for input efficiency, allowing comparison of how effectively each treatment converts inputs into retained yield.
- Benefit–cost ratio (BCR): calculated as gross income divided by total variable costs. A BCR greater than 1.0 indicates economic viability, while differences among treatments reflect relative efficiency rather than scale-dependent profitability.

Financial calculations were based on a weighted average farmgate price of 2.385 USD kg^{-1} for dry CCN-51 cacao beans. This reference price was derived from historical records of the System of Public Agricultural Information (MAGAP-SIPA 2025) for Guayas Province during the 2015–2016 period. The selected value integrates the price volatility observed during the experimental period, when market prices fluctuated between 85 and 123 USD per quintal (qq), thereby providing a realistic and conservative estimate of economic performance under variable market conditions.

3. RESULTS

3.1. Treatment effects on yield and disease variables.

3.1.1. Yield

Dry bean yield. A pronounced and statistically robust Year × Treatment interaction was detected for dry bean yield ($F = 5.33$, $p < 0.0001$), indicating that treatment effects were not consistent across the two production years. In addition, a significant main effect of Year was observed ($F = 18.74$, $p < 0.0001$). Across all treatments, mean dry bean yield declined from 1.32 kg $plant^{-1}$ in 2015 to 1.10 kg $plant^{-1}$ in 2016, corresponding to an average reduction of 0.22 kg $plant^{-1}$. Post-hoc analyses using year-wise simple contrasts with FDR adjustment revealed that this decline was primarily driven by the conventional input regimes (Fig. 4). Both, the Conventional and Conventional–Optimized treatments exhibited a significant reduction in dry bean yield from Year 1 to Year 2 ($p \leq 0.0001$). In contrast, all Biol-based strategies showed a no significant decrease in yield over time (data not shown).

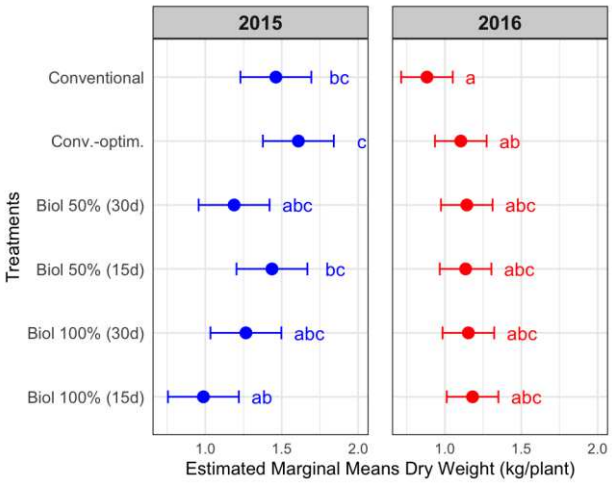


Figure 4. Estimated marginal means and 95% confidence intervals for dry bean yield (kg plant^{-1}) by treatment and year. The overlap of confidence intervals provides a visual approximation of treatment differences within each year. Formal pairwise comparisons were performed using FDR-adjusted contrasts; treatments not sharing a common letter are significantly different ($p < 0.05$).

Analysis of random effects revealed substantial plant-to-plant variability in baseline productivity, reflected by a standard deviation of 0.59 kg plant^{-1} for the random intercept. Moreover, a strong negative correlation ($r = -0.78$) was observed between baseline yield and the temporal change in yield, indicating that plants with higher initial productivity tended to experience greater yield declines over time. Inspection of the relationship between random intercepts and slopes showed no systematic association with either treatment or block (Supplementary Fig. 1), suggesting that this variability reflects intrinsic plant-level differences rather than experimental artefacts.

Pod number. Pod production exhibited patterns closely aligned with those observed for dry bean yield. A significant Year × Treatment interaction was detected ($F = 5.77$, $p = 0.0001$). The Conventional and Conventional–Optimized treatments showed significant reductions in pod number, decreasing by an average of 5.7 ($p = 0.0017$) and 6.6 pods plant^{-1} ($p = 0.0003$), respectively. In contrast, the Biol 100%–15d treatment was the only management strategy to exhibit a significant increase in pod number, with an average gain of 4.6 pods plant^{-1} from Year 1 to Year 2 ($p = 0.0104$). The remaining Biol-based treatments maintained stable pod production across years, with no significant temporal change. Despite the clonal propagation of CCN-51, substantial plant-to-plant variability in baseline pod number was observed, as indicated by a standard deviation of 7.88 pods plant^{-1} for the random intercept.

Pod Index. The Pod Index increased significantly from Year 1 to Year 2 ($F = 40.84$, $p < 0.0001$), indicating a general decline in the efficiency with which pods were converted into dry cacao beans over time. In contrast to

dry bean yield and pod number, no significant Year × Treatment interaction was detected (F = 1.12, p = 0.354), suggesting that this decline in conversion efficiency was consistent across all management strategies. No significant differences in Pod Index were observed among treatments (p = 0.630) or blocks (p = 0.752).

3.1.2. Diseases

Frosty Pod Rot (FPR). The linear mixed-effects model detected no significant main effect of Treatment (F = 0.87, p = 0.502) and no significant Year × Treatment interaction (F = 1.77, p = 0.121) on the number of pods affected by FPR (Fig. 5A). These results indicate that input substitution strategies did not significantly modify disease incidence, and treatment responses remained consistent across the two production cycles. Pairwise comparisons confirmed no significant differences among treatments in either year (FDR-adjusted p > 0.05 for all contrasts). Although numerical variation in FPR counts was observed between years, only the Biol 50%–30d treatment showed a significant temporal increase (from 6.97 to 10.10 pods plant^{-1}; p = 0.037). For all remaining treatments, FPR levels remained statistically stable over time.

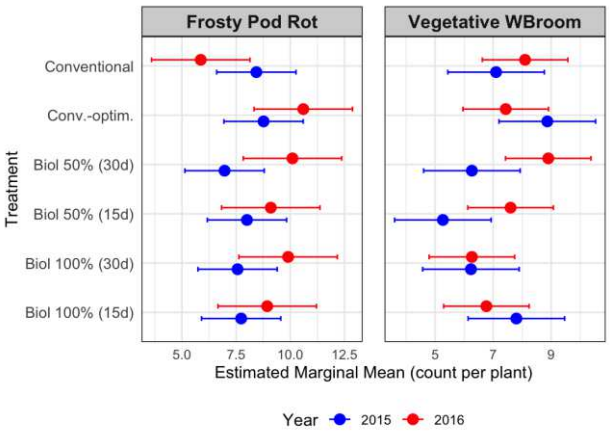


Figure 5. Estimated marginal means of pods with Frosty Pod Rot (*M. roreri*, A) and vegetative Witches' Brooms (*M. perniciosa*, B) by treatment and year. The plot shows point estimates with 95% confidence intervals. Differences between treatments within each year can be assessed by examining the overlap of confidence intervals. Pairwise comparisons were performed within each year using the FDR adjustment for multiple comparisons. No significant differences were observed among treatments in either year for FPR. For WB, treatments were not significantly different except for the comparison between Conventional optim. and B50%-13d in 2015, which showed a significant difference (p=0.0437).

Substantial plant-to-plant variability was observed, reflected in a large random intercept standard deviation (SD = 4.43). Examination of random effects revealed a negative correlation (r = -0.60) between baseline disease levels and temporal change, indicating that plants with higher initial FPR incidence tended to show smaller increases—or greater reductions—over time. This pattern was independent of treatment and block effects, suggesting that

inherent plant-level variability and natural regression dynamics were dominant drivers of disease patterns rather than differences among input strategies. From an input-efficiency perspective, these findings indicate that partial substitution of synthetic inputs with Biol-based regimes did not compromise phytosanitary stability against *M. roreri* under endemic disease pressure during the study period.

Vegetative Witches' Brooms (VWB). Analysis of VWB revealed a significant Year × Treatment interaction ($F = 2.57$, $p = 0.0283$), indicating differential temporal responses among treatments. In Year 1, the Conv.-optim. treatment had a higher number of VWB compared with Biol 50%–15d ($p = 0.0437$), while no significant differences among treatments were detected in Year 2 (Fig. 5B). Simple contrasts within treatments showed a significant increase in VWB counts from Year 1 to Year 2 in two Biol-based regimes (Biol 50%–15d: from 5.27 to 7.60 brooms plant^{-1}, $p = 0.0288$; Biol 50%–30d: from 6.27 to 8.90 brooms plant^{-1}, $p = 0.0138$). All other treatments maintained stable levels of VWB over time.

As observed for FPR, high plant-level variability was detected, with a substantial random intercept standard deviation (SD = 4.19). This variability highlights the strong influence of individual plant factors and inherent disease heterogeneity within the plantation. Overall, disease responses suggest that most input substitution strategies maintained comparable phytosanitary performance relative to conventional management. However, two Biol-based treatments showed differential patterns. The Biol 50%–30d regime was the only strategy associated with significant increases in both FPR (+3.13 pods plant^{-1}, $p = 0.037$) and VWB (+2.63 brooms plant^{-1}, $p = 0.014$) from Year 1 to Year 2. The Biol 50%–15d treatment also showed a significant increase in VWB (+2.33 brooms plant^{-1}, $p = 0.029$), but not in FPR. All remaining treatments maintained stable disease levels across years.

Notably, despite these localized increases in disease incidence for specific Biol-based regimes, neither treatment exhibited corresponding yield penalties in dry bean production (Section 3.1). These patterns reinforce the interpretation that productivity responses in Biol-based treatments were achieved without systematic increases in disease pressure for most strategies—an important consideration for evaluating input efficiency under endemic pathogen conditions.

3.2. Climatic drivers of yield variability and disease expression

To contextualize the treatment effects reported above, we examined the influence of climatic variables on yield, disease incidence, and cherelle wilt using mixed models that incorporate lagged climate predictors. These analyses complement the agronomic results by identifying the environmental windows that modulate productivity and

disease dynamics.

Yield. Linear mixed model (LMM) analysis identified climatic conditions occurring 4–5 months prior to harvest as significant drivers of dry bean yield variability ($P < 0.05$), aligning with key reproductive phases of cacao. Temperature effects were strongly stage-dependent. Higher temperatures during flowering onset (5-month lag) were positively associated with yield ($\beta = 38.15$, $P < 0.001$), whereas elevated temperatures during early fruit-set (4-month lag) negatively impacted final productivity ($\beta = -25.85$, $P = 0.005$; Fig. 6A). Although consecutive time lags are temporally correlated and should be interpreted cautiously, this opposing pattern is biologically consistent with the vulnerability of young pods to heat-induced physiological stress and abortion. Precipitation patterns further contributed to yield variability. Excess rainfall during the flowering period (5-month lag) showed a significant negative association with final yield ($\beta = -20.99$, $P = 0.03$; Fig. 6A), likely reflecting reduced pollination efficiency and increased flower loss under saturated conditions.

Frosty Pod Rot. GLMM analysis identified moisture-related variables as the principal environmental drivers of FPR incidence. Accumulated rainfall one month prior to evaluation (t−1) emerged as the strongest positive predictor ($\beta = 0.38$, $P < 0.001$; Fig. 6B), consistent with the latent period of *M. roreri* and the delayed appearance of symptoms following infection during susceptible pod stages. Relative humidity also exhibited a significant positive association with disease intensity ($\beta = 0.10$, $P < 0.001$), reinforcing the importance of sustained moisture for sporulation and pathogen establishment. In contrast, temperature during the same period showed no significant effect ($\beta = 0.004$, $P = 0.892$), underscoring the predominant role of moisture over thermal conditions in driving FPR dynamics. Concurrent rainfall displayed a negative coefficient ($\beta = -0.19$, $P = 0.006$), suggesting a potential short-term 'spore washing' effect whereby intense rainfall events may temporarily reduce aerial dispersal or limit conidial adhesion on pod surfaces. These results indicate that disease pressure was primarily governed by antecedent moisture conditions rather than immediate rainfall at the time of evaluation.

Vegetative Witches' Broom. The GLMM revealed strong temporal structuring in the environmental drivers of VWB expression. Precipitation accumulated two months prior to evaluation (t−2) was the dominant positive predictor of symptom emergence ($\beta = 0.51$, $P < 0.001$; Fig. 6C), corresponding closely with the known incubation dynamics of *M. perniciosa* infections. Concurrent precipitation displayed a significant negative coefficient ($\beta = -0.47$, $P < 0.001$). This inverse association reflects temporal displacement rather than a suppressive climatic effect: symptom expression peaked during relatively drier periods following infection events initiated under

earlier high-moisture conditions. Precipitation at a one-month lag showed no significant effect (β = −0.07, P = 0.373), further supporting the specificity of the two-month incubation window.

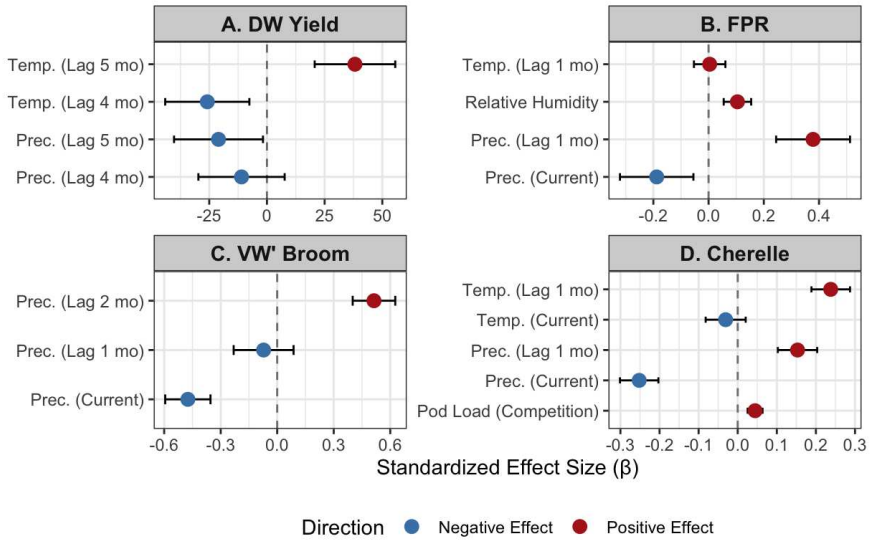


Figure 6. Figure 6. Standardized effects of climatic and physiological drivers on cacao yield and disease incidence. Points represent regression coefficients (β) from linear mixed models (yield) and generalized linear mixed models (disease counts). Horizontal bars indicate 95% confidence intervals. Drivers with confidence intervals crossing the vertical dashed line at zero are not statistically significant ($p \geq 0.05$). Positive coefficients (right of zero) indicate a promoting effect on the response variable; negative coefficients (left of zero) indicate a suppressive effect.

These findings support the interpretation that disease expression followed predictable epidemiological time windows, reinforcing the need to interpret treatment responses within the broader climatic and phenological context rather than as isolated management effects. The contrasting lag structures between FPR (1 month) and VWB (2 months) reflect species-specific differences in their hemibiotrophic life cycles and provide a framework for understanding why disease dynamics in Section 3.1 showed limited treatment effects: under endemic pressure, climatic drivers establish infection windows that affect all plots similarly, overriding potential treatment-specific suppression.

Cherelle wilt. Cherelle dynamics were primarily driven by short-term climatic variability, with physiological load exerting an additional but smaller influence. The GLMM indicated that higher temperatures one month prior to evaluation (t−1) significantly increased abortion rates (β = 0.24, P < 0.001; Fig. 6D), consistent with the sensitivity of young developing pods to thermal stress. In contrast, concurrent rainfall showed a strong protective effect (β = −0.25, P < 0.001), likely reflecting improved plant water status and reduced physiological stress during

early fruit development. However, rainfall one month prior (t−1) exhibited a positive association with cherelle wilt (β = 0.15, P < 0.001), suggesting that wet conditions preceding fruit set may predispose flowers or young pods to subsequent abortion—possibly through reduced pollination efficiency or increased pathogen pressure on reproductive structures.

A moderate density-dependent component was also detected. The concurrent number of developing pods (N_Pods) was positively associated with cherelle wilt intensity (β = 0.04, P < 0.001), confirming that trees bearing heavier fruit loads exhibited slightly increased rates of natural thinning due to internal competition among sinks. Current temperature showed no significant effect (β = −0.03, P = 0.238), indicating that thermal stress operates with a lag rather than instantaneously. Together, these findings highlight that early fruit retention was shaped by the interaction between climatic stress (temperature at t−1, rainfall at t−1 and concurrent) and intrinsic reproductive load, rather than by treatment effects alone.

3.3. Economic Efficiency of Biological Substitution Strategies versus Conventional Management

Building on the agronomic and climatic analyses presented above—which showed that Biol-based treatments-maintained yields comparable to conventional management despite similar disease pressure—we assessed whether these biological strategies translated into economically sustainable outcomes. Given that yield stability (Section 3.1) and disease suppression (Section 3.2) were largely comparable across treatments, the economic viability of input substitution hinges critically on production costs, particularly those associated with application frequency and labour. Economic performance was closely aligned with disease management outcomes, as treatments achieving comparable FPR suppression with reduced application frequency (e.g., T6) maintained profitability while lowering production costs.

Intensive use of biological inputs proved economically unsustainable under the conditions evaluated. Treatment T3 (100% Biol applied every 15 days) incurred the highest production costs (6,501 USD ha^{-1}), resulting in a negative net margin (−884 USD ha^{-1}) and a BCR below unity (0.9), indicating that yield gains were insufficient to offset increased input and labour expenditures. In contrast, reducing both application frequency and dosage substantially improved financial viability. Notably, treatment T6 (50% Biol applied every 30 days) exhibited economic performance comparable to the conventional control (T1). Both treatments achieved a BCR of 2.2 and similar unit production costs (≈1.10 USD kg^{-1}), with net margins of 3,262 USD ha^{-1} and 3,296 USD ha^{-1},

respectively. These results suggest that partial substitution of synthetic inputs with biological formulations can be economically competitive when applied at moderate intervals (30-day schedule), maintaining profitability while reducing reliance on conventional agrochemicals.

Table 2. Economic and financial performance of CCN-51 cacao under different management regimes during 2015 and 2016 production cycles in a commercial farm in Guayas, Ecuador [1].

Treatment-ID	Management Description	Estimate yield (kg/ ha) 2015+2016	Total Variable Cost (USD / ha) 2015-2016	Unit Production Cost (USD / kg)	Net Margin (USD / ha)	Benefit-Cost Ratio (BCR)
T1	Conventional	2,55	2,78	1.09	3,30	2.2
T2	Conv. optimized	2,95	4,29	1.46	2,73	1.6
T3	Biol 100% (15d)	2,35	6,50	2.76	-884	0.9
T4	Biol 100% (30d)	2,63	3,83	1.46	2,44	1.6
T5	Biol 50% (15d)	2,79	4,42	1.58	2,24	1.5
T6	Biol 50% (30d)	2,53	2,78	1.10	3,26	2.2

[1] Financial indicators were calculated using a weighted average farmgate price of 2,385 USD kg^{-1} for dry beans. This value reflects regional price volatility in Guayas during the study period, where prices fluctuated between 123 USD and 85 USD per quintal (qq) (MAGAP-SIPA 2025). A conversion factor of 45.36 kg qq^{-1} was applied.

Intermediate strategies showed mixed outcomes. Treatments T4 (100% Biol every 30 days) and T5 (50% Biol every 15 days) generated acceptable yields but displayed reduced economic efficiency (BCR 1.6 and 1.5, respectively), largely due to increased operational costs relative to yield gains. Overall, the results highlight that the economic success of biological substitution strategies depends less on maximizing application intensity and more on optimizing application frequency to balance production costs and agronomic benefits.

4. DISCUSSION

4.1. Agronomic performance and disease dynamics

Partial substitution of foliar synthetic inputs with a fermented Biol maintained pod and dry bean yields comparable to conventional management, provided that baseline soil fertilization was kept constant. The optimized Biol strategy (50% concentration, 30-day interval; T6) yielded 2,532 kg ha^{-1}, statistically equivalent to the conventional control (T1: 2,546 kg ha^{-1}) and to the high-input optimized treatment (T2: 2,946 kg ha^{-1}). However, a significant Year × Treatment interaction was observed ($p < 0.0001$): conventional treatments declined sharply from 2015 to 2016, whereas most Biol-based regimes remained stable. This inter-annual decline was not treatment-specific. Farm management records indicate that a severe pruning was applied to all plots in December 2015, removing a

large proportion of productive branches and floral cushions. This systemic practice uniformly reduced fruiting potential in the second year, explaining the general yield drop. Additionally, historical spatial variability within the farm placed the experimental plot (Lote 3) in a low-yielding group, which contextualizes the moderate absolute yields (17–18 QQ ha^{-1}) relative to the reported potential of CCN-51 (>40 QQ ha^{-1}).

Disease incidence of Frosty Pod Rot (FPR) and Witches' Broom (VWB) was not significantly affected by treatment (Fig. 5). Neither conventional fungicides nor Biol sprays produced consistent reductions. Instead, the farm's routine sanitary harvesting (monthly removal of infected pods, and weekly when pressure is high) likely played a dominant role in inoculum reduction (Krauss and Soberanis 2002; Evans 2016a, b). Climatic drivers overwhelmingly determined disease expression: rainfall one month prior to evaluation was the strongest predictor for FPR ($\beta = 0.38$, $p < 0.001$), and rainfall two months prior for VWB ($\beta = 0.51$, $p < 0.001$), matching the latent periods of both pathogens (Leandro-Muñoz et al. 2017). Concurrent rainfall showed negative coefficients, reflecting temporal displacement rather than true suppression. Therefore, under endemic pressure, infection windows are set by climate, and calendar-based sprays—whether chemical or biological—cannot interrupt pathogen establishment. Effective management must be prophylactic, timed to climate forecasts, and integrated with rigorous sanitation.

4.2. **Economic trade-offs of biological substitution**

The economic analysis revealed a clear "labor trap": high-frequency Biol applications (T3, 100% every 15 days) generated a negative net margin (−884 USD ha^{-1}) and a Benefit-Cost Ratio (BCR) of 0.9, despite maintaining yields. In contrast, reducing frequency to 30 days (T6, 50% Biol) restored profitability to levels indistinguishable from conventional management (BCR = 2.2, unit production cost ≈1.10 USD kg^{-1}). This outcome challenges the assumption that biological inputs are inherently low-cost. Instead, it illustrates the "labor trap" frequently observed in transitions toward biologically based management systems, where increased labor requirements offset potential agronomic benefits (Riar et al. 2024). The key determinant was not yield maximization but labor efficiency and application logistics.

Similar cost structures have been reported in Ecuadorian cacao systems, where fertilization and energy inputs represent a substantial share of production costs (Pérez Neira 2016). Our results extend this view by showing that input substitution shifts costs from agrochemicals to labor; if not optimized, the transition becomes economically unsustainable. Meta-analyses of biological control in sub-Saharan Africa (Ratto et al. 2022) and Latin America

(van Lenteren et al. 2025) confirm that economic viability depends on genuine displacement of synthetic inputs rather than additive use. Broader analyses of biocontrol adoption likewise emphasize that economic viability emerges only when these strategies are effectively integrated into pest management systems and displace chemical inputs (Colmenarez and Vasquez 2024; van Lenteren et al. 2025). From a systems perspective, improving the sustainability of cacao production requires not only reducing agrochemical inputs but also optimizing energy and labor efficiency across the production system (Pérez-Neira et al. 2020).

Thus, the monthly Biol protocol (T6) represents a practical compromise that reduces chemical dependency while maintaining profitability. However, our findings also underscore that input substitution alone is not sufficient—it must be embedded within an integrated management framework that includes rational application schedules, climate-informed sanitation, and disciplined canopy management. Without these complementary practices, even biologically based inputs can become economically unviable.

4.3. Limitations and scope

The study has several limitations. First, the trial lasted only two years in a single full-sun CCN-51 monoculture on sandy-loam soil, limiting extrapolation to other systems, varieties, or shaded agroforestry. Second, the severe pruning of December 2015 was a confounding systemic factor that reduced yields across all treatments; therefore, the second year was a conservative test of treatment differences. Third, soil fertilization was kept constant across treatments, so the substitution concerned only foliar inputs and fungicides, not complete nutrient replacement. Fourth, the economic analysis used 2015–2016 prices and short-term returns, excluding long-term soil health benefits. Fifth, the Biol used in this study was produced on-farm following a standardized protocol; however, detailed chemical characterization (e.g., nutrient content, phytohormones) was not performed, and its efficacy may vary with fermentation conditions (Gil Ramírez et al. 2023). Despite these limitations, the monthly 50% Biol strategy offers a viable, cost-effective entry point for reducing synthetic input dependency in intensive cacao systems, provided that soil fertility is maintained and climate-informed sanitation is practiced.

5. CONCLUSIONS

1. Agronomic viability: Partial substitution of synthetic foliar fertilizers and fungicides with a fermented Biol (50% concentration, applied every 30 days) maintained dry bean yield (2,532 kg ha^{-1}) statistically equivalent to conventional high-input management, without significantly increasing the incidence of Frosty Pod Rot or Witches' Broom.

2. Effect of application frequency: High-frequency Biol applications (every 15 days) were economically unsustainable (BCR = 0.9) due to prohibitive labor costs, whereas monthly applications restored profitability (BCR = 2.2), matching conventional systems. Economic viability therefore depends on labor efficiency, not yield maximization.

3. Climatic and phenological modulation: Yield was primarily determined by temperature and rainfall during flowering and early fruit set (4–5 months before harvest), while disease incidence was driven by rainfall with lags of one (FPR) or two (VWB) months. These climatic effects overrode treatment differences, emphasizing the need for climate-informed, prophylactic management.

Acknowledgements: We extend our sincere gratitude to Ronald León, Víctor Hernández, Eduardo Chávez, and Diego Rojas for their invaluable technical assistance during the field trial. We also thank the staff of Hacienda San Rafael and Aquiles Bohórques for their logistical support and for providing essential information. Finally, we are grateful to Jörn Pons-Kühnemann for his is advice on statistical analysis.

Author's contributions:

SP.-M., Writing—Original Draft, Writing—Review & Editing.

C.A.-V., Acquisition of data, Methodology, Project administration.

C.A.R., Acquisition of data, Analysis and Interpretation of data

B.R.R-.R. Data curation, Analysis and Visualization.

D.S.C., Conceptualization and Design, Funding acquisition, Writing—Review & Editing.

Data availability: The data that support the findings of this study are openly available in Zenodo at https://doi.org/10.5281/zenodo.17980239

Funding: This research was jointly funded by ESPOL (CIBE) and the NOBIS Consortium (Hacienda San Rafael S.A.) through the Project: "Evaluation of the effect of the bioproduct developed by CIBE on the phytosanitary status and productivity of cacao CCN-51" (February 2015 - January 2017).

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