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Impact of *Rhizophagus irregularis* and *Trichoderma sp.* Inoculation on Cassava (*Manihot esculenta* Crantz) Agronomic Parameters

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Abstract

Background: In Côte d'Ivoire, cassava (*Manihot esculenta* Crantz) is cultivated throughout the country and represents an important source of income and food security. However, its production commonly involves chemical inputs that may contribute to declining soil fertility.
Aims: This study evaluated the effects of *Rhizophagus irregularis* and *Trichoderma sp.* on cassava growth and yield.

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Place and Duration of Study: The study was conducted in two stages: a two-month *in vivo* cultivation phase at the Ecology Research Centre in Abidjan, southern Côte d'Ivoire, followed by a 10-month field phase in Azaguié, south-eastern Côte d'Ivoire.
Methodology: The Yacé and TMS30572 cultivars were grown *in vivo* and under field conditions and inoculated at planting with *R. irregularis* (RI) and *Trichoderma sp.* (TRI). The fungi were applied separately and in combination (RI + TRI). Under field conditions, NPK (10-18-18) fertiliser served as the positive control for yield evaluation.
Results: After two months, stem diameter, plant height, number of leaves, and leaf area were enhanced by RI and RI + TRI, whereas the effect of TRI varied with the plant organ and cultivar. For each cultivar, mycorrhizal colonisation intensity and frequency did not differ significantly between the RI and RI + TRI treatments, confirming the mycotrophic response of cassava. The RI, NPK, and RI + TRI treatments produced statistically comparable yields within each cultivar. For Yacé, yields were 18.56 t/ha with RI, 18.87 t/ha with NPK, and 19.07 t/ha with RI + TRI. The corresponding yields for TMS30572 were 28.76, 28.96, and 29.22 t/ha, respectively.
Conclusion: Under the tested conditions, RI alone and RI + TRI produced cassava yields comparable to those obtained with NPK fertiliser and therefore showed potential for supporting more sustainable cassava production.

Keywords: Rhizophagus irregularis; Manihot esculenta; treatment; Trichoderma sp.; yield.

1. Introduction

Cassava is a tropical crop grown mainly for its tuberous roots and leaves. Global production was estimated at 315 million tonnes in 2023, of which 65% was produced in Africa (Abessolo et al., 2025). In Côte d'Ivoire, cassava production was approximately 7.44 million tonnes in 2022 (Thiémélé et al., 2024).

Cassava is a staple food (N'Zué et al., 2014) for more than 500 million people in Africa (Vernier et al., 2018) and represents an important source of income for farming communities (Bamba & Kouadio, 2022). In Côte d'Ivoire, it is the second most important food crop after yam and is consumed in several forms, including attiéké, placali, and foutou (Yéboué et al., 2017; Soro & Soro, 2022; Abessolo et al., 2025). Cassava is also used in livestock feed, starch production, and numerous industrial applications (Parmar et al., 2017; Fasae & Yusuf, 2022). Despite these advantages, sustainable cassava production remains limited.

To increase crop production, farmers commonly apply chemical fertilisers to improve yields. However, excessive use of these products can cause long-term problems, including groundwater contamination and the accumulation of toxic residues (Mangoua et al., 2020; Koua et al., 2023; Kouamé et al., 2025), which may adversely affect consumer health.

In this context, the development of reliable and environmentally sound strategies has become increasingly important. The use of beneficial microbial agents therefore represents a promising approach for cassava cultivation. Among these agents, arbuscular mycorrhizal fungi (AMF) and *Trichoderma sp.* have been investigated in several studies (Séry et al., 2016; Chukwuka et al., 2017; Gutiérrez-Moreno et al., 2025).

Recent evidence indicates that AMF can enhance nutrient acquisition, plant growth, and disease management, including through interactions with other beneficial rhizosphere microorganisms such as *Trichoderma spp.* (Umer et al., 2025). By establishing symbiotic associations with plant roots, AMF can improve plant health and yield (Samri et al., 2021; Gomathy et al., 2024). This association increases the availability of mineral nutrients required for plant growth (Oyem & Okubor, 2020; Sawadogo et al., 2021; Bennett & Groten, 2022).

In cassava, inoculation with *R. irregularis* has recently been reported to improve early vegetative growth, root colonisation, biomass accumulation, and nitrogen-use efficiency during the acclimatisation of micropropagated plants (Wahyuni et al., 2026). Recent reviews and field evidence also indicate that AMF-based practices can improve soil fertility, plant biomass, yield, and root colonisation, although their performance depends on the crop, amendment, and soil conditions (Nwoke et al., 2026; Astiko et al., 2026). The genus Trichoderma comprises predominantly saprophytic species and has been widely investigated for its plant growth-promoting mechanisms (da Costa et al., 2022; Mahmoodian et al., 2022; Olojugba et al., 2025). These fungi can improve plant mineral nutrition and stimulate plant defences against biotic stressors. However, their use in cassava cultivation in Côte d'Ivoire has received limited attention. Nevertheless, the individual and combined effects of *R. irregularis* and *Trichoderma sp.* on cassava growth, root colonisation, and tuberous-root yield under field conditions in Côte d'Ivoire remain insufficiently documented.

Therefore, this study aimed to evaluate the effects of the AMF *R. irregularis* and *Trichoderma sp.* on the sustainable production of cassava.

2. Material and Methods

2.1 Plant Material

Stem cuttings of the *Manihot esculenta* cultivars Yacé and TMS30572 were used in this study. They were supplied by the National Centre for Agronomic Research (CNRA) and propagated on an experimental plot in Azaguié, 45 km from Abidjan. Yacé is a local cultivar that is well known and highly valued for attiéké production. By contrast, TMS30572 is an improved cultivar developed by the International Institute of Tropical Agriculture (IITA), Nigeria, and is recognised for its agronomic performance. These cultivars had not previously been specifically evaluated with the fungal inoculants used in this study.

2.2 Fungal Material

The fungal material comprised two fungi. The mycorrhizal strain PTB297, *R. irregularis* (RI), was supplied by PREMIER TECH AGRICULTURE, Canada, whereas *Trichoderma sp.* (TRI) was obtained through single-spore culture. Through its hyphae, *R. irregularis* explores a large volume of soil and can thereby improve mineral and water acquisition, plant growth, resistance to water stress, and soil fertility. *Trichoderma sp.* can colonise the plant rhizosphere, inhibit several soil-borne pathogens, stimulate root development, promote nutrient uptake, and enhance the plant's natural defences.

2.3 Methods

2.3.1 Preparation of *in vivo* Cultivation Material and Obtaining the Plants

In vivo cultivation was conducted in nursery bags measuring 19 cm in height and 13 cm in diameter. The bags were filled with 5 kg of soil collected from the Ecology Research Centre in Abidjan, Côte d'Ivoire. The soil was cleared of coarse material and steam-sterilised in a SELECTA oven at 100 °C for 48 h. After filling, the bags were watered to field capacity. Stem cuttings of the Yacé and TMS30572 cassava cultivars were disinfected with 3.6% (w/v) sodium hypochlorite and 70% (v/v) ethanol. After three rinses with sterile distilled water, the stems were cut into 20-cm sections containing at least three nodes. The stakes were placed vertically in the prepared bags. Sprouting and growth occurred under a shelter covered with transparent roofing sheets and illuminated by natural daylight.

2.3.2 Treatment of Plants Grown *in vivo*

Four treatments were applied: TRI, RI, RI + TRI, and an untreated control. The individual TRI and RI treatments were applied at planting. For TRI, 5 mL of spore suspension (10^5 spores/mL) was deposited as close as possible to the cutting at a depth of 5 cm using a micropipette. For RI, 0.25 g of inoculum powder containing 1,600 spores was placed at a depth of 5 cm in the planting hole, after which the cutting was immediately inserted and the surrounding soil was replaced to retain moisture and support the cutting. For the combined treatment, RI was applied at planting and TRI was applied 10 days later. The bags were watered regularly to maintain field capacity. Twelve plants were used per treatment in each replicate. With three replicates, this resulted in 36 plants per treatment and 144 plants per cultivar across the four treatments.

2.3.3 Field Treatment and Plant Production

The field experiment was established in a randomised block design with three replicates to reduce variation associated with soil heterogeneity. Each replicate constituted one block. The experimental plot covered 1,092 m²

(42 m × 26 m), and adjacent blocks were separated by 2 m. Each block contained five elementary plots separated by 1 m. Each elementary plot covered 42 m² (7 m × 6 m) and contained 30 cassava plants, comprising 15 plants of each cultivar. One cassava cutting was placed horizontally in each planting hole, which measured 25 cm in length and 8 cm in depth. Planting holes were spaced 1 m within rows and 1 m between rows. To control termites, Furadan was applied to each planting hole at 0.5 g per hole seven days before planting. No additional inputs were applied after planting, and the plot was weeded manually with a hoe as required.

The field treatments involved applying RI and TRI at the planting point at rates of 0.5 g and 10 mL, respectively. The application procedures were as described above, except that TRI was applied 30 days after planting in the combined RI + TRI treatment. NPK fertiliser (10-18-18) was applied once, two months after planting, at 24 g per planting hole. The untreated control (TE) received no treatment. Each elementary plot represented one treatment.

2.3.4 Measurement of Agronomic Parameters *in vivo*

Agronomic parameters were measured in two-month-old plants and included plant height, stem diameter, number of fully expanded leaves, and leaf area. Measurements were recorded for all plants in each treatment. The assessment procedures and calculation formulae are presented in Table 1.

Table 1. Methods for measuring growth parameters of cassava plants grown *in vivo*

Growth parameters	Assessment method	calculation formulas
Average stem height (H)	Stem height was determined using a tape measure, measuring from the point of stem insertion on the cutting to the shoot apex	$\mathbf{H} = \frac{\sum \mathbf{H_i}}{\boldsymbol{NP}}$; H_i = height of each plant; NP = total number of plants
Average stem diameter (D)	Stem diameter was measured 2 cm from the point of stem insertion on the cutting using a digital caliper (Mitutoyo).	$\mathbf{D} = \frac{\sum \mathbf{D_i}}{\mathbf{NP}}$; Di = diameter of each stem; NP = total number of plants
Average number of leaves (NF)	The number of leaves per plant was assessed by counting the fully expanded leaves.	$\mathbf{NF} = \frac{\sum \mathbf{NF_i}}{\mathbf{NP}}$; NFi =nombre leaves per plant NP = total number of plants
Mean leaf area (LA)	The average leaf area was determined using the method described by Gogbeu et al. (2015).	$\mathbf{SF} = \frac{\sum \mathrm{SFi}}{\mathrm{NF}}$ $\mathrm{SFi} = \mathrm{S} - \left(\frac{\mathrm{S} \times \mathrm{M}'}{\mathrm{M}}\right)$; NF = total number of leaves; M = initial mass of the tracing paper; S = paper area; M'= mass of the tracing paper cut to the leaf dimension

2.3.5 Determination of the Length and Diameter of Tuberous Roots

The length and diameter of the tuberous roots were measured using a tape measure. Tuber length was measured from the point of tuberisation to the apex, whereas diameter was measured at the midpoint of the tuber. Mean tuberous-root length and diameter were then calculated.

2.3.6 Determination of Yield

Cassava storage-root yield was determined 10 months after planting. The storage roots from each treatment were weighed in kilograms using a Roberval balance. Yield expressed in kg/m² was subsequently converted to tonnes per hectare (t/ha).

2.3.7 Estimation of Root Colonization of Plants by *Rhizophagus irregularis* under Field Conditions

Root colonisation was assessed in terms of the frequency (F%) and intensity (I%) of mycorrhizal colonisation. Fine cassava roots were collected from the field 90 days after planting and washed under running water using a 500-µm-mesh sieve. The roots were cut into 1-cm fragments, immersed in 20 mL of KOH solution (10%, w/v), and heated at 80 °C for 1 h. For bleaching, the roots were placed in 1 mL of H_2O_2 (30%, v/v) for 5 min. They were then immersed directly in 5 mL of HCl (1%, v/v) for 30 min and rinsed under running water. Subsequently, the roots were heated at 80 °C for 30 min in 0.05% (v/v) trypan blue stain (Voko et al., 2013). Ten treated root fragments per plant were mounted in 50% (v/v) glycerol for microscopic observation following Kormanik and McGraw (1982). Mycorrhizal colonisation was assessed using the classification scale of Trouvelot et al. (1986). Infection was scored from 0 to 5, with each score corresponding to a defined colonisation class.

Score 0: 0 %; score 1: 0 to 1 %; score 2: 1 to 10 %; score 3: 10 to 50 %; score 4: 50 to 90 %; score 5: > 90 %. Mycorrhization frequency F (%) and intensity I (%) are calculated using the formulas proposed by Trouvelot et al. (1986, cited by Séry et al., 2016) :

F (%) = 100 x (Number of mycorrhizal fragments/ total number of fragments observed)
I (%) = 100 x (95N5+70N4+30N3+5N2+N1/Total number of fragments observed)

N5: number of fragments corresponding to score 5; N4: number of fragments corresponding to score 4; N3: number of fragments corresponding to score 3; N2: number of fragments corresponding to score 2; N1: number of fragments corresponding to score 1.

2.4 Statistical Analysis

The data were subjected to analysis of variance (ANOVA) to determine the individual and combined effects of the fungal treatments and NPK on the parameters measured for each cassava cultivar. When significant differences were detected ($P < 0.05$), means were separated using Duncan's multiple range test. Differences among means were evaluated at the 5% significance level. Percentage data were arcsine-transformed before ANOVA to improve conformity with the assumptions of analysis for proportional data.

3. Results and Discussion

3.1 Effects of the Fungi *Rhizophagus irregularis* and *Trichoderma sp.* on the Agronomic Parameters of Cassava Grown *in vivo*

Table 2 shows that the application of *R. irregularis* (RI) and *Trichoderma sp.* (TRI) to the cassava growth medium generally improved the measured agronomic parameters. For some traits, the combined RI + TRI treatment produced greater responses than either fungus applied alone. RI increased stem diameter and plant height in both Yacé and TMS30572. The combined treatment increased leaf number and leaf area relative to the control, and significant treatment effects were detected ($P < 0.05$; Duncan's test). TRI increased stem diameter in both cultivars and promoted stem elongation and leaf production in TMS30572. For other cultivar–trait combinations, TRI did not produce significant differences at the 5% level (Table 2). The values obtained with RI + TRI were generally similar to those obtained with RI alone, except for stem diameter in both cultivars and plant height in Yacé. In Yacé, stem diameter was 8.10 ± 0.17 mm with RI + TRI and 7.95 ± 0.11 mm with RI alone. In TMS30572, the corresponding values were 8.17 ± 0.58 and 7.86 ± 0.68 mm. Plant height in Yacé was 15.76 ± 2.18 cm with RI and 16.75 ± 1.13 cm with RI + TRI (Table 2).

3.2 Root Colonization of Yacé and TMS30572 Cassava Cultivars by *Rhizophagus irregularis* after Three Months of Field Cultivation

Root colonisation by *R. irregularis* (RI) was expressed as mycorrhizal intensity (I) and frequency (F) after three months of field cultivation. As shown in Table 3, mycorrhizal colonisation intensity did not differ significantly between the RI and RI + TRI treatments in either Yacé or TMS30572. Similarly, colonisation frequency did not differ significantly between the two treatments within either cultivar ($P > 0.05$).

Table 2. Evolution of agronomic parameters of cassava cultivars Yacé and TMS30572 according to treatments

Cultivars	Parameters	Treatments			
		TE	RI	TRI	RI+TRI
Yacé	Dm (mm)	5.85± 0.11d	7.95± 0.11ab	6.35± 0.33c	8.10± 0.17a
	Hm (cm)	14.38 ±2.12b	15.76±2.18ab	14.68 ±1.51b	16.75 ±1.13a
	SmF (cm^2)	39.27±2.01b	41.62 ±1.05a	39.11±1.24b	41.72 ±1.03a
	NmF	9 ±0.44b	9±0.15b	9 ± 0.11b	11 ± 0.21a
TMS30572	Dm (mm)	6.65± 0.23c	7.86±0.68b	7.19± 0.19b	8.17± 0.58a
	Hm (cm)	13.65± 1.42c	17.35±2.30a	14.09± 0.98b	17.62±1.45a
	SmF (cm^2)	39.68± 1,74c	44.89±1.24a	41.37± 1.68b	45.66± 2.14a
	NmF	10± 0.67b	11±0.63ab	10 ±0.26b	12±0.29a

Within the same row, values followed by the same letter do not differ statistically at the 5 % level (Duncan test). TE: control; RI: Rhizophagus irregularis; TRI: Trichoderma sp.; RI + TRI: co-treatment with Rhizophagus irregularis and Trichoderma sp.; Dm: mean stem diameter; Hm: mean stem height; SmF: mean leaf area; NmF: mean number of leaves produced by the stems

Table 3. Mycorrhizal colonization status (%) of three-month-old cassava cultivar plants in the fiel

Cassava cultivars	Mycorrhization parameters (%)	Plants treatments	
		RI	RI + TRI
YACE	I	20.08±1.45a	22.19± 0.96a
	F	63.16±0.65a	65.01±1.56a
TMS30572	I	27.00±0.87a	25.18±1.77a
	F	64.10±2.04a	62.37±3.08a

For each cultivar, values in the same row followed by the same letter do not differ significantly at the 5 % level (Duncan test). RI: Rhizophagus irregularis; TRI : Trichoderma sp.; I: Mycorrhization intensity; F: Mycorrhization frequency

3.3 Influence of Mycorrhization on the Length and Diameter of Tuberous Roots of Cassava Cultivars Yacé and TMS30572

The mean length (LmRT) and mean diameter (DmRT) of tuberous roots produced by plants receiving the fungal treatments or NPK (10-18-18) are presented in Table 4. The results indicated that the fungal inoculants and NPK increased both parameters relative to the control. NPK fertiliser and the RI + TRI treatment produced the greatest tuberous-root lengths and diameters. These responses were statistically comparable within each cultivar, except for tuber diameter in TMS30572, which was greater with RI + TRI (36.70 ± 1.55 cm) than with NPK fertiliser (30.20 ± 2.96 cm) (Table 4).

Table 4. Variation in the length and diameter of tuberous roots according to treatments

Treatments	Cultivars			
	Yacé		TMS30572	
	LmRT (cm)	DmRT (cm)	LmRT (cm)	DmRT(cm)
TE	25.90 ± 4.78c	16.26 ± 2.77c	30.67 ± 3.36c	23.16 ± 2.84c
RI	29.30 ± 2.11ab	18.12 ± 1.47ab	41.26 ± 2.44a	32.86 ± 5.52b
TRI	26.50 ±2.59c	17.10 ±1.47bc	31.63 ± 3.09b	25.50 ± 2.96c
RI+TRI	31.37 ± 3.64a	20.17 ± 2.50a	40.63 ±1.44 a	36.70 ± 1.55a
NPK	33.10 ± 4.05a	18.74 ±2.03ab	41.73 ± 1.55a	30.20 ± 2.96b

Within the same column, values followed by the same letter do not differ statistically at the 5 % level (Duncan test)
LmRT : mean length of tuberous roots ; DmRT : mean diameter of tuberous roots

3.4 Effect of Treatments on Tuberous Root Yield of Cassava Cultivars Yacé and TMS30572

The tuberous-root yields of the Yacé and TMS30572 cultivars are presented in Table 5. The RI, NPK, and RI + TRI treatments produced the highest yields, with no significant differences among these three treatments according to Duncan's test. By contrast, the yield obtained with TRI alone did not differ significantly from that of the untreated control in either cultivar (Table 5).

Table 5. Tuberous root yield of cassava cultivars Yacé and TMS30572 according to treatments

Treatments	Cultivar yields (t/ha)	
	Yacé	TMS30572
TE	13.05 ± 0.12b	20.33 ± 0.41b
RI	18.56 ± 0.29a	28.76 ± 0.38a
TRI	13.58 ± 0.20b	20.77 ± 0.29b
RI+TRI	19.07 ± 0.19a	29.22 ± 0.32a
NPK	18.87 ± 0.21a	28.96 ± 0.26a

Within the same column, values followed by the same letters do not differ statistically at the 5 % level (Duncan test). TE: control; RI: Rhizophagus irregularis; TRI: Trichoderma sp.; RI+TRI: co-treatment with Rhizophagus irregularis and Trichoderma sp.; NPK: NPK chemical fertilizer

3.5 Discussion

The results indicated that *R. irregularis* (RI) and *Trichoderma sp.* (TRI) promoted cassava growth. Plants receiving either fungus alone or the combined inoculation generally grew better than the untreated controls, as reflected by increases in stem diameter, plant height, leaf number, and leaf area. These responses were most evident with RI alone and RI + TRI in both cultivars, whereas the effect of TRI alone was more apparent in TMS30572. RI was therefore more effective than TRI when the fungi were applied individually. The growth responses may be associated with improved water and mineral nutrition. When mycorrhizal fungi such as RI colonise plant roots, their external mycelium explores a greater soil volume and can enhance the absorption of water and nutrients. Similar findings have been reported by Weisany (2018), Aliyu et al. (2019), and Bossou et al. (2019), who observed increased nutrient concentrations in mycorrhizal plants. The growth-promoting effect of TRI has been associated with secondary metabolites produced by Trichoderma, as reported by Chagas Junior et al. (2022) and Woo et al. (2023). These metabolites may function as growth regulators (Dutta et al., 2024; Chen et al., 2025). Trichoderma can also produce organic acids that lower soil pH and facilitate the solubilisation of phosphate and micronutrients such as iron and magnesium (Bononi et al., 2020). Thus, improved rhizosphere conditions resulting from TRI, together with the expanded water- and nutrient-absorbing surface provided by RI mycelium, may explain the favourable response to combined inoculation. Hussein et al. (2024) similarly reported beneficial interactions between Trichoderma and AMF. TRI alone did not significantly affect tuberous-root yield in either cultivar. However, RI alone and RI + TRI produced yields that were statistically comparable to those obtained with NPK fertiliser. This response may be related to the enhanced acquisition of water and mineral nutrients by mycorrhizal roots. Whereas mineral fertiliser supplies readily available nutrients, mycorrhizal hyphae explore a larger soil volume and transfer nutrients to roots. Rostamikia et al. (2017) also reported increased photosynthetic pigment concentrations in inoculated plants, which may support carbohydrate accumulation in storage roots. Increased biomass production and water-use efficiency in mycorrhizal cassava have likewise been reported by Thanni et al. (2024). The colonisation observed in the present study supports the mycotrophic nature of cassava, consistent with Voko et al. (2013) and Séry et al. (2016). The comparable yields obtained with RI, RI + TRI, and NPK indicate that the fungal treatments may provide a biological contribution to cassava nutrition under the tested conditions. Positive effects of AMF on cassava have also been reported by Ndeko et al. (2020) and Oyetunji et al. (2023).

4. Conclusion

This study demonstrated that *Rhizophagus irregularis* improved the vegetative growth and tuberous-root yield of the Yacé and TMS30572 cassava cultivars under the evaluated conditions. Inoculation with *R. irregularis* alone increased several agronomic parameters, while combined inoculation with *R. irregularis* and *Trichoderma* sp. produced favourable growth responses and yields statistically comparable to those obtained with NPK fertiliser. The comparable mycorrhizal colonisation frequencies and intensities recorded for RI and RI + TRI indicate that the addition of *Trichoderma* sp. did not reduce root colonisation by *R. irregularis*. By contrast, *Trichoderma* sp. applied alone had a limited and cultivar-dependent effect on vegetative growth and did not significantly improve tuberous-root yield relative to the untreated control. Overall, the findings indicate that *R. irregularis*, either alone or in combination with *Trichoderma* sp., has potential as a biological input for cassava production. Nevertheless, the results apply specifically to the cultivars, soil, management conditions, and production cycle assessed in this study.

5. Limitations

The findings are limited to two cassava cultivars, one sheltered cultivation phase, and one field location evaluated during a single production cycle. Treatment performance may therefore vary with soil type, season, indigenous microbial communities, and crop management. The study compared the biological treatments with one NPK regime but did not assess longer-term soil effects or economic feasibility. Multi-location and multi-season trials are required to evaluate consistency, persistence, and practical applicability.

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Declaration of AI Use

This manuscript was prepared through the combined contributions of all author(s), including contributions to the study design, data, content development, results, interpretation, and related scholarly work. The author(s) acknowledge the use of Grammarly and ChatGPT to assist with grammar checking, language refinement, reference formatting. These AI-assisted tools were not used as authors and did not replace the intellectual contributions or scholarly judgment of the author(s). All AI-assisted outputs, including content, references, and interpretations, were carefully reviewed, revised, verified, and approved by the author(s). The author(s) accept full responsibility for the accuracy, integrity, and final content of the manuscript.

Competing Interests

Authors have declared that no competing interests exist.

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