

Is seedling rescue a viable strategy for producing *Virola surinamensis* plants with controlled-release fertilizer?

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SILVICULTURE

ABSTRACT

Background: The rapid expansion of deforestation and forest degradation in the Amazon has intensified the demand for native seedlings for restoration programs, while seed and seedling availability remains a major difficulty. Although seedling rescue has emerged as a promising alternative propagation strategy, its application and management remain poorly studied for most Amazonian species, including *Virola surinamensis* (Rol. ex Rottb.) Warb., a vulnerable species with restoration potential. This study evaluated the viability of seedling rescue for *V. surinamensis* and assessed the effects of controlled-release fertilizer doses applied during nursery production on survival, growth, and physiological performance.

Results: Seedling survival was high (97%) and was not affected by fertilizer application. In contrast, controlled-release fertilization significantly improved morphological and physiological attributes, including biomass accumulation, Dickson Quality Index, SPAD index, and gas exchange parameters. However, responses tended to saturate at higher doses, with no additional gains beyond 4.1 g dm⁻³. Effect size analysis indicated a large magnitude of fertilization effects on seedling quality traits.

Conclusions: Overall, seedling rescue represents a viable propagation strategy for *V. surinamensis*, and the application of controlled-release fertilizer at 4.1 g dm⁻³ is recommended to optimize seedling quality without unnecessary increases in production costs, supporting the use of this technique in Amazonian forest restoration programs.

Keywords: Amazonian species; plant propagation; natural regeneration; forest nursery.

HIGHLIGHTS

Seedling rescue is a viable strategy to produce *Virola surinamensis* seedlings.
Controlled-release fertilizer does not influence the survival of rescued seedlings.
A dose of 4.1 g dm⁻³ of fertilizer is recommended for high-quality seedling production.
Higher doses saturate physiology without additional gains in plant growth or biomass.

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INTRODUCTION

It is estimated that approximately 18% of the Amazon has already been deforested, while a significant portion of the remaining forests is degraded (Berenguer *et al.*, 2024). To address this scenario, several forest restoration projects have been developed, in which seedling planting constitutes the main technique employed (da Cruz *et al.*, 2021). Nevertheless, the availability of seeds and seedlings remains one of the primary bottlenecks for forest restoration and reforestation with native species in the Amazon (Nunes *et al.*, 2020).

The use of seeds is the most widely employed strategy of plant propagation of native forest species (Werden *et al.*, 2024). However, to meet the growing demand for native seedlings, other alternatives can be explored, such as seedling rescue. This technique consists of the collection, transport, and transplantation of seedlings from the natural regeneration of forest areas for nursery production (Calegari *et al.*, 2011). According to the literature, seedling rescue has shown promising results for woody plants (Schorn *et al.*, 2024).

Despite its potential, seedling rescue remains poorly explored for most Amazonian species, including *Virola surinamensis* (Rol. ex Rottb.) Warb., a vulnerable species (Brasil, 2021) with multiple uses (Paes *et al.*, 2023; Holanda *et al.*, 2024) and potential for forest restoration (Braga *et al.*, 2025). Furthermore, despite the considerable attention given to controlled-release fertilizers in Amazonian forest species (Sousa *et al.*, 2023; Borges *et al.*, 2025; Rocha *et al.*, 2026), the influence of fertilizer doses on seedling rescue remains unexplored.

Thus, with our work, we sought to answer three research questions: 1) Is seedling rescue a viable propagation strategy for producing *V. surinamensis* seedlings? 2) Does the use of controlled-release fertilizer influence the survival of rescued seedlings? 3) What is the appropriate dose to produce seedlings of this species?

Naturally germinated seedlings of *Virola surinamensis* were rescued from a forest fragment and submitted to four fertilizer doses (0.0, 4.1, 8.2, and 12.3 g dm⁻³) of a controlled-release fertilizer. At the end of the experimental period, we evaluated survival, as well as physiological and quality characteristics of the seedlings. The use of controlled-release fertilizer did not influence survival but, in general, ensured a considerable improvement in the characteristics of the seedlings produced.

MATERIAL AND METHODS

The experiment was conducted over a 110-day period in a greenhouse covered with plastic and 50% shade cloth in 2021, at the Institute of Agricultural Sciences (ICA), Federal Rural University of the Amazon (UFRA) – in Belém, Pará, Brazil. According to the Köppen climate classification, Belém is classified as type Af (tropical), with an average annual temperature above 26 °C and average monthly precipitation exceeding 60 mm (Alvares *et al.*, 2013).

The rescue of *V. surinamensis* seedlings (Figure 1) was performed following the steps described by Calegari *et al.* (2011), with adaptations. Thus, during the morning, *V. surinamensis* seedlings with a height of up to 10 cm and intact cotyledons were collected without the use of tools or leaf area reduction. Subsequently, the collected material was placed in open buckets with water and transported to the forest nursery. In the afternoon, each individual was transplanted into 280 mL tubes filled with coconut fiber and vermiculite in a 1:1 ratio, where the treatments were applied.

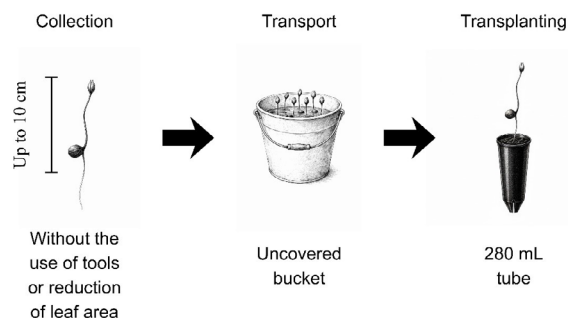


Figure 1: Details of the *Virola surinamensis* seedling rescue methodology.

The treatments consisted of doses (0.0, 4.1, 8.2, and 12.3 g dm⁻³) of the controlled-release fertilizer Osmocote®, which features an NPK 15-09-12 formulation, plus 1.3% Mg, 5.9% S, 0.02% B, 0.05% Cu, 0.46% Fe, 0.06% Mn, 0.02% Mo, and 0.05% Zn, with an expected release time of five months. These doses corresponded to different percentages (0%, 50%, 100%, and 150%) of the fertilization regimen typically adopted in this nursery for woody seedlings. Throughout the experimental period, irrigation was performed daily, and monitoring and, if necessary, pest and disease control were carried out.

The experimental design was randomized blocks, with four replicates and 10 plants per replicate. At the end of the experimental period (110 days), we evaluated seedling survival (%), in addition to the following biometric variables: stem diameter – SD (mm); height – H (cm); shoot dry mass – SDM (g); and root dry mass – RDM (g). Leaf area – LA (cm²) was obtained using a leaf area analyzer (model LI-3100C Area Meter). From the collected data, we calculated the height (cm)/stem diameter (mm), total dry matter – TDM (g), the shoot dry matter (g) / root dry matter (g), and the Dickson Quality Index (Dickson *et al.*, 1960), as follows equation 1:

$$DQI = \frac{TDM}{\frac{H}{CD} \cdot \frac{SDM}{RDM}} \quad (1)$$

Additionally, we also evaluated the following physiological variables: SPAD index, obtained with a portable meter (model SPAD-502); the assimilation rate – A (μmol CO₂ m⁻² s⁻¹), stomatal conductance – g_s (mol H₂O m⁻² s⁻¹), intercellular CO₂ concentration – C_i (μmol CO₂ mol⁻¹), and transpiration rate – E (mmol H₂O m⁻² s⁻¹), obtained with an infrared gas analyzer (IRGA, model LI-6400). From these data, we calculated the water-use

efficiency – A/E ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1} / \text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) and the carboxylation efficiency – A/C_i ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1} / \mu\text{mol CO}_2 \text{ mol}^{-1}$).

Survival, height, and stem diameter in all available seedlings were evaluated. For the SPAD index, leaf area, and gas exchange analyses, one representative plant with the average stem diameter of each plot was selected. For shoot and root dry mass analyses, we evaluated the five plants closest to the average stem diameter of each plot. These plants were dried in a forced-air circulation oven at 65 °C until constant mass was reached. We emphasize that the differences in the number of plants per evaluation were strictly operational. Photographic records of some of these steps are presented in Figure S1.

All data obtained were subjected to evaluation for the assumptions of normality of residuals and homogeneity of variances, respectively, using the Shapiro-Wilk and Bartlett tests. When one or more assumptions were violated,

the data were transformed (x^2). Once the assumptions were met, we performed Analysis of Variance (ANOVA) and, if necessary, applied the Tukey test for mean comparisons.

Furthermore, we calculated the effect size, omega squared (Ω^2), for the controlled-release fertilizer doses, according to Kroes and Finley (2023). For all tests and analyses, a significance level of 5% ($\alpha = 0.05$) was adopted. All statistical activities, as well as graph generation, were performed in RStudio 4.4.3 – Trophy Case (RTeam, 2026), using the AgroR (Shimizu *et al.*, 2023), MBESS (Kelley, 2025), and ggplot2 (Wickham, 2016) packages.

RESULTS

There was a significant effect ($p < 0.05$) of the controlled-release fertilizer doses for all analyzed variables, except for survival. On average, the survival of *V. surinamensis* seedlings at 110 days was 97%.



Figure S1: Recently collected *Virola surinamensis* seedlings (A), comparison between plants grown without and with controlled-release fertilizer at 110 days (B), and gas exchange measurements performed at 110 days (C).

Regarding the seedling quality characteristics (Figure 2 and Figure S2), in general, the means of the treatments with controlled-release fertilizer (4.1 to 12.3 g dm⁻³) did not differ from each other but were superior to the control treatment (0.0 g dm⁻³). On average, the effect size (Ω^2) for the seedling quality variables was 0.7622 (Table S1).

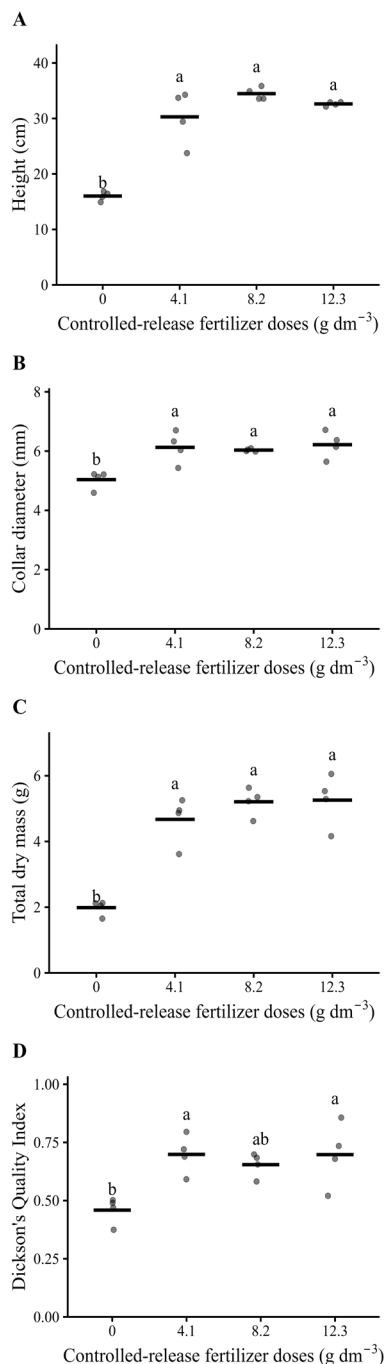


Figure 2: Height (A), collar diameter (B), total dry mass (C), and Dickson's Quality Index (D) of *Virola surinamensis* seedlings produced by seedling rescue and different controlled-release fertilizer doses at 110 days. Averages (crossbars) followed by the same lowercase letter do not differ significantly according to Tukey's test ($\alpha = 0.05$).

Regarding the main physiological characteristics of the seedlings shown in Figure 3 (SPAD index, net carbon assimilation rate, stomatal conductance, and transpiration rate), treatments with controlled-release fertilizer from 4.1 g dm⁻³ onwards showed higher SPAD index and assimilation rate compared with the control treatment (0.0 g dm⁻³).

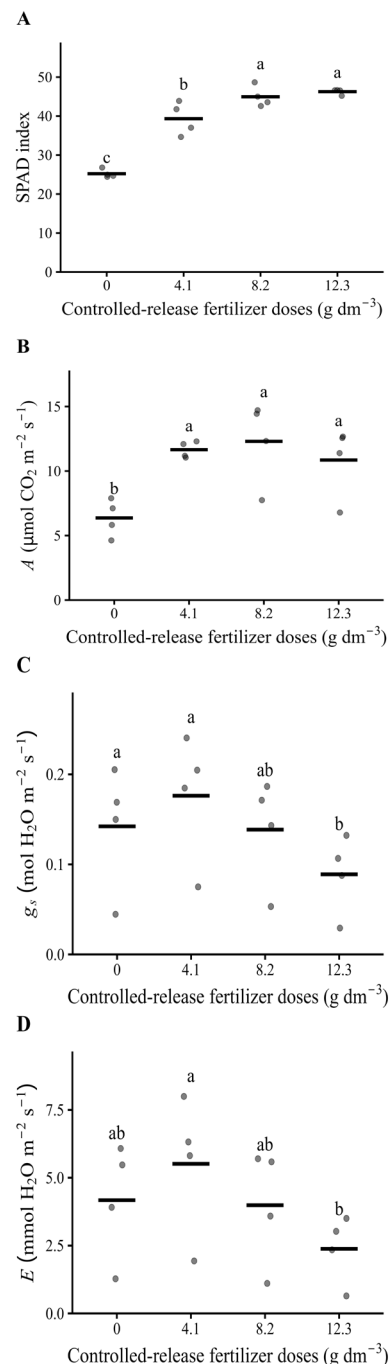


Figure 3: SPAD index (A), assimilation rate - A (B), stomatal conductance - g_s (C), and transpiration rate - E (D) of *Virola surinamensis* seedlings produced by seedling rescue and different controlled-release fertilizer doses at 110 days. Averages (crossbars) followed by the same lowercase letter do not differ significantly according to Tukey's test ($\alpha = 0.05$).

Differences in stomatal conductance and transpiration rate were mainly observed between the doses of 4.1 and 12.3 g dm⁻³. On average, the effect size (Ω^2) for the physiological variables was 0.4461 (Table S1 and Figure S1, S2 and S3). Additional physiological variables are presented in Figure 3.

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DISCUSSION

In this study, we evaluated the survival and the morphological and physiological characteristics of *V. surinamensis* seedlings produced via seedling rescue and treated with controlled-release fertilizer to address our three research questions. We consider seedling rescue a viable propagation strategy for this species and, although it does not affect survival, the use of controlled-release fertilizer at a dose of 4.1 g dm⁻³ is recommended.

We consider seedling rescue a viable strategy because the survival rate at the end of the evaluation period was high and comparable to the best results reported for other native species rescued from forest areas (Schorn *et al.*, 2024).

This result is likely related to the minimal damage caused to the rescued plants (Reis *et al.*, 2025). At the time of rescue, the seedlings had no secondary roots, were in soil that offered little resistance to collection, and were transported over a short distance.

However, the controlled-release fertilizer did not influence the survival of the rescued seedlings. This finding aligns with the literature, as it is known that the initial establishment of seedlings is determined by cotyledon reserves, and fertilization cannot replace them in this function (Nunes *et al.*, 2024). This indicates that for the rescue of *V. surinamensis* seedlings, it is crucial to collect individuals with intact cotyledons.

Considering our results, the recommended dose is 4.1 g dm⁻³ of controlled-release fertilizer. This is because, for all evaluated characteristics except survival, the controlled-release fertilizer doses showed an omega-squared value (Ω^2) above 0.138, indicating a large effect magnitude (Kirk, 1996).

In the case of morphological characteristics, although specific and updated recommendations for *V. surinamensis* do not exist, higher values are desired for the main indicators, as they are interrelated and linked to greater hardiness and initial field establishment (Guimarães *et al.*, 2024).

Regarding physiological characteristics, the SPAD index indirectly represents the total leaf chlorophyll content (Nurbaiti *et al.*, 2025). Meanwhile, the transpiration rate expresses the water vapor flux from the leaf to the atmosphere, stomatal conductance is the diffusive capacity of stomata to water vapor, and the assimilation rate defines the net CO₂ flux assimilated by the leaf (Busch *et al.*, 2024).

Table S1: Effect size of controlled-release fertilizer on the characteristics of *Virola surinamensis* seedlings produced by seedling rescue.

Characteristics	df _e	MS _e	F	Ω^2p	Confidence interval limits	
					Lower	Upper
Survival	9	33.3333	0.5000	-0.0938	0.0000	0.3097
Collar diameter	9	0.1698	7.0954	0.5617	0.0751	0.7679
Height	9	5.1394	54.8846	0.8946	0.7265	0.9571
Total dry mass	9	0.4081	23.6344	0.8267	0.4813	0.9083
Dickson's Quality Index	9	0.0095	5.4928	0.4871	0.0154	0.7267
Shoot dry mass	9	0.1719	34.5565	0.8663	0.6042	0.9344
Root dry mass	9	0.0582	8.8202	0.6379	0.1371	0.8001
Shoot-to-root dry mass ratio	9	0.0145	80.7913	0.9142	0.8042	0.9702
Height-to-collar diameter ratio	9	0.0513	95.8035	0.9094	0.8319	0.9747
SPAD index	9	6.2024	59.8191	0.9126	0.7457	0.9604
A	9	2.3655	12.1895	0.5278	0.2441	0.8426
E	9	1.0437	6.3042	0.2277	0.0457	0.7493
gs	9	0.0005	9.7397	0.2244	0.1683	0.8139
Ci	9	617.7046	12.2344	0.3806	0.2454	0.8430
A/E	9	1.0472	10.8469	0.3089	0.2039	0.8281
A/Ci	9	0.0002	6.5908	0.5410	0.0564	0.7564

df:degrees of freedom of error; MS: mean square of error; F: F statistics of the treatments (controlled-release fertilizer doses); Ω^2p : partial omega squared of the treatments (controlled-release fertilizer doses). A: assimilation rate; E: transpiration rate; gs: stomatal conductance; Ci: intercellular CO₂ concentration; A/E: water use efficiency; A/Ci: carboxylation efficiency.

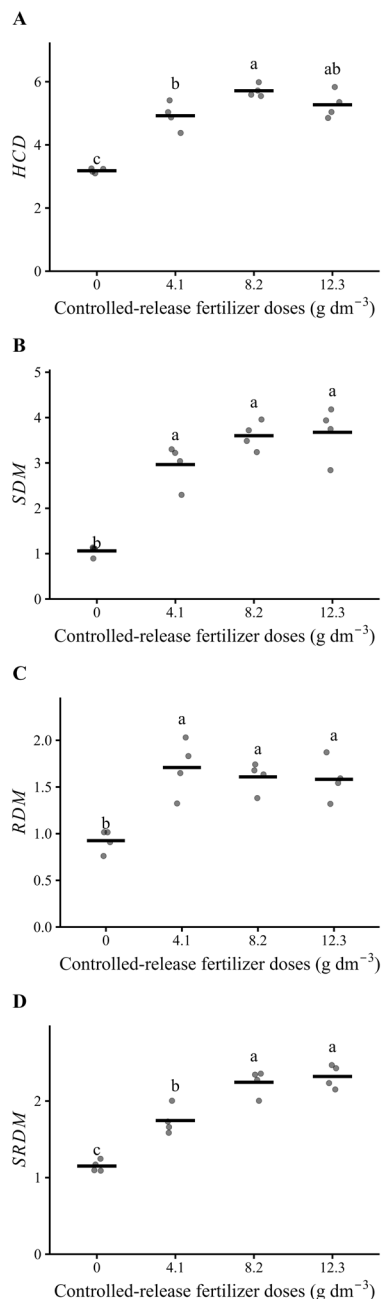


Figure S2: Height-to-collar diameter ratio – HCD (A), shoot dry mass – SDM (B), root dry mass – RDM (C), and shoot-to-root dry mass – SRDM (D) of *Virola surinamensis* seedlings produced by seedling rescue and different controlled-release fertilizer doses. Averages (crossbars) followed by the same lowercase letter do not differ significantly according to Tukey's test ($\alpha = 0.05$).

In the absence of other limitations, increments in the SPAD index, transpiration rate, and stomatal conductance are expected to be accompanied by a higher assimilation rate (Wang *et al.*, 2022; Zhang *et al.*, 2025). However, this relationship rarely remains linear in practice, as photosynthesis can be limited by factors other than photosynthetic structure and stomatal control, such as

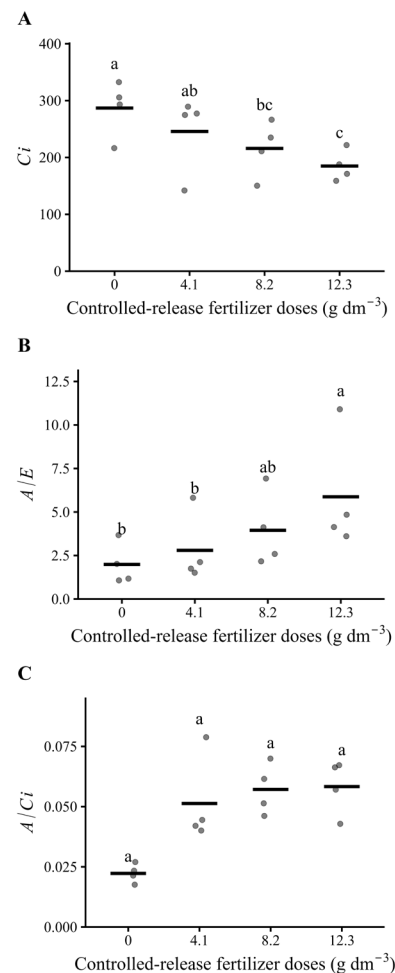


Figure S3: Intercellular CO₂ concentration – Ci (A), water use efficiency – A/E (B), and carboxylation efficiency – A/Ci (C) of *Virola surinamensis* seedlings produced by seedling rescue and different controlled-release fertilizer doses. Averages (crossbars) followed by the same lowercase letter do not differ significantly according to Tukey's test ($\alpha = 0.05$).

anatomical and biochemical restrictions (Nadal-Sala *et al.*, 2021; Knauer *et al.*, 2022)

Thus, although the highest dose promoted a more developed photosynthetic apparatus and greater efficiency in stomatal control, these adjustments did not translate into increases in carbon assimilation rate or growth, which remained similar from the dose of 4.1 g dm⁻³ of controlled-release fertilizer onwards. This indicates that the plants reached a luxury consumption step (Penn *et al.*, 2023; Sá *et al.*, 2024). Therefore, using doses higher than recommended would not tend to provide additional benefits to the seedlings and could merely increase the costs associated with this input.

Although it was not the objective of this study, practical observations during the rescue operations suggest that minor procedural adjustments can be adopted without compromising survival. Under our operational conditions, the absence of collection tools, leaf area reduction, and transport bucket covering did not negatively affect plant

survival. However, these findings should be interpreted as context-dependent observations rather than controlled experimental evidence, as outcomes may vary according to species and handling conditions.

This study was conducted exclusively under nursery conditions; therefore, the field performance of the produced seedlings remains unknown. Future studies should evaluate post-transplant survival and growth under field conditions to confirm the practical effectiveness of the rescue technique. Additionally, we did not assess forest dynamics in the collection area following the removal of individuals. Understanding the ecological consequences of seedling rescue is essential when applying this approach in natural forest areas intended for conservation or long-term maintenance.

Despite this, we emphasize that our study was the first to evaluate the use of controlled-release fertilizer to produce *V. surinamensis* seedlings via seedling rescue. In doing so, we provide important perspectives for applying this propagation technique to an Amazonian species with immense potential and suggest a fertilizer dose for seedling production. Furthermore, we report effect size values, which are uncommon in articles on this subject. With this, we hope to contribute to expanding the use of the species in seedling production and forest restoration programs, while also encouraging other researchers to move beyond the p-value.

CONCLUSION

Seedling rescue is a viable strategy for producing *V. surinamensis* seedlings within 110 days, effectively utilizing natural regeneration to support the propagation and supply of this vulnerable species. While the application of controlled-release fertilizer did not influence initial survival, it significantly improved the morphological and physiological traits of the rescued seedlings. For optimal seedling quality and resource efficiency, a dose of 4.1 g dm^{-3} is recommended, as higher doses lead to luxury consumption without providing additional growth benefits.

AUTHORSHIP CONTRIBUTION

Project Idea: DPSF; MAPG; DGA
Database: DPSF; MAPG; DGA
Processing: DPSF
Analysis: DPSF
Writing: DPSF; BSS; ACS; KJSC
Validation: DPSF; BSS; ACS; KJSC
Review: DPSF; MAPG; DGA; BSS; ACS; KJSC

DATA AVAILABILITY

The datasets supporting the conclusions are included in the article.

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