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Agronomic Performance of Solid and Liquid Digestate from Neem Fruit Pulp (*Azadirachta indica* A. Juss) on Growth and Yield of Lettuce (*Lactuca sativa* L.) under Tropical Conditions

Djalalou-Dine Memoko [a*], Yao Félicité Amenuti [a]
and Amen Yawo Nenonene [a]

[a] Laboratory for Research on Agroresources and Environmental Health (LARASE), Higher School of Agronomy (ESA); University of Lomé; 10 BP 1515; Lomé, Togo.

Authors' contributions

This work was carried out in collaboration among all authors. Author DDM designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author YFA felicity managed analyses of the study. Author AYN reviewed, proofread, and approved the manuscript. All authors read and approved the final manuscript.

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Abstract

Background and Aims: Anaerobic digestion digestates represent a promising organically derived alternative to mineral fertilizers in sustainable agriculture; however, their agronomic efficiency is highly dependent on post-digestion management practices. This study compared the agronomic performance of the solid and liquid fractions of digestate derived from the anaerobic digestion of neem fruit pulp (*Azadirachta indica* A. Juss) on lettuce (*Lactuca sativa* L.).

**Corresponding author: E-mail: djalmemoko40@gmail.com;*

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Study Design: A randomized complete block design (six treatments, three replications) was established using an isonitrogen application rate of 100 kg N ha^{-1} (i.e. all treatments supplying the same total nitrogen).

Place and Duration of Study: Agronomic Experimental Station of Lomé (SEAL), Togo, between July 2025 and August 2025.

Methodology: Treatments consisted of an unfertilized control (T0), mineral NPK 15-15-15 fertilizer (TN), ENPRO compost (TC), solid digestate (TSD), liquid digestate (TLD), and a mixed digestate combining 50% solid and 50% liquid fractions (TM). Growth parameters recorded included leaf number, leaf spread diameter, leaf color chart index, and final marketable yield. Treatment effects were assessed using a linear mixed model, with means separated by Tukey's HSD test ($P < 0.05$).

Results: Phase separation resulted in contrasting chemical profiles: the liquid digestate exhibited a C/N ratio of 7.9, conducive to rapid ammonium nitrogen release, whereas the solid digestate displayed a C/N ratio of 18.2, associated with slower nitrogen mineralization. Mineral fertilizer (TN) achieved the highest yield (14.93 t ha^{-1}), followed by liquid digestate (TLD) at 13.70 t ha^{-1}, a performance not statistically different from TN ($P > 0.05$), while the overall treatment effect on yield was highly significant ($P < 0.001$). Compost (TC) and mixed digestate (TM) yielded intermediate and statistically similar results, while solid digestate (TSD) recorded the lowest performance among fertilized treatments (9.00 t ha^{-1}), corroborating the mismatch between its slow nitrogen release kinetics and the short growing cycle of lettuce.

Conclusion: The liquid fraction of neem fruit pulp digestate represents an organically derived alternative whose agronomic efficiency is statistically comparable to that of mineral NPK fertilizer under the conditions of this trial. These findings are based on a single short-cycle trial in which only nitrogen was standardized across treatments, and therefore warrant confirmation under multi-season conditions.

Keywords: Digestates; anaerobic digestion; Azadirachta indica; pulp; Lactuca sativa.

1. Introduction

In response to global population growth (UN DESA, 2023), the use of mineral fertilizers has intensified with the aim of increasing agricultural yields and ensuring global food security. Globally, the amount of inorganic fertilizers used in the agriculture was estimated at 185 million ton in 2022, of which approximately 58 % was in nitrogen form (FAO, 2024). While mineral fertilizers remain essential to global food production, growing evidence highlights the need to partially substitute them with organic alternatives to preserve soil health and reduce environmental risks (Xing et al., 2025). This concern is especially acute in sub-Saharan Africa, where most fertilizers are imported and recent price shocks have severely constrained smallholder farmers' access to them (Amankwah et al., 2025).

Despite the yield improvements achieved through the use of chemical fertilizers, studies have shown that they cause several environmental and health impacts, including cancer and hematological disorders (Tagkas et al., 2024). Feng et al. (2022) also reported that the overapplication of nitrogen fertilizers contributes to nitrate contamination of groundwater, exposing populations to thyroid disorders and longer-term health risks. Adding to these concerns are the high prices of synthetic fertilizers and the limited access faced by many smallholders farmers, particularly in sub-Saharan Africa.

In this context, the expansion of market gardening in the peri-urban areas of Lomé, where rapid urbanization is driving increasing demand for fresh vegetables (Graner et al., 2023), particularly involves lettuce (*Lactuca sativa* L), a major leafy vegetable highly valued by consumers for its nutritional qualities. As a short-cycle crop with high nitrogen requirements, lettuce is particularly sensitive to the form of nitrogen supplied through fertilization. Hong et al. (2022) reported that lettuce yield increased by 13.56 % to 22.03 % under increasing nitrogen inputs compared to unfertilized controls, with quadratic relationships between nitrogen rate and yield, underscoring the importance of rapid and well-adjusted nitrogen availability for this crop.

In the search for alternatives to mineral fertilizers that can sustain crop with minimal environment impact, anaerobic digestion has emerged as a promising approach, producing not only biogas as a renewable energy source, but also digestate, an organic fertilizer rich in plant-available nutrients (Doyeni et al., 2021). In Togo, where neem (*Azadirachta indica* A. Juss) is widely distributed (Gadikou et al., 2022), neem fruit pulp, a by-product of neem fruit processing, represent a locally available biomass in significant quantities. The valorization of this biomass through anaerobic digestion and the subsequent use of the resulting digestate align with the broader search for locally adapted alternatives to mineral fertilizers.

Unlike raw manure, digestate from anaerobic digestion offers a high proportion of nitrogen that is immediately plant-available, particularly in ammoniacal form, making it a more efficient fertilizer (Pranckietienė et al., 2023). However, the agronomic efficiency of digestate depends strongly on the feedstock used for anaerobic digestion and on the post-digestion management method, particularly solid-liquid separation (Lamolinara et al., 2022; Romio et al., 2024). Solid fraction acts primarily as slow-release organic soil amendment, whereas the liquid fraction behaves as a fast-acting liquid fertilizer. For the short-cycle, nitrogen-demanding crops such as lettuce, nitrogen release kinetics represent a critical parameter in fertilizer selection and positioning. Yet few studies have directly compared the agronomic effectiveness of the solid and liquid fractions of digestate derived from neem fruit pulp anaerobic digestion, applied separately or in combination, against reference mineral and organic fertilizers on lettuce growth and yield under tropical conditions.

This study aimed to evaluate and compare the agronomic effectiveness of solid and liquid digestate fractions from neem fruit pulp, compost, and synthetic mineral fertilizer, applied at an isonitrogen rate of 100 kg N ha^{-1}. Specific objectives were to: (1) characterize the physicochemical properties of the different digestate fractions and reference fertilizers; (2) evaluate the effects of these treatments on lettuce vegetative growth, as assessed by leaf number, leaf spread diameter, and leaf color chart index (LCC); and (3) compare marketable leaf yields obtained under the different fertilizer treatment.

2. Material and Methods

2.1 Study Site

The trial was conducted at Lomé Agricultural Experiment Station (SEAL) of the Higher School of Agronomy of the University of Lomé, Togo (06°17'N, 001°21'E, approximately 19-60 m above sea level). The station hosts an anaerobic digestion unit, a neem fruit (*Azadirachta indica* A. Juss) processing unit for biopesticide production, and livestock husbandry units (cattle, sheep, and goats). Neem fruit processing generates residual fruit pulp, which constitute the primary substrate for anaerobic digestion, while cattle manure produced on-site serves as co-substrate. The study area has a Guinean-type climate characterized by four alternating seasons, two rainy and two dry.

2.2 Soil Characteristics

The soil at the experimental site is sandy loam with low organic matter content (Toundou, 2016). Physicochemical analyses carried out prior to trial establishment confirmed this low natural fertility, with organic matter (OM) of 1.2 %, total nitrogen of 0.14 %, total organic carbon (TOC) of 0.9 %, pH of 6.1, total phosphorus of 0.06 %, and potassium of 0.1 %.

2.3 Plant Material

Seeds of leaf lettuce (*Lactuca sativa* L.) cultivar "KOKILA" (Technisem, Longué-Jumelles, France), selected for its heat tolerance and suitability for cultivation under tropical conditions, were used as plant material.

2.4 Fertilizers Tested

Four types of fertilizers were evaluated in this trial alongside an unfertilized control:

- **Solid digestate (SD)**: solid fraction obtained after solid-liquid separation of raw digestate from neem fruit pulp anaerobic digestion (see section 2.6.2).
- **Liquid digestate (LD)**: liquid fraction recovered after solid-liquid separation and filtration of the same raw digestate (see section 2.6.2).
- **ENPRO compost**: a commercially available compost produced from municipal solid waste, widely used by market gardeners in Togo, included as an organic fertilizer reference.

- **NPK 15-15-15 mineral fertilizer** : a compound mineral fertilizer included as a mineral reference treatment.

2.5 Digestate Processing and Experimental Equipment

Solid-liquid separation of raw digestate was performed using a 50-liter settling tank followed by filtration through a 0,5 mm nylon mesh sieve. After 48 hours of gravitational settling, the liquid digestate was collected in 25-liter containers. Experimental plots were delineated using a tape measure and string, and identified with numbered stakes. Soil preparation was carried out manually using hoes and rakes. Fertilizers doses and lettuce fresh were weighed using a precision balance (±0,1 g accuracy) and liquid volumes were measured with a graduated cylinder. Liquid fertilizers were applied using a backpack pressure sprayer. Growth variables were measured with a measuring tape.

2.6 Digestate Production and Physicochemical Characterization

2.6.1 Anaerobic Digestion Process and Digestate Production

The digestate used in this trial was produced at Lomé Agricultural Experiment Station (SEAL) anaerobic digestion unit. The substrate introduced into the digester consisted of neem fruit pulp obtained from fruits depulping and fresh cow manure in a ratio 50:50 (dry weight basis). The total load introduced was 167 kg at dry matter content of 15 %. Anaerobic digestion was carried out under mesophilic conditions at an average temperature of 32,5 °C, with a hydraulic retention time of 45 days. At the end of the digestion process, the raw digestate underwent solid-liquid separation as described in section 2.6.2.

2.6.2 Solid-liquid separation of Raw Digestate

Prior to separation, the raw digestate was homogenized by manual stirring and transferred to a 50-liter settling tank placed in a stable location away from sunlight. The digestate was allowed to settle by gravity for 48 hours, during which the densest particles accumulated at the bottom of the tank as the solid fraction, while the aqueous fraction formed a supernatant layer above.

At the end of settling period, the supernatant was collected using a small pitcher into a 25-liter container, taking care not to draw up the sediment. The residual solid fraction was then poured on to 0,5 mm nylon mesh sieve suspended above a collection container. The liquid that drained by gravity through the sieve was added to the previously collected supernatant, constituting total volume of liquid digestate. The solid fraction retained on the sieve constituted the solid digestate.

2.6.3 Physicochemical Characterization of Fertilizers

Representative samples of each fertilizer, solid digestate and liquid digestate and ENPRO compost were collected and analyzed for the following parameters: pH, organic matter (OM), total organic carbon (TOC), total nitrogen (N), total phosphorus (P), total potassium (K), and C/N ratio.

The pH was measured by potentiometry (VIVOSUN pH meter) on a 1:5 (m/v) suspension in distilled water for solid matrices, and directly on liquid digestate. Organic matter content was determined by loss on ignition at 550°C for 4 hours (NF EN 13039). Total organic carbon was calculated from OM content using the Van Bemmelen conversion factor (TOC = OM × 0.58). Total nitrogen was determined by the Kjeldahl method (NF ISO 11261). Total phosphorus and total potassium were quantified after acid mineralization by the molybdate colorimetric method and flame photometry respectively. The C/N ratio was calculated as the TOC/total N ratio. The total nitrogen content of each fertilizer, determined by laboratory analysis and summarized in Table 3, served as the basis for calculating the application doses.

2.7 Experimental Design

The trial was set up as a randomized complete block design (RCBD) comprising six treatments and three replicates, for a total of 18 experimental plots. The factor studied was the type of fertilizer applied. Blocks were established to account for spatial soil heterogeneity. The six treatments were as follows: T0 (unfertilized control), TN (NPK 15-15-15 mineral fertilizer), TC (ENPRO compost), TSD (solid digestate), TLD (liquid digestate), and TM (mixed digestate: 50 % SD + 50 % LD on a nitrogen equivalent basis). Each experimental plot measured 2.0 m × 1.2 m (2.4 m2), with 0.5 m between plots and 1.0 m between blocks.

2.7.1. Nursery Management and Transplanting

KOKILA lettuce seeds were sown in nursery beds 15 days before transplanting. Seedlings were raised under shade with daily irrigation and transplanted at the 15-day stage, corresponding to 3-true-leaf stage, following a 25 cm × 35 cm planting pattern with 20 plants per plot. Growth and yield measurements were restricted to the 8 central plants in each plot to eliminate border effects.

2.7.2 Calculation and Application of Fertilizer Doses

Fertilizer doses were calculated on the basis of uniform isonitrogen application of 100 kg N ha^{-1} (equivalent to 0,024 kg N per 2,4 m^2 plot), using the total nitrogen content determined by laboratory analysis for each fertilizer. The calculated doses and corresponding application methods are summarized in Table 1.

Table 1. Calculated doses and application methods for each treatment

Treatment	Fertilizer	Dose (t or L ha^{-1})	Quantity per plot	Application method	Application schedule
T0	None	-	-	-	-
TN	NPK 15-15-15	0.67 t ha^{-1}	0.16 kg	Soil drench application	DAT7, DAT14, DAT21 (1/3 each)
TC	ENPRO compost	20 t ha^{-1}	4.80 kg	Soil incorporation	Basal dressing (DAT-2)
TSD	Solid digestate	23.81 t ha^{-1}	5.71 kg	Soil incorporation	Basal dressing (DAT-2)
TLD	Liquid digestate	16666.7 L ha^{-1}	4.00 L	Soil drench application	DAT7, DAT14, DAT21 (1/3 each)
TM	SD + LD (50/50)	11,90 t ha^{-1} + 8333.3 L ha^{-1}	2.86 kg + 2.00 L	Soil incorporation + Soil drench application	SD : Basal dressing (DAT-2); LD : DAT7, DAT14, DAT21

DAT = days after transplanting ; DAT-2 = two days before transplanting; DAT7, DAT14, DAT21 = 7th, 14th, and 21st days after transplanting.

Solid fertilizers (solid digestate, ENPRO compost, and the solid fraction of mixed digestate) were evenly broadcast over the plot surface and incorporated into the top 10 cm of soil using a rake two days before transplanting (basal dressing, DAT-2). Liquid fertilizers (TN, TLD, and the liquid fraction of TL) were applied as a soil drench using a backpack sprayer, each diluted in 10 L of water per plot, in three equal fractions at the 7th,14th and 21th days after transplanting (DAT7, DAT14, DAT21).

2.7.3 Crop management and irrigation

All plots received uniform irrigation twice daily (morning and evening) using a watering can at a rate of 6 L $plot^{-1}$ per irrigation event throughout the crop cycle. Weeds were removed manually on a weekly basis, with identical management applied across all plots.

2.7.4 Data Collection

Growth measurements and nitrogen status monitoring were carried out on the eight central plants of each plots at 7, 17, 27, and 37 days after transplanting (DAT). Harvesting took place at 40 DAT. The variables measured, measurement dates, and data collection protocols are summarized in Table 2.

Table 1. Variables measured, measurement dates, and data collection protocols

Variable	Measurement dates (DAT)	Measurement protocol
Leaf number	7; 17; 27; 37	Count of fully expanded leaves on the 8 central plants per plot
Leaf spread diameter (cm)	7; 17; 27; 37	Measurement with a tape measure along the largest horizontal axis of the foliage of the 8 central plants per plot
Leaf color chart index (LCC)	7; 17; 27; 37	Visual comparison of the most recently fully expanded leaf of each of 8 central plants with the IRRI Leaf Color Chart. Three readings per leaf; retained value = mean of three readings. Ordinal scale 1 to 6
Marketable yield (t ha^{-1})	40 (harvest)	All plants per plot harvested at collar level and weighed using a precision balance; yield calculated as follow: $Yield\ (t\ ha^{-1}) = \frac{Fresh\ weight\ per\ plot\ (kg)\times 10}{Plot\ area\ (m^2)}$

DAT = days after transplanting

2.8 Statistical Analysis

Data were analyzed using R software (version 4.5.1). Growth variables (leaf number, leaf spread diameter, and leaf color chart index), recorded at 7, 17, 27, and 37 days after transplanting, were analyzed using a linear mixed model in which treatment, measurement date, and their interaction were treated as fixed effects, and block as a random effect, a structure appropriate for repeated measurements taken on the same plots within a randomized complete block design. Marketable yield, measured once at harvest, was analyzed with the same model including treatment as a fixed effect and block as a random effect. Leaf color chart (LCC) index data, although measured on a discrete ordinal scale from 1 to 6, were analyzed within the same framework after verifying residual normality, in accordance with standard practice in agronomic literature when the number of ordinal classes is sufficient. Prior to analysis, the assumptions of normality of residuals and homogeneity of variances were verified using the Shapiro-Wilk and Levene tests, respectively. When the treatment effect was significant ($P < 0.05$), means were separated at each date using Tukey's Honest Significant Difference (HSD) test. Results are presented as means ± standard error.

The IRRI Leaf Color Chart (LCC) is a reference tool for visually estimating plant nitrogen status by comparing foliage color with a calibrated colorimetric scale ranging from 1 (pale green, indicating nitrogen deficiency) to 6 (dark green, indicating adequate nitrogen status).

3. Results And Discussion

3.1 Results

3.1.1 Physicochemical Characterization of Fertilizers

Laboratory analysis of the four fertilizers revealed contrasting physicochemical properties, as summarized in Table 3.

Organic matter content, total nitrogen content, and C/N ratios varied considerably among fertilizers. Solid digestate (SD) was characterized by the highest organic matter content (76.2 %) and a slightly acidic pH of 6.8, but exhibited the lowest total nitrogen content among the organic fertilizers (0.42 %), with a C/N ratio of 18.2. Liquid digestate (LD) had the highest total nitrogen content among the organic fertilizers (0.60 %), the lowest C/N ratio (7.9), and a neutral pH of 7.2. ENPRO compost exhibited an alkaline pH of 8.25, an intermediate total nitrogen content (0.50 %), and a C/N ratio of 24.7. The NPK 15-15-15 mineral fertilizer, used as the mineral reference, contained 15 % each of the three major nutrient (N, P and K).

3.1.2 Effects of Treatments on Leaf Number

Fig. 1 illustrates the temporal dynamics of leaf number in lettuce plants throughout the growing cycle according to fertilizer treatment.

Table 3. Physicochemical characteristics of the fertilizers used in the trial

Fertilizers	pH	OM (%)	N (%)	P (%)	K (%)	C/N ratio
Liquid digestate (LD)	7.2	4.3	0.6	0.15	0.38	7.9
Solid digestate (SD)	6.8	76.2	0.42	0.68	0.17	18.2
ENPRO compost	8.25	26.1	0.5	0.03	0.40	24.7
NPK 15-15-15	-	-	15.0	15.0	15.0	-

OM = organic matter; C/N = carbon-nitrogen ratio; (-) = not applicable

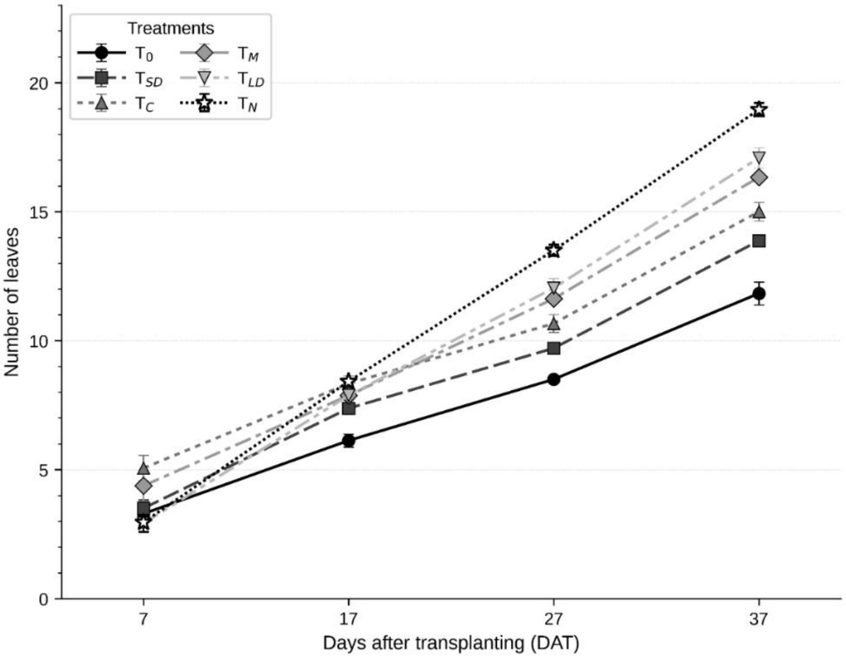

Fig. 1. Changes in leaf number of lettuce plants throughout the growing cycle according to fertilizer treatment

T_0 = control (no fertilization); TSD = solid digestate; TC = ENPRO compost; TM = mixed digestate (50 % SD + 50 % LD); TLD = liquid digestate; T_N = NPK 15-15-15 mineral fertilizer. Bars represent standard errors of the mean (n = 3)

During the first week after transplanting, leaf number remained relatively low across all treatments. Analysis of variance revealed a significant effect of treatments on leaf production at 7 DAT ($P = 0.031$), with values ranging from 2.92 ±0.34 leaves for TLD (liquid digestate) to 5.06 ±0.49 leaves for TC (ENPRO compost) (Table 4).

Table 4. Leaf number per treatment across the growing cycle (7, 17, 27, and 37 DAT)

Traitement	Number of leaves			
	7 DAT	17 DAT	27 DAT	37 DAT
T0	3.29 ± 0.54 a	6.13 ± 0.25 c	8.50 ± 0.12 d	11.83 ± 0.44 d
TSD	3.50 ± 0.32 a	7.38 ± 0.07 b	9.71 ± 0.11 c	13.88 ± 0.22 c
TC	5.06 ± 0.49 a	8.34 ± 0.29 a	10.67 ± 0.34 c	15.00 ± 0.36 c
TM	4.38 ± 0.62 a	7.88 ± 0.19 a	11.63 ± 0.14 b	16.34 ± 0.11 b
TLD	2.92 ± 0.34 a	7.88 ± 0.26 a	12.05 ± 0.37 b	17.09 ± 0.40 b
TN	2.96 ± 0.37 a	8.42 ± 0.04 a	13.50 ± 0.22 a	18.96 ± 0.25 a
p-value	0.031	< 0.001	< 0.001	< 0.001

T_0 = control (no fertilization); TSD = solid digestate; TC = ENPRO compost; TM = mixed digestate (50 % SD + 50 % LD); TLD = liquid digestate; T_N = NPK 15-15-15 mineral fertilizer; Means ± standard error; different letters within the same column indicate significant differences (Tukey's test, $p < 0.05$)

Beyond 7 DAT, leaf production increased markedly and differentially among treatments. At 17 DAT, analysis of variance revealed a highly significant treatment effect ($P < 0.001$) on leaf production. The NPK 15-15-15 (8.42 ± 0.04 leaves) and ENPRO compost (8.34 ± 0.29 leaves) treatments recorded the highest leaf numbers, significantly greater than that of the unfertilized control T0 (6.13 ±0.25 leaves), which recorded the lowest leaf number at this date (Table 4).

This trend became more pronounced at 27 and 37 DAT, with highly significant differences among treatments at both dates ($P < 0.001$). At 37 DAT, Tukey's HSD test identified five distinct homogeneous groups. The highest leaf number was recorded in the TN treatment (18.96 ± 0.25 leaves), followed by TLD (17.09 ±0.40 leaves), TM (16.34 ± 0.11 leaves), TC (15.00 ± 0.36 leaves), TSD (13.88 ± 0.22 leaves), and T0 (11.83 ± 0.44 leaves). The treatment hierarchy established at 17 DAT was thus maintained and reinforced throughout the remainder of the growing cycle.

3.1.3 Effect of Treatments on Leaf Spread Diameter

Changes in leaf spread diameter according to treatment are illustrated in Figure 2 and Table 5. A highly significant effect of fertilizer type on leaf spread diameter was detected at 7 DAT ($P < 0.001$). The ENPRO compost treatment (6.73 ±0.18 cm) was significantly greater than all other treatments, which formed a homogeneous group with values ranging from 5.29 ±0.04 cm (TN) to 5.35 ±0.07 cm (T0).

At 17 DAT, a highly significant treatment effect on leaf spread diameter was observed ($P < 0.001$). The unfertilized control T0 (7.52 ±0.31 cm) recorded significantly lower values than all fertilized treatments, which formed a homogeneous group with values ranging from 8.64 ±0.22 cm (TSD) to 9.65 ±0.05 cm (TN).

At 27 and 37 DAT, the hierarchy among treatments was fully differentiated, with highly significant differences among all treatments ($P < 0.001$). At 37 DAT, the highest leaf spread diameter was recorded for TN (19.89 ±0.41 cm), followed by TLD (18.81 ±0.10 cm). The TM (17.54 ±0.12 cm) and TC (16.85 ±0.12 cm) treatments formed a homogeneous intermediate group, while TSD (15.04 ±0.38 cm) and T0 (13.24 ±0.56 cm) recorded the lowest values, each forming a statistically distinct group.

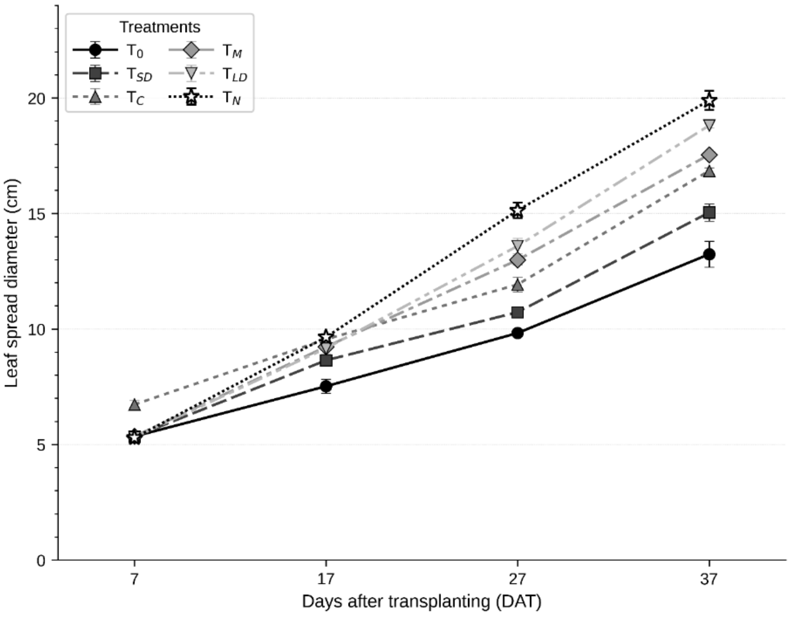

Fig. 2. Changes in leaf spread diameter of lettuce plants throughout the growing cycle according to fertilizer treatment

T0 = control (no fertilization); TSD = solid digestate; TC = ENPRO compost; TM = mixed digestate (50 % SD + 50 % LD); TLD = liquid digestate; TN = NPK 15-15-15 mineral fertilizer; Bars represent standard errors of the mean (n = 3)

Table 5. Leaf spread diameter (cm) per treatment across the growing cycle (7, 17, 27, and 37 DAT)

Treatment	Leaf spread diameter (cm)			
	7 DAT	17 DAT	27 DAT	37 DAT
T0	5.35 ± 0.07 b	7.52 ± 0.31 b	9.82 ± 0.13 d	13.24 ± 0.56 d
TSD	5.34 ± 0.05 b	8.64 ± 0.22 a	10.73 ± 0.10 c	15.04 ± 0.38 c
TC	6.73 ± 0.18 a	9.55 ± 0.25 a	11.93 ± 0.32 c	16.85 ± 0.12 b
TM	5.35 ± 0.02 b	9.23 ± 0.14 a	12.99 ± 0.20 b	17.54 ± 0.12 b
TLD	5.31 ± 0.02 b	9.16 ± 0.25 a	13.59 ± 0.33 b	18.81 ± 0.10 a
TN	5.29 ± 0.04 b	9.65 ± 0.05 a	15.13 ± 0.34 a	19.89 ± 0.41 a
p-value	< 0.001	< 0.001	< 0.001	< 0.001

T_0 = control (no fertilization); TSD = solid digestate; TC = ENPRO compost; TM = mixed digestate (50 % SD + 50 % LD); TLD = liquid digestate; T_N = NPK 15-15-15 mineral fertilizer; Means ± standard error (cm); different letters within the same column indicate significant differences (Tukey's test, p < 0.05)

3.1.4 Effect of Treatments on Leaf Color Chart (LCC) Index

Monitoring of the leaf color chart (LCC) index throughout the growing cycle provided insight into changes in the nitrogen status of lettuce plants under the different fertilizer treatments (Fig; Table 6). Analysis of variance revealed a significant fertilizer type effect on LCC index at all measurement dates.

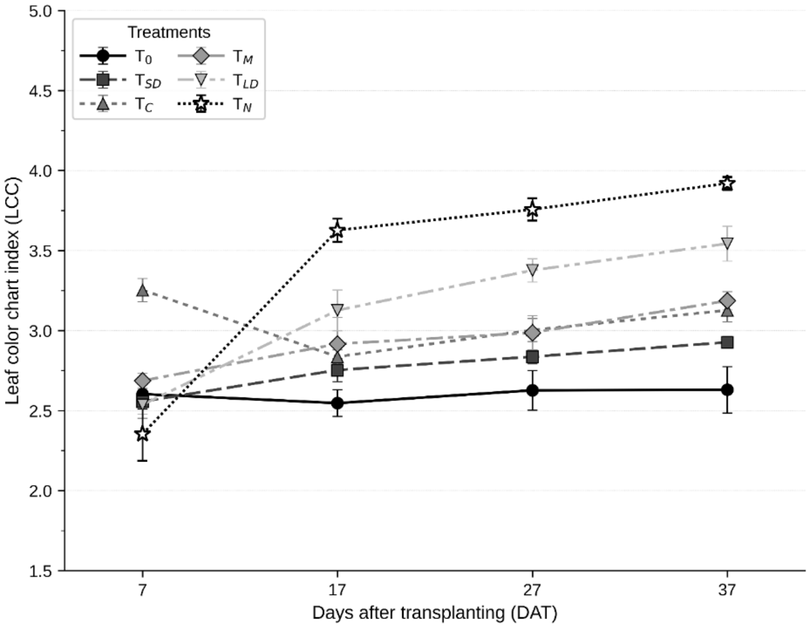

Fig. 3. Changes in leaf color chart (LCC) index of lettuce plants throughout the growing cycle according to fertilizer treatment

T0 = control (no fertilization); TSD = solid digestate; TC = ENPRO compost; TM = mixed digestate (50 % SD + 50 % LD); TLD = liquid digestate; TN = NPK 15-15-15 mineral fertilizer. Bars represent standard errors of the mean (n = 3)

At 7 DAT, the LCC index was significantly influenced by fertilizer type ($P = 0.003$). The ENPRO compost (TC) treatment recorded the highest LCC value (3.25 ±0.07), significantly greater than all other treatments, which formed a homogeneous group with values ranging from 2.35 ±0.17 (NPK 15-15-15, TN) to 2.69 ±0.05 (mixed digestate, TM).

From 17 DAT onward, a clearly differentiated trend emerged among treatments, with highly significant differences ($P < 0.001$). TN (NPK 15-15-15 mineral fertilizer) recorded the highest LCC value (3.63 ±0.07), significantly greater than all other treatments, while T0 had the lowest value (2.55 ±0.08), significantly different from all fertilized treatments.

At 27 and 37 DAT, the highly significant treatment effect on plant nitrogen status was maintained ($P < 0.001$). At the final measurement date, the highest LCC values were recorded for TN (3.92 ±0.04) and liquid digestate TLD (3.54 ±0.11), which formed a distinct homogeneous group, ENPRO compost TC (3.13 ±0.07), mixed digestate TM (3.19 ±0.06), and solid digestate TSD (2.93 ±0.04) constituted an intermediate homogeneous group, while T0 (2.63 ±0.14) recorded the lowest LCC value throughout the cycle.

Table 6. Leaf color chart (LCC) index per treatment across the growing cycle (7, 17, 27, and 37 DAT)

Traitement	**LCC index**			
	7 DAT	**17 DAT**	**27 DAT**	**37 DAT**
T0	2.60 ± 0.09 b	2.55 ± 0.08 c	2.63 ± 0.12 b	2.63 ± 0.14 c
TSD	2.56 ± 0.10 b	2.75 ± 0.07 b	2.84 ± 0.04 b	2.93 ± 0.04 b
TC	3.25 ± 0.07 a	2.84 ± 0.04 b	3.00 ± 0.07 b	3.13 ± 0.07 b
TM	2.69 ± 0.05 b	2.92 ± 0.07 b	2.99 ± 0.11 b	3.19 ± 0.06 b
TLD	2.54 ± 0.06 b	3.13 ± 0.13 b	3.38 ± 0.07 a	3.54 ± 0.11 a
TN	2.35 ± 0.17 b	3.63 ± 0.07 a	3.76 ± 0.07 a	3.92 ± 0.04 a
p-value	0.003	< 0.001	< 0.001	< 0.001

T_0 = control (no fertilization); TSD = solid digestate; TC = ENPRO compost; TM = mixed digestate (50 % SD + 50 % LD); TLD = liquid digestate; T_N = NPK 15-15-15 mineral fertilizer; Means ± standard error; different letters within the same column indicate significant differences (Tukey's test, p < 0.05)

3.1.5 Effect of Treatments on Marketable Yield

Marketable yield results according to fertilizer treatment are presented in Fig. . Analysis of variance revealed a highly significant treatment effect on lettuce marketable yield ($P < 0.001$). Yields ranged from 6.10 ±0.08 t ha^{-1} for T0 (control) to 14.93 ±0.18 t ha^{-1} for TN (NPK 15-15-15 mineral fertilizer). Compared to the unfertilized control, yield gains were 144.8 % for TN, 124.6 % for TLD (liquid digestate), 86.2 % for TM (mixed digestate), 85.7 % for TC (ENPRO household compost), and 47.5 % for solid digestate TSD (Fig.).

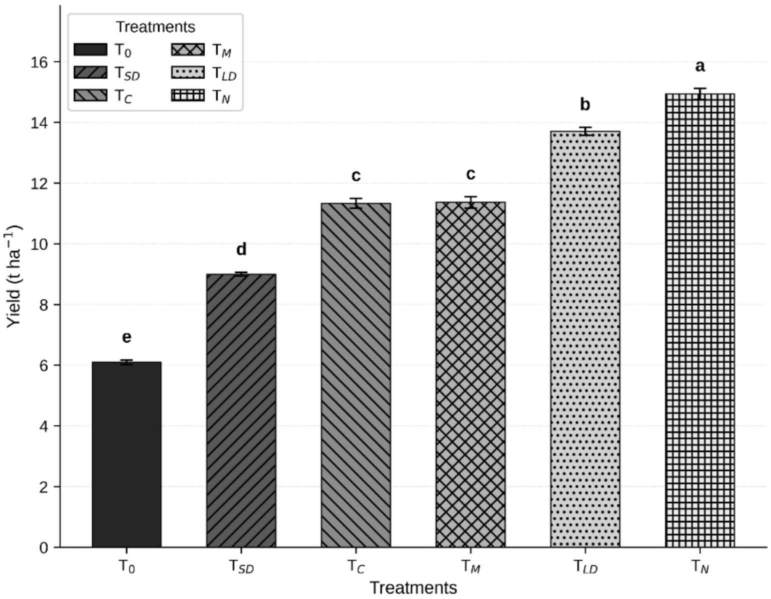

Fig. 4. Effect of fertilizer treatments on marketable yield of lettuce plants

T0 = control (no fertilization); TSD = solid digestate; TC = ENPRO compost; TM = mixed digestate (50 % SD + 50 % LD); TLD = liquid digestate; TN = NPK 15-15-15 mineral fertilizer. Bars represent standard errors of the mean (n = 3)

3.2 Discussion

3.2.1 Physicochemical Characteristics of Fertilizers and Their Agronomic Implications

The physicochemical analysis of fertilizers revealed markedly contrasting compositions that help to explain the observed agronomic responses. The total nitrogen contents of solid digestate (0.42 %), liquid digestate (0.60 %), and ENPRO compost (0.50 %) fall within the range reported in the literature for similar organic fertilizers (Akhiar et al., 2017; Alan et al., 2025; Czekała, 2022; Horta & Carneiro, 2022). These values are, however, significantly lower than that of NPK 15-15-15 mineral fertilizer (15 % N), which means that much larger volumes and quantities of organic fertilizer are required to reach the target nitrogen rate of 100 kg N ha^{-1}.

Solid-liquid separation of the raw digestate concentrated ammoniacal nitrogen in the liquid fraction and organic carbon in the solid fraction, generating markedly contrasting C/N ratios: 7.9 for liquid digestate, 18.2 for solid digestate, and 24.7 for ENPRO compost (Lamolinara et al., 2022; Romio et al., 2024). The C/N ratio is a key indicator of rate at which organic nitrogen is mineralized and therefore of its availability to plants. Liquid digestate, with the lowest C/N ratio among the organic fertilizers tested (C/N = 7.9), exhibited the most favorable conditions for rapid nitrogen mineralization, consistent with the finding of Lazicki et al., (2020), who established a strong predictive relationship between C/N ratio and plant-available nitrogen after 84 days of incubation (R^2 = 0.92), with net mineralization prevailing below a threshold of approximately 14 to 19 depending on amendment type. In contrast, solid digestate (C/N = 18.2) and ENPRO compost (C/N = 24.7) exhibited slower mineralization kinetics, consistent with values typically reported in the literature for these types of amendments (El Janati et al., 2025; Geisseler et al., 2021). Van der Sloot et al. (2022) further confirmed that organic amendments with C/N ratios above 20 reduce crop biomass during the ongoing cycle while accumulating mineral nitrogen in the soil, suggesting that solid digestate and ENPRO compost are better suited as basal amendments for subsequent cropping seasons than as immediate nitrogen sources for short-cycle crops such as lettuce.

The measured pH values also revealed meaningful differences among fertilizers. Liquid digestate (pH = 7.2) and solid digestate (pH = 6.8) fall within the slightly acidic to neutral ranges typically reported for digestates produced by anaerobic digestion of plant-based substrates (Alan et al., 2025). The slightly higher pH of liquid digestate is attributable to the preferential concentration of ammoniacal nitrogen (NH_4^+/NH_3) in the aqueous fraction during the separation phase, as free ammonia in solution consumes protons and thereby raises pH (Camilleri-Rumbau et al., 2021; Romio et al., 2024). ENPRO compost had the highest pH (8.25), attributable to the accumulation of carbonates and bicarbonates resulting from the decomposition of organic matter during composting (Piccolo & Drosos, 2025). While this alkaline pH can improve phosphorus availability in slightly acidic soils such as that of the experimental site (pH = 6.1), it also shows a risk of ammoniacal nitrogen volatilization during application, which may reduce the apparent nitrogen use efficiency of the amendment (Chen et al., 2023; Debicka et al., 2023).

3.2.2 Effect of Treatments on Vegetative Growth

At 7 DAT, a statistically significant effect on leaf number was already detected (P = 0.031), although the differences among treatments remained modest at this early stage. In contrast, a highly significant effect on leaf spread diameter was observed at the same date (P < 0.001), with TC (ENPRO compost) recording a substantially greater value than all other treatments.

These two responses dynamics reflect the different sensitivities of the variables considered. Leaf numbers, which depends primarily on plant's internal morphogenetic processes, responded more gradually to fertilizer applications, whereas leaf spread diameter, directly linked to leaf cell expansion, reacted more rapidly to the supply of plant-available mineral nutrients.

The early advantage of TC in leaf spread diameter at 7 DAT is attributable to the high pH of ENPRO compost (pH = 8.25), which may have promoted the initial solubilization of certain nutrients, combined with the stimulatory effect of partially humified organic matter on root activity (Piccolo & Drosos, 2025). However, this initial lead did not persist through the crop cycle. By 37 DAT, liquid digestate (TLD) had emerged as the best-performing organic fertilizer for both vegetative growth variables, reflecting a sustained and progressive availability of plant-available nitrogen from the liquid digestate throughout the growing cycle.

From 17 DAT onward, the hierarchy among treatments stabilized in the order $TN > TLD > TM \approx TC > TSD > T0$ for both growth variables, a ranking directly consistent with nitrogen availability of each fertilizer as predicted by their respective C/N ratios. Similar results were reported by Jimenez et al. (2020) and Iocoli et al. (2019), who demonstrated in lettuce cultivation that C/N ratio of digestate is the primary determinant of plant nitrogen uptake, regardless of the climatic conditions of the study site. The statistical equivalence observed between TM (mixed digestate) and TC (ENPRO compost) suggest that combining equal parts of the two digestate fractions on a nitrogen equivalent basis did not produce a synergistic effect on vegetative growth beyond that achieved by compost alone

3.2.3 Effect of Treatments on Plant Nitrogen Status

Changes in the leaf color chart (LCC) index provided a direct illustration of the nitrogen uptake dynamics inducted by the different fertilizer treatments. At 7 DAT, the high LCC value recorded for ENPRO compost, TC (3.25) contrasted with the lowest value observed for NPK 15-15-15, TN (2.35). This pattern reflects the absence of any nitrogen input prior to transplanting for the split mineral fertilizer treatment, whose first application occurred precisely at 7 DAT. The high pH of ENPRO compost (pH = 8.25) likely stimulated the activity of soil ammonifying bacteria upon application (Breugem et al., 2024; Lucia et al., 2026), thereby accelerating early organic mineralization and explaining the relatively high LCC values observed for TC at this date.

The steady and progressive increase in LCC values for liquid digestate (TLD) throughout the crop cycle, from 2.54 at 7 DAT to 3.54 at 37 DAT, confirmed the gradual yet sustained availability of plant-available nitrogen from the liquid digestate, consistent with its low C/N ratio of 7.9. This nitrogen release pattern corresponds to the expected behavior of a rapidly mineralizing organic fertilizer, as described by van Midden et al. (2024), who reported that anaerobic digestion mineralize between 15 % and 82 % of substrate-bound organic nitrogen into ammonium, a form directly available to plants. In contrast, the relatively stable and modest LCC values recorded for solid digestate (TSD) throughout the cycle confirmed the slower nitrogen mineralization kinetics of this fraction, constrained by its C/N ratio of 18.2, which places it within the immobilization zone identified by Lazicki et al. (2020).

The LCC values remaining below 4 for all treatments, including TN (3.92 at 37 DAT), suggest that the applied rates, although equivalent to 100 kg N ha^{-1}, did not fully meet the crop's nitrogen requirements under the experimental conditions. This may be partly attributable to nitrogen losses through ammonia volatilization during soil drench application, a phenomenon particularly likely at temperatures exceeding 30°C (Fan et al., 2023; Govindasamy et al., 2023). This observation highlights the need to optimize both application rates and methods for digestate-based fertilizers under tropical conditions.

3.2.4 Effect of Treatments on Marketable Yield

Marketable yield is the key integrative variable of this experiment. The highly significant treatment effect on yield ($P < 0.001$) confirmed that differences in the physicochemical composition of the fertilizers translated into distinct agronomic responses. The yield recorded under NPK 15-15-15 mineral fertilizer TN (14.93 t ha^{-1}) represent the best overall performance, consistent with the results of numerous studies demonstrating the superiority of soluble mineral fertilizers for short-cycle crops such as lettuce. At comparable doses to those used in the present trial, García-Rández et al. (2025) reported yield increases of 243.97 % and 182.49 % with NPK 15-15-15 compared respectively to the unfertilized control and sewage sludge. Meskelu et al. (2024) similarly reported a 21.72 % yield increase with mineral fertilizers over the unfertilized control.

Among organic fertilizers, liquid digestate (TLD) recorded the highest marketable yield (13.70 t ha^{-1}), a value not statistically different from that of TN according to Tukey's HSD test, representing a 124.6 % increase over the unfertilized control. This performance is attributable to the combination of a high total nitrogen content (0.60 %), a low C/N ratio (7.9) promoting rapid nitrogen mineralization, and a near-neutral pH (7.2) limiting the risk of ammonia volatilization during application. The superior performance of liquid digestate relative to other organic fertilizers is consistent with the findings of Fedeli et al. (2023), who reported a 22.9 % increase in fresh leaf biomass under controlled greenhouse conditions using liquid digestate of maize, pomace, and turkey manure. The 124.6 % yield gain recorded for TLD in the present trial is substantially greater than the 22.9 % reported by (Fedeli et al., 2023), a discrepancy attributable to differing experimental conditions. Tropical conditions at the study site likely promoted accelerated mineralization of ammoniacal nitrogen from the liquid digestate, unlike the controlled temperature of 15 ±2 °C used by the authors. Furthermore, the isonitrogen rate of 100 kg N ha^{-1} applied in the present study ensured optimal coverage of the crop's nitrogen requirements, whereas Fedeli et al. (2023) applied an arbitrary dose of 3 % of the total potting mix mass.

The statistical equivalence between mixed digestate TM (11.36 t ha^{-1}) and ENPRO compost TC (11.33 t ha^{-1}) indicated that the mixed digestate combining 50 % SD and 50 % LD equivalent basis produced no synergistic effect on yield compared to compost alone. This absence of synergy may be explained by the dilution of the liquid fraction within the mixture, which reduced the beneficial effects specific to liquid digestate without the solid fraction providing sufficient nitrogen supplementation over such a short crop cycle. This result aligns with the observations of Ronga et al, (2019), who recorded the lowest biomass production among fertilized treatments with the solid digestate + liquid digestate combination in hydroponic lettuce, attributing this underperformance to ammonia toxicity and the high pH of the mixture. In the present field trial, an additional mechanism can be proposed: the slow nitrogen mineralization kinetics of solid digestate (C/N = 18.2), incompatible with the 40-day crop cycle of lettuce, likely prevented this fraction from contributing meaningfully to plant nitrogen nutrition during the growing period.

Solid digestate (TSD) recorded the lowest yield among fertilized treatments (9.00 t ha^{-1}), with a yield gain only 47.5 % over the unfertilized control. This limited performance is attributable to the slow mineralization kinetics of solid digestate (C/N = 18.2), which is incompatible with the short growing cycle of lettuce and prevented this fraction from reaching peak nitrogen release before harvest. The C/N ratio of solid digestate places this fertilizer within the net immobilization zone identified by Lazicki et al. (2020), who established a strong predictive relationship between C/N ratio and mineralizable nitrogen after 84 days ($R^2 = 0.92$), with the threshold between net mineralization and net immobilization falling between C/N 14 and 19 depending on amendment type. These results indicate that TSD is better suited as a long-term soil amendment, either as a basal dressing prior to a long-cycle crop or as a soil preparation input for subsequent growing season, than as an immediate nitrogen source for short-cycle crops such as lettuce.

3.2.5 Study Limitations

This study was conducted over a single cropping cycle (July-August 2025), a relatively warm period with reduced but non-negligible rainfall on the Togolese coast, on a sandy loam soil of low natural fertility characteristic of the Lomé area. Several limitations of this study should be acknowledged. First, fertilizer doses were standardized solely on a total nitrogen basis; phosphorus and potassium inputs were therefore not balanced across treatments and varied according to the intrinsic composition of each fertilizer, so a partial contribution of these nutrients to the observed yield differences cannot be entirely excluded. Second, the trial was conducted over a single 40-day cropping cycle during one growing season, which limits extrapolation to other seasons, soil types, or repeated digestate applications.

4. Conclusion

This study compared the agronomic effectiveness of the solid and liquid fractions of neem fruit pulp digestate against mineral and organic reference fertilizers on lettuce. Among the organic fertilizers tested, the liquid fraction was the most effective, supporting the best vegetative growth and a marketable yield statistically equivalent to that of NPK 15-15-15 mineral fertilizer, owing to its low C/N ratio and rapid supply of plant-available nitrogen. In contrast, the solid fraction, with its slower mineralization kinetics, proved poorly suited to the short cycle of lettuce and would be better valorized as a soil amendment for longer-cycle crops or as a basal input ahead of subsequent seasons. The mixed digestate offered no synergistic advantage over compost.

These results stem from a single short cropping cycle (July-August) under specific coastal conditions, with treatment doses standardized on nitrogen alone; phosphorus and potassium inputs were therefore not balanced across treatments. Multi-season trials balancing all major nutrients, extending to other short-cycle crops, and including an economic assessment of the neem–biogas–digestate value chain would help confirm these findings and evaluate their scalability for smallholder market gardeners in Togo.

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Disclaimer (Artificial Intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

Competing Interests

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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