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Evaluation of the Performance of a Nitrogen Treatment Plant in a Continental Mediterranean Climate: A Spanish Pig Farm Case Study

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Abstract

This study presents a four-year evaluation (2020–2024) of an integrated climate mitigation project on a pig farm in Ávila, Spain, at an elevation of over 1100 m above sea level with continental climate conditions. The project aimed to reduce greenhouse gas emissions (GHG) and nitrogen pollution by implementing solid–liquid filtration followed by biological treatment in a 625 m³ Sequencing Batch Reactor (SBR) operating under a nitrification–denitrification (N-DN) regime. The SBR carried out four daily cycles, alternating aerobic and anoxic phases, with 5 and 8 m³ inlets. Aeration intensity and redox potential were continuously monitored to optimize bacterial activity. Analytical parameters (pH, electrical conductivity, solids content, nitrogen, phosphorus, and potassium) were measured using ISO methods and tracked frequently. Annual emission reductions were 75% for N₂O, up to 97% for NH₃, and 80% for N₂. In the summer months, we observed higher efficiency reduction for N₂, NH₃, and NO₂. Additionally, there was a 75% average reduction for COD and up to 92% for total GHG emissions. This real-world case study highlights the effectiveness of SBR-based N-DN systems for nutrient removal and emission reduction in high-altitude, climate-sensitive regions, contributing to EU nitrate directive compliance and circular economy practices.

Keywords: pig slurry; nitrification–denitrification; nitrogen removal; wastewater treatment; agro-industrial effluents



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1. Introduction

The intensification of livestock farming has led to a significant rise in manure production, especially from pig farms, where slurry management has become a pressing environmental and regulatory concern. Spain is currently the leading producer of pig meat in the European Union (EU), accounting for 24% of EU production, and ranks third globally after China and the United States [1]. Castilla y León is a major contributor among Spanish

regions, generating over 3.5 million pigs annually [2]. This level of production results in an estimated 7 million cubic meters of pig slurry each year [3].

Pig slurry is typically characterized by a high water content and low dry matter, making its direct application to agricultural fields both logistically challenging and environmentally risky. While it is a valuable source of nitrogen, phosphorus, potassium, and organic matter, its high ammonium nitrogen content poses a risk to water bodies due to leaching and runoff, particularly when applied in nitrate vulnerable zones (NVZs). In accordance with the EU Nitrates Directive (91/676/EEC) [4], nitrogen application from organic waste is limited to 170 kg N/ha/year in NVZs. NVZ areas represent a significant extension in Castilla y León. The study NDN plant is located in this Spanish region, encompassing over 14,000 km² (nearly 20% of the region's total area) and including nearly 400 municipalities.

In this context, the challenge of managing livestock waste arises, particularly in transforming the nitrogen in pig slurry into more sustainable and environmentally friendly forms. One effective strategy involves processing the slurry through nitrification–denitrification (NDN) treatment plants, where the ammonium (NH₄⁺) contained in the slurry is biologically converted into molecular nitrogen gas (N₂), which is harmlessly released into the atmosphere [5]. As urea in urine is hydrolyzed to ammonium (NH₄⁺) and subsequently converted to nitrate (NO₃[−]) through microbial nitrification in soils, the excessive or poorly managed application of slurry increases the risk of groundwater contamination and contributes to eutrophication [6]. To address these concerns, pre-treatment of slurry before land application has become increasingly important.

In recent years, Spain has adopted strategies under its national climate policy, such as the “*Clima Projects*” promoted by the Ministry for the Ecological Transition and the Demographic Challenge, which incentivize implementing low-emission technologies in diffuse sectors, including agriculture [7]. Among the main emission sources in pig farming are ammonia volatilization and nitrous oxide emissions derived from slurry management and storage. To reduce these emissions while maintaining nutrient recycling in agriculture, two primary approaches have emerged for nitrogen management in manure: transformation of nitrogen into inert gaseous forms via biological or physicochemical processes [8], and concentration and recovery of nitrogen for use as fertilizer in a more controlled form [9]. Biological treatments integrating solid–liquid filtration and (N-DN) processes offer a technically and economically viable solution for medium to large-scale farms [10,11].

Solid–liquid filtration reduces organic load and suspended solids in the liquid fraction, enhancing the efficiency of biological treatments. The solid fraction can be composted and used as an organic amendment, contributing to soil organic matter restoration—an objective aligned with the EU Soil Strategy and the Farm to Fork strategy [12]. Meanwhile, the liquid fraction can be biologically treated using technologies such as Sequential Batch Reactors (SBRs), which alternate aerobic and anoxic conditions to promote nitrification and subsequent denitrification, effectively reducing total nitrogen content [9,13]. The SBR system has demonstrated robust and cost-effective nitrogen removal for high-strength agro-industrial effluents, such as pig slurry, achieving removal efficiencies ranging from 50% to 90% depending on influent characteristics and operational parameters [14,15].

The province of Ávila, where the Nitrogen Treatment Plant evaluated in this work is located, is characterized by what the Spanish State Meteorological Agency (AEMET) describes as a Continental Mediterranean climate [16]. This climate represents a variation of the typical Mediterranean type but is marked by more pronounced temperature extremes. Due to its inland location and significant elevation, Ávila experiences colder, longer winters—with average temperatures often falling below 3 °C—and warm to hot summers marked by a clear dry period. This continental influence leads to notable daily

and seasonal temperature swings, distinguishing it from the milder Mediterranean climates closer to the coast. A key peculiarity of Ávila is its high altitude: the provincial capital is approximately 1131 m above sea level, making it the highest provincial capital in Spain. This elevation intensifies the continental characteristics of the climate, resulting in cooler temperatures throughout the year and sharper contrasts between seasons compared to other Mediterranean regions at lower altitudes.

These pronounced temperature fluctuations and the generally cooler climate, especially during the extended cold season, pose challenges for biological treatment processes, such as NDN employed in wastewater management. The microbial communities responsible for NDN are highly temperature-sensitive; lower temperatures can slow or inhibit nitrification and denitrification rates, potentially reducing treatment efficiency. Consequently, climatic conditions in Ávila require careful consideration when designing and operating NDN systems to ensure optimal performance throughout seasonal variations.

This study evaluates the implementation of a climate mitigation project titled “Reduction of Greenhouse Gas Emissions in Pig Farms through Integrated Waste Management Technologies,” conducted between 2020 and 2024 on a pig farm in Ávila, Spain. The farm houses 3000 sows and represents a real-case scenario of integrated slurry treatment under Spanish and EU environmental regulations. Notably, this processing plant is situated in a zone with a peculiar climate and high altitude, distinguishing it from many other similar facilities installed in Spain, which are often located in milder, lower-altitude regions. This unique environmental context will influence the plant’s operational dynamics and treatment efficiency. The primary objective of this study was to evaluate the plant’s performance and environmental benefits under different operational configurations, including variations in anoxic and aerobic phase durations, daily treated volumes, reductions in holding times within equalization tanks [17], etc. A secondary objective is to understand how seasonal and interannual climatic variations—reflecting the peculiar climate of the region where the plant is located, with high altitude, sharp temperature shifts in summer, and long, cold winters—influenced its performance over the four-year study period. The conclusions obtained will inform future decision-making regarding plant operation, whose expected optimization will be further analyzed in subsequent research.

2. Materials and Methods

2.1. Farm Description and Project Implementation

This study was conducted at a commercial pig farm in Herreros de Suso (Ávila, Spain) (Figure 1), part of the Kerbest Group, housing approximately 3000 white sows. The treatment system was implemented between 2020 and 2024 under the Clima Project, funded by the Spanish Carbon Fund for a Sustainable Economy (FES-CO₂), with the primary objective of reducing greenhouse gas (GHG) emissions through integrated slurry management.

The facility was designed and installed by Mecàniques Segalés S.L. (Vic, Barcelona, Spain), a company with over 50 years of experience in agricultural waste treatment systems. The treatment line includes mechanical and biological processes recognized as Best Available Techniques (BATs) by Spanish and EU environmental authorities [18].

The integrated nitrogen removal system was monitored at the treatment plant over a four-year period (2020–2024). The facility treats the liquid fraction of pig slurry using a multi-stage process that includes mechanical solid–liquid filtration and biological treatment in a Sequencing Batch Reactor (SBR) configured for NDN.



Figure 1. Localization of Herreros de Suso, Ávila.

2.2. Slurry Pre-Treatment: Equalization and Solid–Liquid Filtration

Pig slurry is initially collected in a reception and equalization tank with a hydraulic retention time (HRT) of approximately 7 days. This tank is equipped with mechanical stirring operated via a timer to ensure homogeneity, reduce fluctuations in composition, and provide a continuous feed for subsequent treatment steps. Before entering the biological stage, the raw slurry undergoes two successive filtration processes designed to reduce suspended solids and optimize downstream reactor performance (see Figure 2):

- **Primary Filtration:** The slurry, homogenized in a reception pit, is pumped to an inclined ramp screen with a 250 μm mesh that retains coarse solids. The screened liquid is transferred to Equalization Tank 1. This first filtration uses the Kompack[®] system (Mecàniques Segalés SL, Barcelona, Spain), which combines a 250 mm ramp filter with a pressure separator. The solid fraction contains approximately 29–30% total solids (TS), $\sim 7 \text{ g}\cdot\text{kg}^{-1}$ total Kjeldahl nitrogen (TKN), and $\sim 1.9 \text{ g}\cdot\text{kg}^{-1}$ total phosphorus (P), making it suitable for composting or off-site agricultural use.
- **Secondary Filtration:** Liquid from Equalization Tank 1 undergoes finer filtration through vibrating sieves with an 80 μm mesh. The resulting clarified liquid is stored in Equalization Tank 2, which acts as the feed reservoir for the SBR. In this step, the liquid fraction undergoes microfiltration via a vibrating screen (FILVI system (Mecàniques Segalés SL, Barcelona, Spain) with a mesh size of 80 μm , yielding a clarified liquid with 10–20 $\text{g}\cdot\text{kg}^{-1}$ TS, $\sim 2.0 \text{ g}\cdot\text{kg}^{-1}$ TKN, and $<0.2 \text{ g}\cdot\text{kg}^{-1}$ P. This clarified effluent is suitable for biological treatment [19,20].

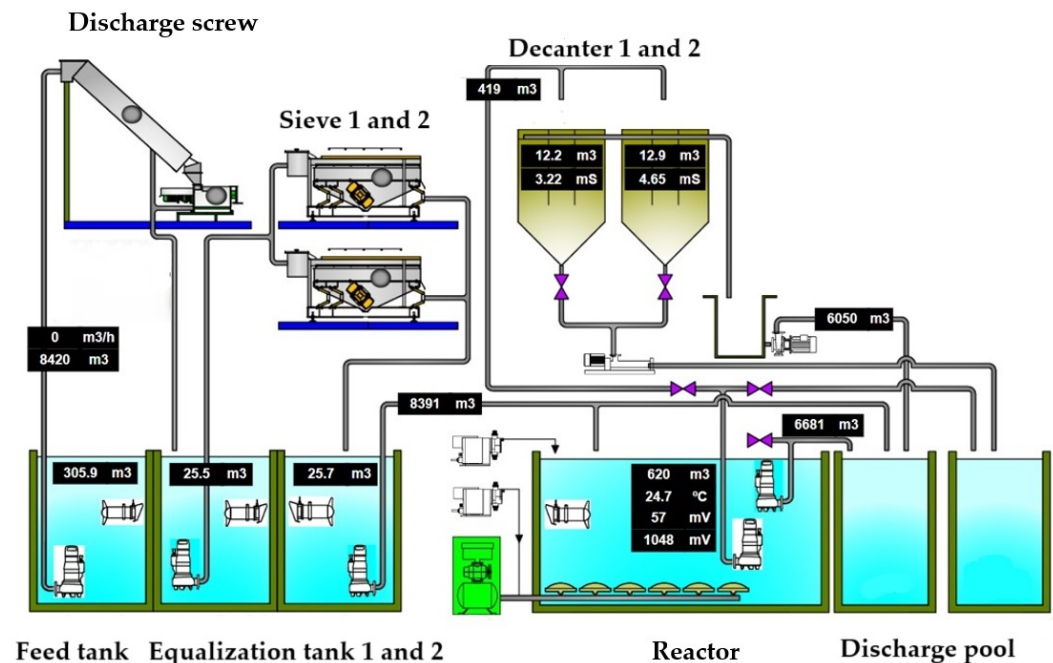
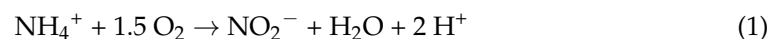


Figure 2. Flow diagram of the SBR-NDN system by Mecàniques Segalés S.L.

2.3. Biological Treatment: Sequencing Batch Reactor (SBR)

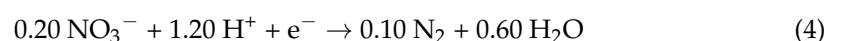
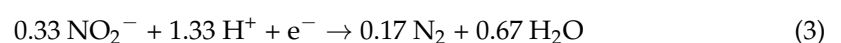
The clarified liquid fraction is biologically treated in an SBR system (Mecàniques Segalés SL, Barcelona, Spain) with a total operational volume of 625 m³, operating in repeated time-based cycles that alternate between aerobic and anoxic phases, thus enabling the sequential removal of nitrogen via coupled nitrification and denitrification processes. Each cycle lasts approximately 6 h, allowing for up to four cycles per day, and is dynamically controlled based on oxidation–reduction potential (ORP). The SBR cycle includes the following phases:

- **Filling:** Influent from Equalization Tank 2 is introduced under agitation but without aeration, establishing an initial anoxic environment.
- **Aerobic Phases (Phases 1 and 3):** Aeration is applied to support biological nitrification. Ammonia-oxidizing bacteria (AOB), such as *Nitrosomonas*, oxidize ammonium (NH₄⁺) to nitrite (NO₂[−]), and nitrite-oxidizing bacteria (NOB), such as *Nitrobacter* or *Nitrospira*, further oxidize nitrite to nitrate (NO₃[−]). The reactions are as follows:



Approximately 75% of the required oxygen is consumed during the nitrification step, generating protons (H⁺) and potentially lowering the pH depending on the system's buffering capacity.

- **Anoxic Phases (Phases 2 and 4):** In the absence of dissolved oxygen, denitrifying heterotrophic bacteria (e.g., *Paracoccus*, *Pseudomonas*) use residual organic matter as an electron donor to reduce nitrate and nitrite to nitrogen gas (N₂), which is then released into the atmosphere. Denitrification proceeds via the following reactions:



Denitrification via nitrite is more energy- and carbon-efficient and helps counteract acidification resulting from nitrification.

- Sedimentation: Biomass is allowed to settle, separating it from the treated supernatant.
- Decanting and Sludge Wasting: A programmed volume of clarified effluent is discharged, and surplus sludge is periodically removed to maintain optimal biomass concentration and ensure stable operation. Treated effluent and waste sludge volumes are quantified via flow meters.
- Batch Refilling: Feed volumes per batch typically range between 5 and 8 m³, adjusted according to real-time monitoring of reactor conditions, such as oxidation–reduction potential and organic load estimated via refractometric readings. Hydraulic stability is maintained by balancing influent and effluent volumes across cycles.

The SBR system has demonstrated robust and cost-effective nitrogen removal for high-strength agro-industrial effluents, such as pig slurry, achieving removal efficiencies ranging from 50% to 90% depending on influent characteristics and operational parameters [21,22].

2.4. Final Effluent Polishing and Sludge Management

Post-SBR, the treated effluent flows through secondary sedimentation channels for final polishing. These channels capture suspended solids and support the growth of natural biofilms (algae, bacteria, protozoa), which enhance the uptake of residual nitrogen and organic matter. The final effluent contains <0.3 g·L^{−1} total nitrogen, representing overall nitrogen removal efficiencies of >85%, and is suitable for fertigation applications.

Excess sludge from the SBR and sedimentation channels is processed in a thickener, concentrating solids to 7–9% TS. The sludge volume represents ~12% of the total treated liquid and is sent off-site for composting, supporting circular nutrient management.

2.5. Methods

2.5.1. Sampling Procedure

To ensure that the analytical results accurately reflect the composition of the pig slurry, all samples collected for characterization were obtained following an internal standardized Sampling and Analysis Protocol to ensure that the measured nitrogen content accurately reflected the composition of the waste material. Samples were collected to represent the material under study and transported under conditions that preserved their original characteristics. Clean, dry containers of PTFE or polyethylene were labeled with the sample name, date, and time. Composite samples were created by combining grab samples collected within the relevant process interval at different times. The flow was allowed to run for tap sampling before collecting each grab, which was then homogenized to produce the final composite. Each sample was collected in duplicate, with a final volume of 1 L, leaving headspace in the container for gas expansion or freezing. During transport and storage, the samples were kept at 4–8 °C. If the analysis was delayed more than two days, the samples were frozen at −18 °C, except where freezing could affect sample integrity.

2.5.2. GHG Emission Calculation

A standardized calculation tool developed by Spain's Ministry for the Ecological Transition and Demographic Challenge (MITECO) was employed to estimate reductions in greenhouse gas emissions. This tool, provided as an Excel-based workbook [23], quantifies emission reductions by comparing two scenarios: a baseline scenario in which pig slurry is directly applied to agricultural fields without treatment, and a project scenario where specific treatment technologies—such as the nitrification–denitrification (NDN) process—are used to transform the waste and mitigate emissions.

The slurry produced on the farm was characterized, allowing for identifying two distinct types based on their origin and composition.

On the one hand, a slurry with a higher nitrogen content and significant agronomic value was identified, primarily originating from gestating sows. This type of slurry has a total nitrogen (Nt) content ranging from 4 to 7 kg/m³.

On the other hand, the slurry produced in the farrowing units and from piglets was characterized by a higher water content and, consequently, a much lower total nitrogen concentration, typically between 1 and 2 kg/m³. Due to these characteristics, this second type of slurry was treated at the NDN plant as part of the current project.

The calculation must be performed annually and requires comprehensive, verifiable data, including detailed chemical analyses of the waste before and after treatment and thorough documentation of transport logistics in the baseline scenario (e.g., average transport distances, quantities moved, and number of trips).

The project is subject to external verification by an accredited certification body each year. This entity reviews both regulatory compliance and the methodological soundness of the calculations. Official registration with MITECO is only granted if it can be demonstrated that the treatment process achieves at least a 60% reduction in emissions relative to the baseline scenario. Such registration ensures the legal validity, traceability, and formal recognition of the emission reductions achieved by the NDN plant within Spain's national climate change mitigation framework.

2.5.3. Analytical Methods

All physicochemical analyses followed internationally standardized protocols, primarily ISO methods, ensuring traceability and reproducibility. The samples of pig slurry were analyzed in triplicate. Below is a detailed description of each parameter, methodology, and standard applied.

- **pH Measurement:** The pH was measured using a combined glass electrode, previously calibrated with standard buffer solutions (pH 4.00, 7.00, and 10.00). The measurement was conducted under ISO 10523:2008 [24].
- **Density:** Density was determined using a digital oscillation-type densimeter at 20 °C, following ISO 15212-1:1998 [25].
- **Electrical Conductivity (1:9 dilution):** Samples were diluted 1:9 (*w/v*) with deionized water. Conductivity was measured using a conductivity meter with automatic temperature compensation, following ISO 11265:1994 [26].
- **Direct Electrical Conductivity:** Undiluted samples were analyzed using a conductivity meter, as described in ISO 7888:1985 [27].
- **Electrical Conductivity in Solids:** As no ISO standard exists for this matrix, the procedure followed a validated internal protocol based on leachate extraction and conductivity measurement, referencing ISO 11265:1994 [26].
- **Moisture Content:** Moisture was assessed gravimetrically by drying at 105 °C until a constant weight, according to ISO 11465:1993 [28].
- **Total Solids:** Total solids were determined via oven drying at 105 °C, following ISO 11465:1993 [28].
- **Volatile Solids:** The loss on ignition at 550 °C was used to estimate volatile solids (organic matter), following ISO 10694:1995 [29].
- **Ammoniacal Nitrogen (NH₄⁺):** Ammonium nitrogen was determined by spectrophotometry following the indophenol blue method, as per ISO 11732:2005 [30].
- **Total Nitrogen:** Total nitrogen was determined by modified Kjeldahl digestion and distillation, based on ISO 11261:1995 [31].

- Total Phosphorus (P_2O_5): Samples were subjected to acid digestion, and the phosphorus content was quantified spectrophotometrically using the ascorbic acid method (ISO 6878:2004) [32].
- Potassium (K_2O): Potassium was determined via atomic absorption spectrometry (AAS) following sample digestion, by ISO 9964-3:1998 [33].
- Chemical Oxygen Demand (COD): COD was determined via closed reflux with potassium dichromate and spectrophotometric detection, based on ISO 6060:1989 [34].
- Nitrite ($N-NO_2$): Nitrite was measured by colorimetry after reaction with sulfanilamide and N-(1-naphthyl) ethylenediamine, by we ISO 6777:1984 [35].

2.5.4. Statistical Analysis

Statistical analyses were conducted using the R programming environment (version 4.3; R Core Team, Vienna, Austria) [36]. Linear regression models were employed to examine the influence of operational variables on key performance indicators, such as nitrogen removal efficiency and effluent quality. Before modeling, the datasets were evaluated for normality using the Shapiro–Wilk test and for homoscedasticity using Levene’s test. Correlation matrices were constructed to explore potential associations between input parameters—such as treated volume, mixing duration, aeration duration, etc.—and treatment outcomes (total nitrogen efficiency, NO_2^- reduction efficiency, etc.). Pearson’s correlation coefficient (R) was used for normal distribution data, while Spearman’s rank correlation was applied to non-parametric datasets.

Statistical significance was defined as follows: *** ($p \leq 0.001$) indicates highly significant, ** ($p \leq 0.01$) indicates very significant, * ($p \leq 0.05$) indicates significant, ($p \leq 0.1$) indicates marginally significant, and no symbol denotes non-significant results ($p > 0.1$). Although the Pearson correlation coefficient (R) quantifies the strength and direction of a linear relationship between two continuous variables, the associated p -value reflects the statistical likelihood that the observed correlation occurred by chance. All statistical analyses and visualizations were conducted in the R environment, using packages such as *ggplot2* or *GGally* for plotting and *ggpairs* to produce correlation matrix diagrams, which facilitated the interpretation of multivariate relationships across operational and performance variables.

To evaluate differences in key measured indicators (e.g., input–output and efficiency of COD, P_2O_5 , NH_4^+ , etc.) across the different months over the four-year study period, one-way Analysis of Variance (ANOVA) was applied. The ANOVA tested the null hypothesis that mean values did not differ significantly among months. When significant differences were detected ($p < 0.05$), post hoc multiple comparison tests were performed to identify homogeneous groups. Specifically, Tukey’s Honest Significant Difference (HSD) test was used to control the family-wise error rate and to establish which monthly means were statistically distinguishable. The R function *aov()* was used to fit the ANOVA models, and *HSD.test()* was applied to derive pairwise comparisons and identify homogeneous groups of months with similar treatment performance. This approach allows for robust interpretation of seasonal patterns and operational variability, providing insight into how temporal factors influence reactor performance in a Mediterranean continental climate.

3. Results

3.1. Long-Term Efficiency Performance of Typical Operating Cycles

3.1.1. COD Reduction Efficiency

Figure 3 compares monthly mean COD reduction efficiencies over the four-year study period using homogeneous group analysis (ANOVA). Months belonging to the same homogeneous group are indicated in the same color. As observed, the average COD

reduction efficiency is close to 75% for most months, except for April, which exhibits the lowest mean value.

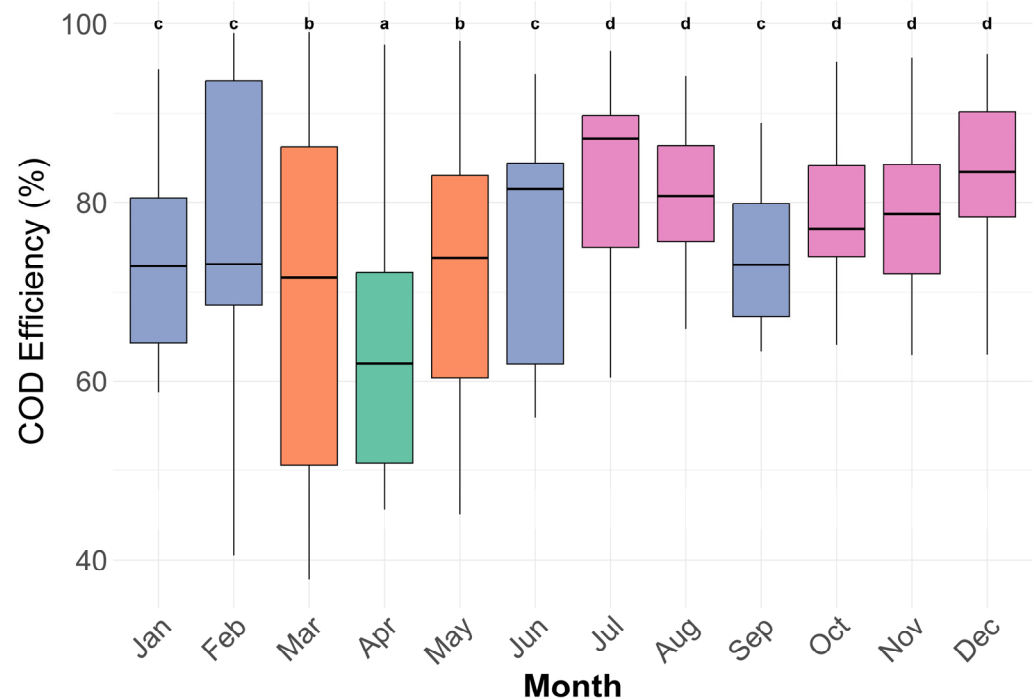


Figure 3. Boxplot of monthly COD removal efficiency over four years. Letters a, b, c, and d represent different homogeneous groups (ANOVA, Tukey HSD, $p < 0.05$).

When performing an annual evaluation of the differences between input and output COD values in the NDN reactor (Figure 4), June through August exhibit the most remarkable COD removal differences. This increase in COD reduction corresponds with the warmest months of the year.

Regarding the analysis of correlations with operational configurations such as variations in anoxic and aerobic phase durations, daily treated volumes, and reductions in holding times within equalization tanks, etc., the efficiency of COD removal shows correlations with several operational variables (see Figure S1): the treated volume (Corr. 0.203 *) and residence time in Equalization Tank 1 (Corr. 0.280 ***). Notably, residence time in Equalization Tanks 1 (Corr. 0.569 ***) and 2 (Corr. 0.435 ***) increases during periods when higher volumes (TreatVol) of slurry are processed and when decreasing the aeration time (Corr. −0.355). This indicates that during these periods, the residence times in the pre-treatment equalization tanks preceding the SBR-NDN process are extended.

These positive correlations suggest that higher inflows result in system-wide adjustments that lead to longer retention times before the biological treatment stage, potentially influencing oxidation–reduction dynamics and microbial activity in subsequent phases.

As will be discussed further when analyzing the performance of phase-specific operation rates (e.g., ORP slope, aeration control), this trend suggests that efficiency is more likely to be compromised during high-load periods due to shifts in hydraulic conditions and process stability.

In addition, residence time in Equalization Tank 2 shows positive correlations with treated volumes (Corr. 0.435 ***) and aeration time (Corr. −0.362 ***), indicating possible compensatory interactions between physical equalization processes and biological activity. Furthermore, correlations with average temperature and wet-bulb temperature (Corr. 0.261 ** and 0.240 ***) highlight the role of ambient climate in influencing tank behavior and microbial dynamics.

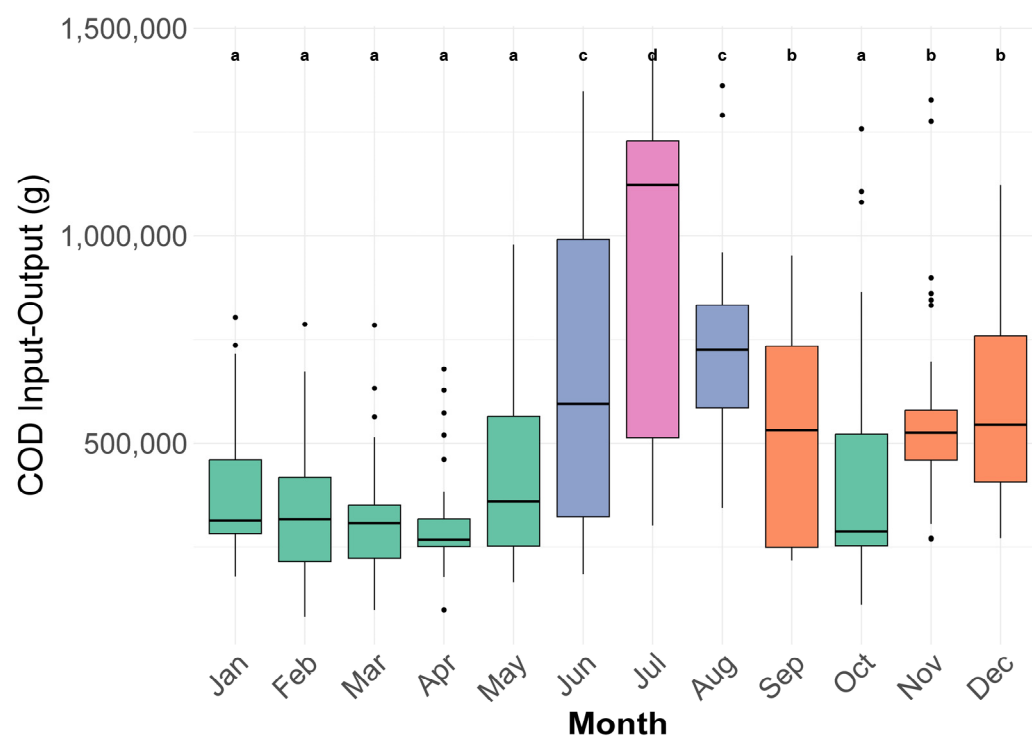


Figure 4. Boxplot of monthly COD input-output differences over a four-year period. Letters a, b, c, and d represent different homogeneous groups. Dots on the plot represent outliers (ANOVA, Tukey HSD, $p < 0.05$).

A particularly notable result is the strong negative correlation between mixing time and aeration time (Corr. -0.371^{***}), which may reflect an operational balancing strategy, where longer mixing phases are offset by reduced aeration times, or vice versa, depending on system demands.

A robust inverse correlation was observed between relative humidity and temperature (Corr. -0.805^{***}), confirming the expected meteorological pattern: as temperature increases, relative humidity decreases. This interdependence is essential for interpreting how climatic fluctuations may indirectly influence biological treatment performance.

These correlations, derived from Pearson's coefficient (R) for normally distributed variables and Spearman's rank for non-parametric data, were interpreted in light of their statistical significance thresholds, as defined previously. Together, they support the identification of interdependent variables that can affect the system's treatment performance under variable climatic and operational loads.

3.1.2. NH_4^+ Reduction Efficiency

Figure 5 compares monthly mean NH_4^+ removal efficiencies over the four years, grouped by homogeneous subsets using ANOVA analysis. As observed, NH_4^+ removal efficiency remained consistently high throughout the year, with average values close to 97% for most months. However, July and August exhibited significantly lower mean efficiencies, around 95%, forming their own distinct homogeneous group. This seasonal decline corresponds to the year's hottest months and will be further examined in subsequent correlation analyses with temperature data.

When performing an annual evaluation of the differences between the input and output NH_4^+ values in the NDN reactor, it is observed that the months from May to July exhibit the most remarkable NH_4^+ removal differences (Figure 6), coinciding with the warmest months of the year.

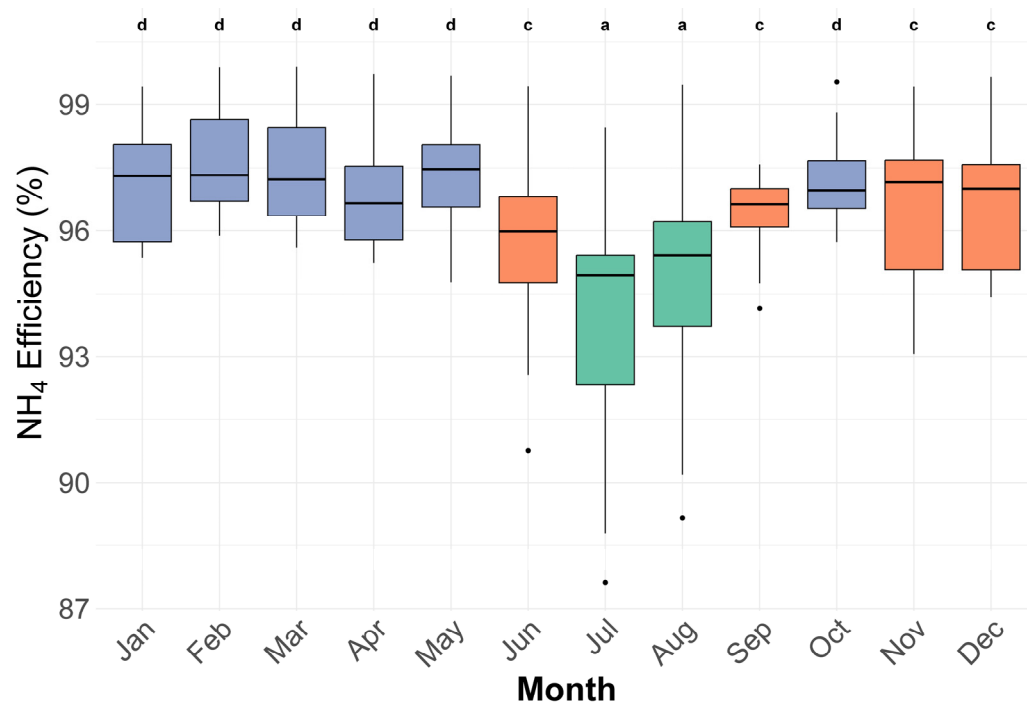


Figure 5. Boxplot of monthly NH_4^+ removal efficiency across a four-year period. Letters a, c, and d represent different homogeneous groups. Dots on the plot represent outliers (ANOVA, Tukey HSD, $p < 0.05$).

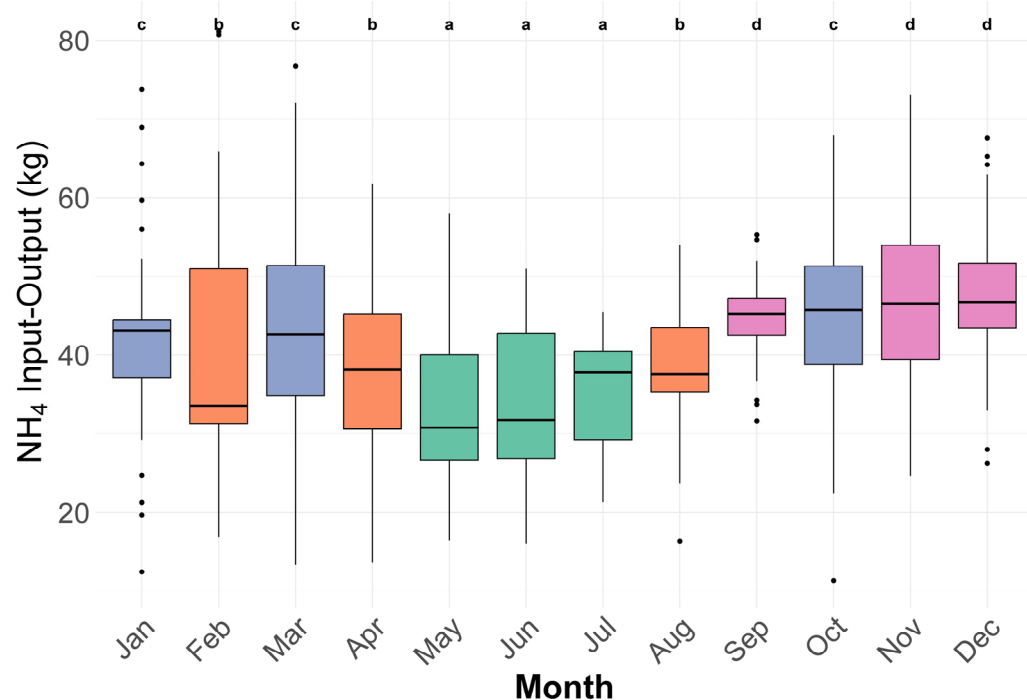


Figure 6. Boxplot depicting monthly differences in NH_4^+ input and output over a four-year period. Letters a, b, c, and d represent different homogeneous groups. Dots on the plot represent outliers (ANOVA, Tukey HSD, $p < 0.05$).

Regarding the analysis of correlations with operational configurations, the NH_4^+ reduction efficiency also demonstrated significant correlations with several operational and environmental variables (see Figure S2). A high negative correlation was found with residence time in Equalization Tank 2 (Corr. -0.472^{***}), suggesting that prolonged retention in this tank may adversely affect ammonium removal. Conversely, mixing time (Corr.

0.176 *), aeration time (Corr. 0.387 *), and relative humidity (Corr. 0.256 **) showed a positive correlation, indicating that enhanced equalization may promote more effective ammonium reduction, likely due to improved substrate distribution and contact with nitrifying microorganisms.

In terms of environmental conditions, both mean temperature and wet-bulb temperature were negatively correlated with NH_4^+ removal efficiency (Corr. -0.309^{***} and -0.2562^{**} , respectively), while relative humidity displayed a positive correlation (Corr. 0.306^{***}), suggesting that cooler, more humid conditions favor ammonium removal.

Concerning treated volume, significant positive correlations were observed for both Equalization Tank 1 (Corr. 0.569^{***}) and Equalization Tank 2 (Corr. 0.435^{***}), consistent with patterns noted in the analysis of COD efficiency and pointing to volumetric loading as a key driver in system behavior. Interestingly, aeration time exhibited a weak negative correlation with NH_4^+ reduction efficiency (Corr. -0.350^{***}).

3.1.3. NO_2^- Reduction Efficiency

Figure 7 shows the results of an ANOVA-based homogeneous group comparison of the monthly average NO_2^- reduction efficiencies recorded over the four-year study period. The data reveal that the reduction efficiency averages around 55% between November and April. In contrast, during July and August, the efficiency markedly improves, with values near 95%, constituting a separate homogeneous group. This pattern again highlights the influence of the warmest months on NO_2^- reduction performance, a relationship that will be further explored through temperature correlation analyses.

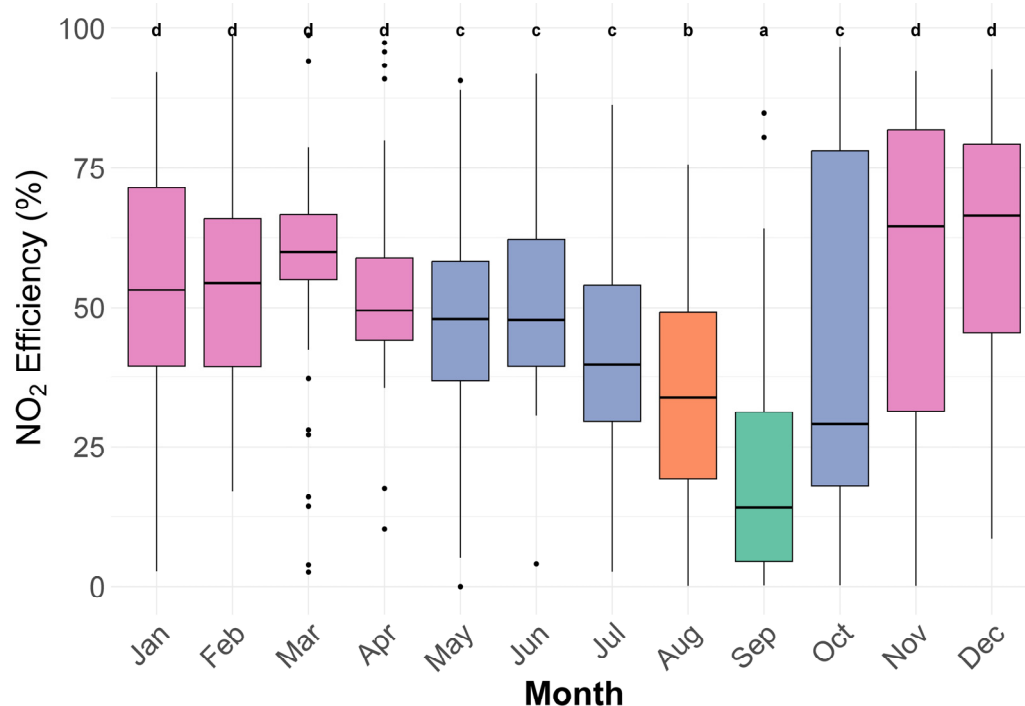


Figure 7. Boxplot of monthly NO_2^- reduction efficiency over a four-year period. Letters a, b, c, and d represent different homogeneous groups. Dots on the plot represent outliers (ANOVA, Tukey HSD, $p < 0.05$).

The annual analysis of NO_2^- input–output differences in the NDN reactor indicates that the lowest reduction differences are observed from July to September, the hottest months of the year (Figure 8).

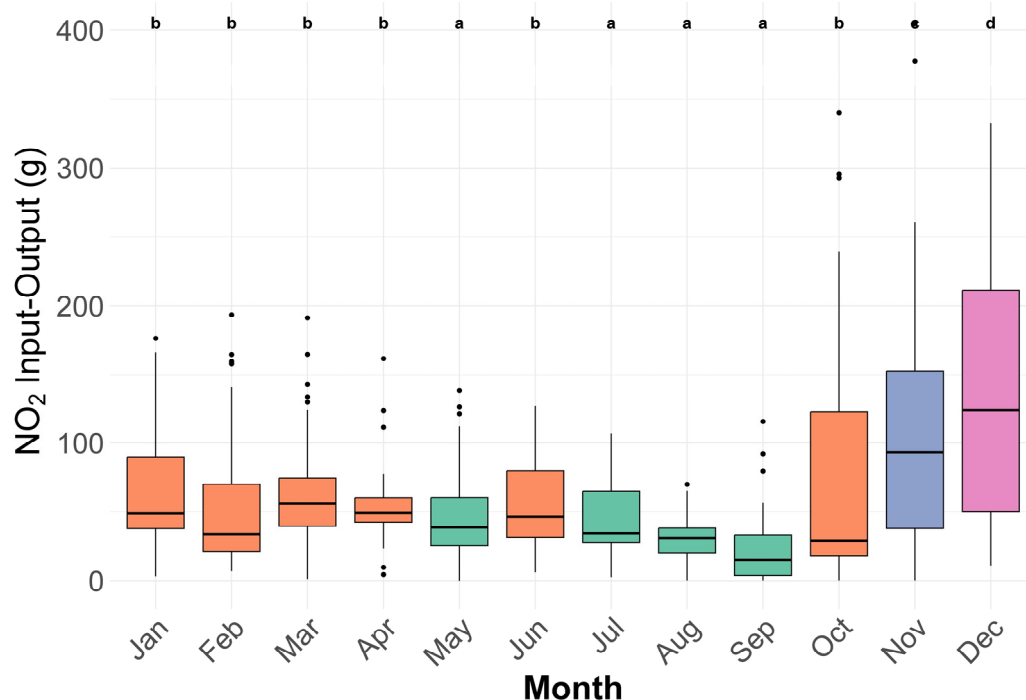


Figure 8. Boxplot of monthly NO_2^- input-output differences over a four-year period. Letters a, b, c, and d represent different homogeneous groups. Dots on the plot represent outliers (ANOVA, Tukey HSD, $p < 0.05$).

Regarding the analysis of correlations with operational configurations, the NO_2^- reduction efficiency exhibited several statistically significant correlations with operational and climatic variables (see Figure S3). No correlation was found with the treated volume (Corr. 0.030). At the same time, residence time in Equalization Tank 2 showed a strong negative correlation (Corr. -0.303^{***}), indicating that longer retention in this tank may lead to conditions unfavorable for complete nitrite reduction, possibly due to partial nitrification or substrate imbalances.

Regarding process dynamics, aeration time displayed a moderate positive correlation with NO_2^- removal efficiency (Corr. 0.177^*), highlighting the role of oxygen availability in promoting the oxidation of nitrite to nitrate during the aerobic phase. In contrast, mixing time was weakly or not correlated (Corr. -0.142), which may suggest that excessive mixing without concurrent aeration provides limited benefit for nitrite conversion.

Environmental conditions did not play a notable role: both mean temperature and wet-bulb temperature showed no correlations (Corr. -0.097 and -0.053 , respectively), indicating no relation between higher temperatures and the inhibition of optimal NO_2^- reduction. On the other hand, temperature differential presented a moderate positive correlation (Corr. 0.183^*), suggesting that greater thermal variation between the reactor and the atmosphere may be associated with improved NO_2^- removal, possibly due to diurnal shifts in oxygen demand or process rebalancing during alternating operational phases.

The results reveal that nitrite reduction is highly responsive to both operational parameters and environmental factors, reinforcing the importance of integrated system management to accommodate diverse climatic conditions.

3.1.4. Total Nitrogen Reduction Efficiency

Figure 9 shows a comparison, by homogeneous groups (ANOVA), of the mean monthly results for the total N reduction efficiency over the four years of study. As can be seen, the total N reduction efficiency shows average values close to 90% between February and

May. It is reduced to average values close to 80% from June to September. Once again, the hottest months of the year affect efficiency, as will be shown later in the correlation diagrams with temperature.

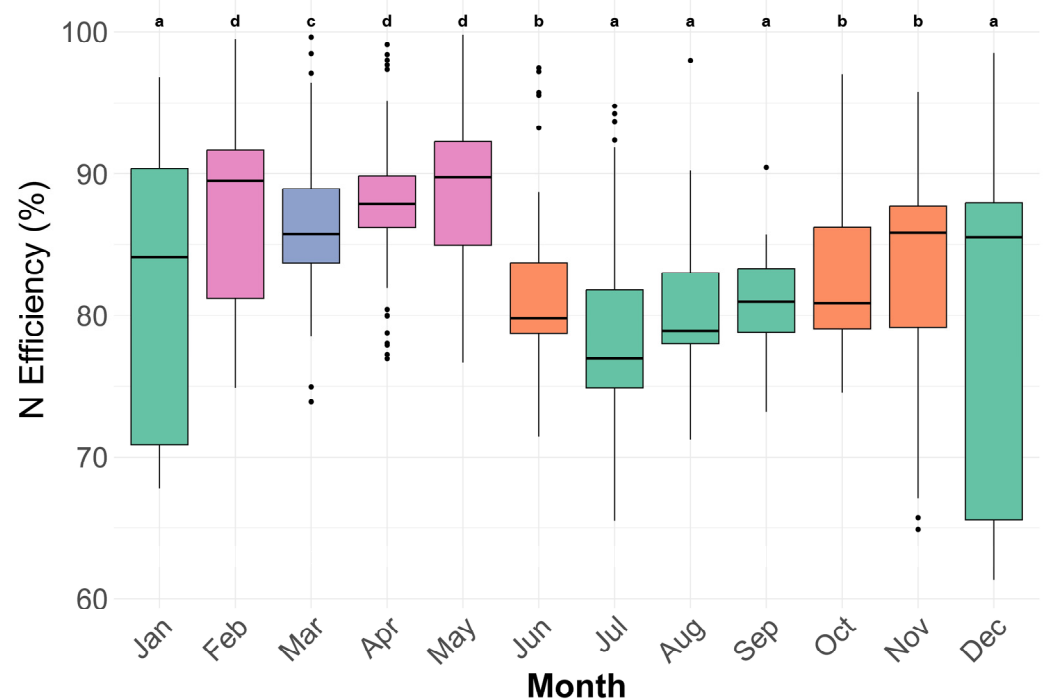


Figure 9. Boxplot of monthly total nitrogen reduction efficiency over a four-year period. Letters a, b, c, and d represent different homogeneous groups. Dots on the plot represent outliers (ANOVA, Tukey HSD, $p < 0.05$).

An annual evaluation of the differences between the total nitrogen input and output values in the NDN reactor reveals that the smallest reduction differences occur from July to September, coinciding with the year's warmest months. This variability in input–output results may explain why the reduction efficiency observed in January and December does not follow the typical pattern observed in the other colder months (Figure 10).

Regarding the analysis of correlations with operational configurations, the total nitrogen reduction efficiency exhibited significant correlations with several operational and environmental variables (see Figure S4). A negative correlation was observed with the treated volume (Corr. -0.172^*), indicating that increased hydraulic loading negatively impacts nitrite removal efficiency. Similarly, residence times in Equalization Tank 1 (Corr. -0.189^*) and Equalization Tank 2 (Corr. -0.308^{***}) showed negative correlations, suggesting that longer retention before biological treatment reduces the system's effectiveness in nitrite reduction.

Conversely, aeration time was positively correlated with total nitrogen removal efficiency (Corr. 0.401^{***}), highlighting the importance of sufficient oxygen supply during the aerobic phase for effective nitrite oxidation. Additionally, relative humidity exhibited a negative correlation (Corr. -0.276^{***}), implying that lower humidity conditions may favor nitrite removal, potentially through effects on microbial activity or oxygen transfer efficiency. This last factor is related to average temperature (Corr. 0.214^{**}) and medium wet-bulb temperature (TbH Avg) (Corr. 0.206^*), with a negative correlation between the total nitrogen reduction efficiency and higher temperature values.

These findings underscore the sensitivity of nitrite reduction to both operational control and ambient environmental conditions, further emphasizing the need for integrated system management under varying climatic scenarios.

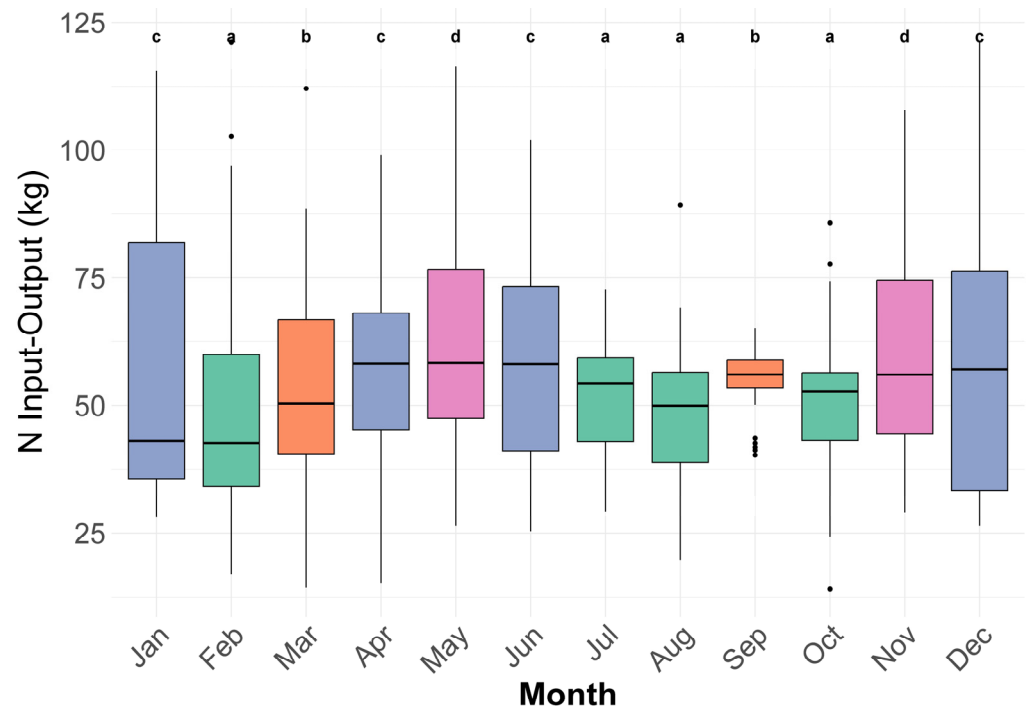


Figure 10. Boxplot of monthly total nitrogen input-output differences over a four-year period. Letters a, b, c, and d represent different homogeneous groups. Dots on the plot represent outliers (ANOVA, Tukey HSD, $p < 0.05$).

3.1.5. P_2O_5 Reduction Efficiency

Figure 11 shows the results of an ANOVA-based homogeneous group comparison of the monthly average P_2O_5 reduction efficiency over the four-year study period. The data indicate that the average reduction efficiency is close to 30% for most months. However, from May to August, the efficiency increases to average values near 45%. Once again, the year's warmest months appear to influence efficiency, a relationship that will be further examined in the correlation diagrams with temperature.

An annual evaluation of the differences between P_2O_5 input and output values in the NDN reactor shows that the smallest reduction differences occur between November and January, the coldest months of the year (Figure 12). Conversely, the most considerable P_2O_5 removal differences are observed from May to August.

Regarding the analysis of correlations with operational configurations in an SBR-NDN system (see Figure S5), the reduction efficiency of P_2O_5 (phosphorus, typically present as phosphate) does not appear to be significantly influenced by the treated volume or the residence time in Equalization Tank 1, suggesting that these early-stage operational variables do not strongly affect phosphorus dynamics in this setup. However, the negative correlation with the residence time in Equalization Tank 2 (Corr. -0.307^{***}) indicates that longer retention in this later-stage equalization tank may harm phosphorus removal. This could be due to the release of phosphorus from organic matter under anoxic or slightly reducing conditions, which can occur if the tank operates without sufficient oxygen or mixing, leading to phosphorus solubilization rather than its retention or biological uptake. Conversely, the positive correlation with aeration time (Corr. 0.286^{***}) suggests that longer aerobic phases in the SBR enhance phosphorus removal, likely through enhanced biological phosphorus uptake by polyphosphate-accumulating organisms (PAOs). These microorganisms assimilate phosphorus most effectively under aerobic conditions after exposure to prior anaerobic conditions, a core mechanism in enhanced biological phosphorus removal (EBPR) [37].

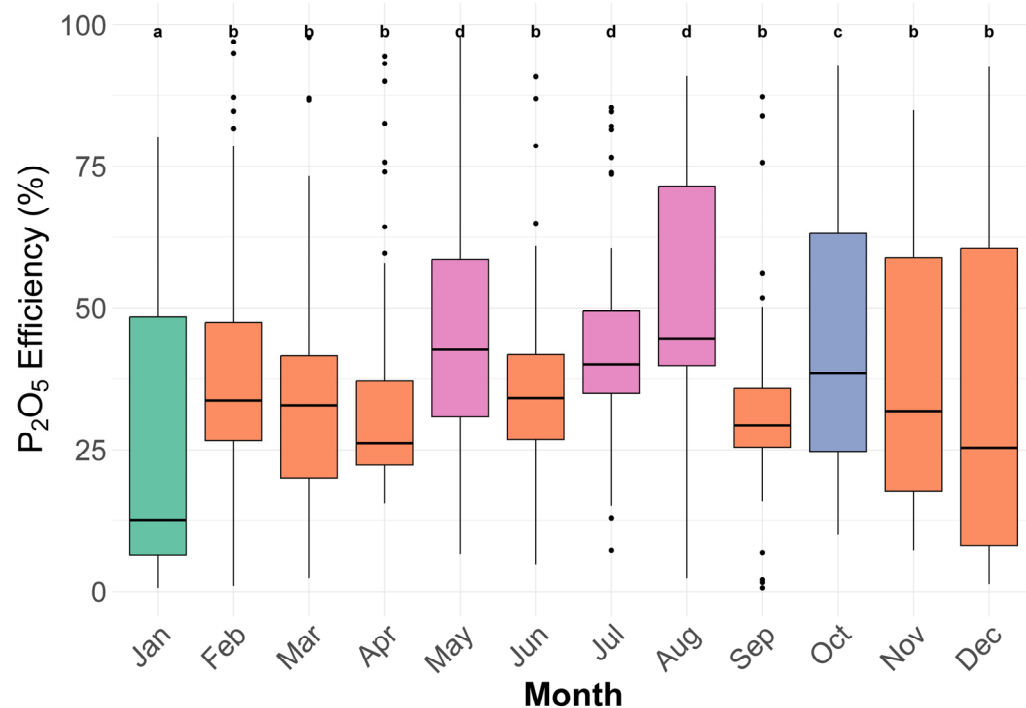


Figure 11. Boxplot of monthly P_2O_5 reduction efficiency over a four-year period. Letters a, b, c, and d represent different homogeneous groups. Dots on the plot represent outliers (ANOVA, Tukey HSD, $p < 0.05$).

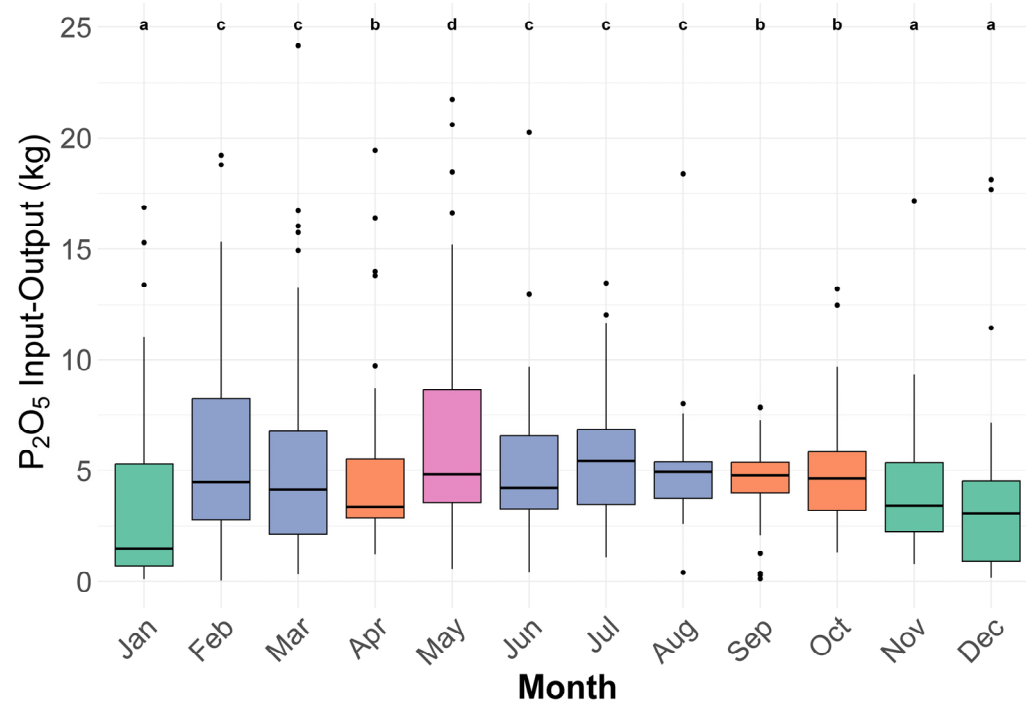


Figure 12. Boxplot of monthly P_2O_5 input-output differences over a four-year period. Letters a, b, c, and d represent different homogeneous groups. Dots on the plot represent outliers (ANOVA, Tukey HSD, $p < 0.05$).

3.1.6. GHG Emission Analysis

Regarding the analysis of GHG emissions, Tables 1 and 2 summarize N_2O and other GHG emissions, respectively. These values were computed using the tool provided by MITECO [23]; the results obtained show an approximate annual reduction of 75% in

N₂O emissions and up to 92% in total greenhouse gas (GHG) emissions following the implementation of the NDN plant. In their study, the authors propose optimizing the conventional SEP = NDN configuration by incorporating a prior nutrient recovery stage. This enhancement enables additional resource valorization and significantly reduces the potential for N₂O formation within the NDN process.

Table 1. N₂O emission reductions before (conventional practices) and after applying the NDN technology (WITH NDN). Data obtained using the MITECO calculator.

Year	Conventional Practice (t CO ₂ e)	With NDN (t CO ₂ e)	%
2020	70	27	61%
2021	199	50	75%
2022	278	33	88%
2023	220	52	76%

Table 2. GHG emission reductions before (conventional practices) and after applying the NDN technology (WITH NDN). Data obtained using the MITECO calculator.

Year	Conventional Practice (t CO ₂ e)	With NDN (t CO ₂ e)	Emission Reduction (t CO ₂ e)	%
2020	152	19	133	87%
2021	522	28	494	94%
2022	675	17	658	97%
2023	293	34	259	88%

A comparison between both approaches suggests that integrating nutrient recovery prior to biological treatment could further enhance the overall GHG mitigation efficiency, potentially surpassing the reductions observed in this study. In this context, integrating complementary technologies with NDN represents a promising technological pathway for developing more sustainable systems with a lower climate footprint in agro-industrial and waste treatment applications.

3.2. Analysis of Oxidation–Reduction Potential Performance During Aerobic and Anoxic Phases

Figure 13 shows the distribution of the velocity variation in the ORP during Phases 1 and 3 (aerobic phases), analyzing each month individually. The color of the graphics represents the group result of an ANOVA analysis over the four-year study period. The ORP slope in these phases provides insight into the system's recovery rate throughout the year, indicating that higher slopes reflect a better capacity to respond to flow variations under different climatic conditions.

Oxidation–Reduction Potential (ORP) Slope Analysis During Aerobic Phases:

The density plots for ORP in Phases 1 and 3 reveal key patterns in the distribution of ORP slope values throughout the year. When a single prominent peak is observed in the density curve, it indicates that Phases 1 and 3 share similar ORP values, as is evident in March, April, and May. Conversely, when the peaks are more pronounced and located further from the origin, this suggests improved aeration system performance and enhanced oxygen solubility, conditions more frequently observed during the colder months from September to December.

Additionally, the density peaks in April and May tend to cluster around ORP values of approximately 1.5, forming a statistically distinct homogeneous group. The observed shift in the density peaks from the left side of the graph (February to May) to the right side as temperatures rise in June suggests that ORP values progressively increase with

warmer conditions before decreasing again in the colder months. However, the formation of a separate homogeneous group for January cannot be fully explained with the current dataset. It may be influenced by variations in influent quality, such as differences in manure composition.

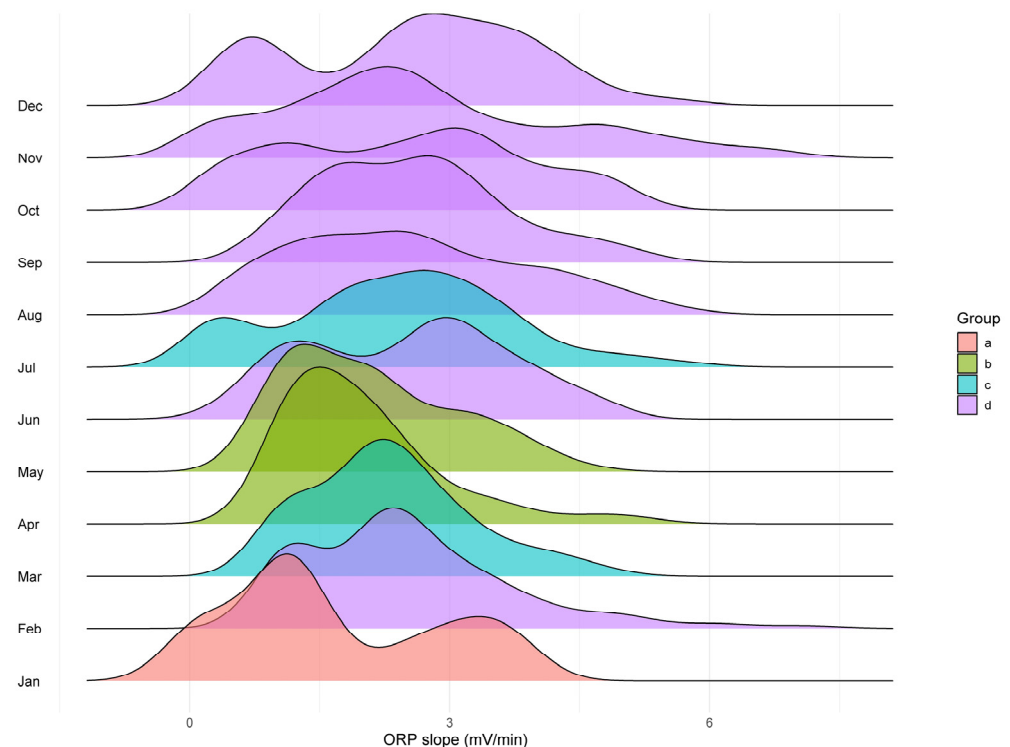


Figure 13. Distribution of the ORP slope for aerobic phases (pH 1 and 3) per month. Letters a, b, c, and d represent different homogeneous groups (ANOVA, Tukey HSD, $p < 0.05$).

The slope between the maximum and minimum ORP values observed during Phases 1 and 3 of each operational cycle indirectly indicates the rate at which the system reaches the target redox conditions. This slope reflects the biological activity and system responsiveness under aerobic conditions.

In both Phase 1 and Phase 3, the ORP slope positively correlates with the treated volume (Phase 1: Corr. 0.199 ***; Phase 3: Corr. 0.308), indicating that higher inflows may enhance oxidative processes, possibly due to increased substrate availability (see Figures S6 and S7, respectively). Additionally, residence time in Equalization Tank 3 positively correlates with the OR slope in both phases (Phase 1: Corr. 0.333 ***; Phase 3: Corr. 0.304 ***), suggesting that extended retention times before aeration phases may improve redox transition efficiency.

The strongest positive correlations are observed with aeration time (Phase 1: Corr. 0.649 ***; Phase 3: Corr. 0.748), emphasizing the importance of sufficient oxygen supply in accelerating oxidative reactions and achieving optimal redox states during aerobic treatment.

No significant correlations were found between the ORP slope and climatic variables such as temperature, humidity, or wet-bulb temperature, suggesting that operational rather than environmental factors predominantly govern aerobic redox dynamics in this system.

Oxidation–Reduction Potential (ORP) Slope Analysis During Anoxic Phases (phases 2 and 4):

Figure 14 presents a comparison, by homogeneous groups (ANOVA), of the average monthly ORP slope results for the two anoxic phases of the NDN processes over the four years of study. The slopes of these phases allow us to understand the ORP recovery rate

during the different months of the year. When there is a higher velocity, the system recovers better from flow variations for the different climatic variations throughout the year. The graph shows that June through July generate a homogeneous group, for which the recovery rate from the anoxic phases decreases. This would imply that temperature affects gas dissolution and bacterial metabolism, while in the cold months, this recovery rate increases.

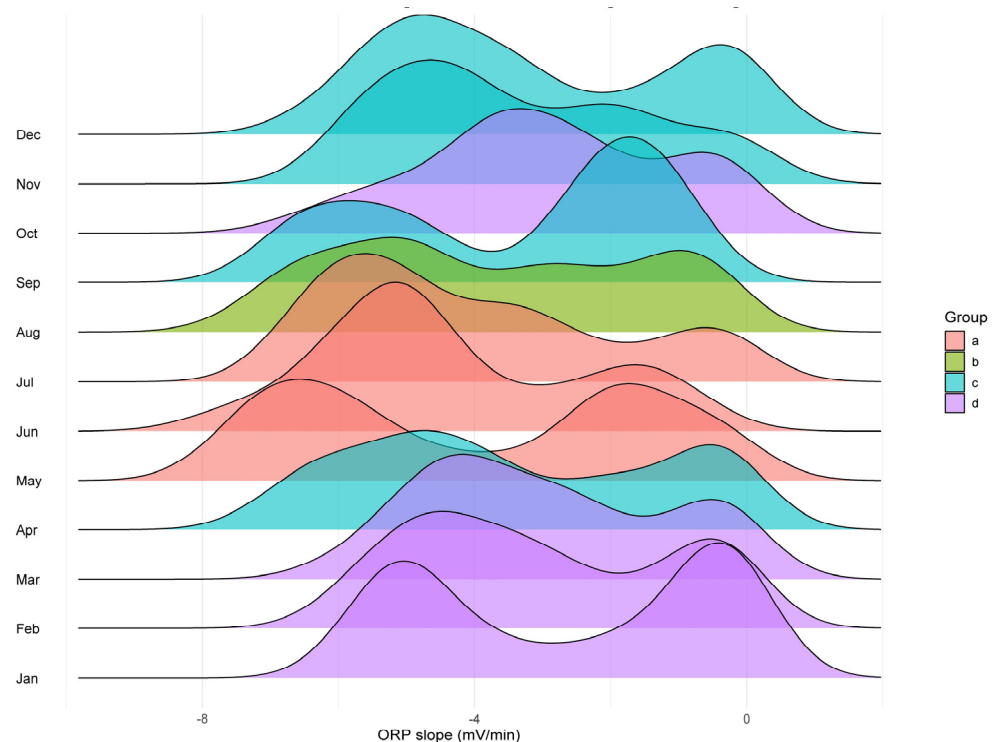


Figure 14. Distribution of the ORP slope for anoxic phases (pH 2 and 4) per month. Letters a, b, c, and d represent different homogeneous groups (ANOVA, Tukey HSD, $p < 0.05$).

Regarding the analysis of correlations with operational configurations in contrast to the aerobic stages, the ORP slope during the anoxic phases (Phases 2 and 4) does not exhibit significant correlations with the treated volume or the residence times in Equalization Tanks 1 and 2. However, it does show a positive correlation with mixing time (Phase 2: Corr. 0.366; Phase 4: Corr. 0.225), suggesting that improved equalization during these phases supports more consistent redox transitions under anoxic conditions (see Figures S8 and S9, respectively).

Conversely, the ORP slope correlates negatively with aeration time (Phase 2: Corr. -0.274^{***} ; Phase 4: Corr. -0.461^{***})—a result that likely reflects residual oxygen inhibiting denitrification efficiency during the subsequent anoxic steps, thereby flattening the redox gradient.

Environmental variables also appear to influence anoxic performance [38]. Specifically, average temperature (Phase 2: Corr. -0.551^{***} ; Phase 4: Corr. -0.470^{***}) and wet-bulb temperature (Phase 2: Corr. -0.534^{***} ; Phase 4: Corr. -0.440^{***}) exhibit strong negative correlations with the ORP slope, indicating that higher ambient temperatures may reduce the efficiency of achieving the desired anoxic redox potential, potentially due to increased microbial respiration rates that alter redox stability.

These findings underline the sensitivity of anoxic phases to operational mixing strategies and ambient thermal conditions, reinforcing the need for precise process control during denitrification stages to ensure optimal nitrogen removal performance.

4. Discussion

Interpretation of Long-Term Efficiency Performance of Typical Operating Cycles:

The treatment system exhibited clear correlations between operational parameters, environmental factors, and the removal efficiencies of key pollutants—namely, chemical oxygen demand (COD), ammonium (NH_4^+), nitrite (NO_2^-), and total nitrogen. The COD removal efficiency was positively correlated with increased residence times in the equalization tanks and higher treated volumes, suggesting that prolonged pre-treatment phases may enhance downstream microbial activity and favorable redox conditions. However, COD removal performance declined under peak loading scenarios, likely due to transient process imbalances. This mirrors the findings of Weon et al. [39], who reported temperature-driven reductions in nitrification and COD removal efficiency at suboptimal conditions.

Ammonium (NH_4^+) reduction efficiency showed significant sensitivity to residence time and mixing intensity, with ambient temperature and relative humidity exerting a strong influence. Cooler, more humid conditions favored ammonium removal, while extended retention in Equalization Tank 2 correlated negatively with performance. These observations are consistent with previous studies indicating that nitrification rates decrease below 15 °C and collapse under prolonged hydraulic retention without adequate aeration control [40].

In the case of nitrite (NO_2^-), higher flow rates and prolonged retention times impeded removal efficiency, whereas adequate aeration facilitated nitrite oxidation. The inverse relationship between relative humidity and temperature highlights the climatic sensitivity of biological processes—a phenomenon well-documented in SBR systems relying on controlled DO and ORP profiles [41].

Total nitrogen reduction efficiency, reflecting successful denitrification, showed moderate dependence on flow and retention time but required sufficient aeration for optimal performance. Studies on SBR systems confirm that precise aerobic–anoxic cycling and dissolved oxygen control are essential for sequential nitrification and denitrification pathways [37].

Collectively, these results support the consensus that hydraulic loading, retention time, temperature, and humidity are critical drivers of biological nutrient removal performance. They echo the existing literature that underscores the importance of adaptive strategies to maintain efficient COD and nitrogen pollutant removal under variable operating conditions [38].

In addition to nitrogen compounds, the system's behavior in relation to phosphorus removal was also evaluated, explicitly focusing on P_2O_5 reduction efficiency. The results revealed no significant correlation between P_2O_5 removal and treated volume or residence time in Equalization Tank 1. However, a negative correlation was observed with retention time in Equalization Tank 4 (Corr. -0.307^{***}), and a positive correlation emerged with aeration time (Corr. 0.286^{***}).

These findings suggest that longer residence times in the final equalization stage may lead to phosphorus release or re-solubilization, potentially due to anaerobic conditions or microbial cell lysis under suboptimal redox states. Conversely, increased aeration time appears to favor P_2O_5 removal, likely by promoting phosphorus uptake by polyphosphate-accumulating organisms (PAOs) during aerobic phases—a well-known mechanism in enhanced biological phosphorus removal (EBPR) systems [39].

This behavior underscores the importance of optimizing redox transitions and maintaining appropriate oxygenation during aerobic phases to sustain phosphorus removal performance. In SBR–NDN systems, where biological phosphorus removal is not always the primary design goal, these observations highlight the potential for integrating EBPR strategies to enhance P_2O_5 control under specific operational regimes.

Interpretation of Redox Slope Dynamics During Aerobic and Anoxic Phases:

The analysis of oxidation–reduction potential (ORP) slopes across the aerobic (Phases 1 and 3) and anoxic (Phases 2 and 4) segments of the SBR–NDN process revealed distinct behavior linked to operational variables and environmental conditions.

In the aerobic phases, the ORP slope—defined as the rate of change between the maximum and minimum values—showed positive correlations with treated volume (Corr. 0.199 ***, 0.308), retention time in Equalization Tank 3 (Corr. 0.333 ***, 0.304 ***), and aeration time (Corr. 0.649 ***, 0.748). These patterns suggest that greater organic and nitrogen loading during high-volume operations accelerates microbial oxidative activity, reflected in steeper redox gradients. This aligns with findings from the literature, where faster redox transitions during aerobic phases are associated with increased nitrification rates and microbial respiration under sufficient oxygen supply [40]. In contrast, the anoxic phases exhibited no correlation between the ORP slope and treated volume or residence time in Equalization Tanks 1 and 2. However, a positive correlation was observed with mixing time (Corr. 0.225, 0.366), and a significant negative correlation was observed with aeration time (Corr. −0.461 ***, −0.274 ***), average temperature (Corr. −0.470 ***, −0.551), and wet-bulb temperature (Corr. −0.440 ***, −0.534). These results highlight that under cooler, more humid conditions, denitrifying activity becomes more pronounced, leading to more dynamic redox transitions even in the absence of oxygen—a behavior consistent with optimal anoxic performance observed in previous SBR studies under controlled temperature and mixing regimes [41]

Interpretation of GHG emission analysis:

The plant studied by Vingerhoets et al. [8] demonstrates higher removal efficiencies than the one analyzed in our work. It is clear that the recovery of nitrogen (N) and phosphorus (P) prior to the NDN treatment significantly improves overall performance. In our case, we achieved average removal efficiencies of 84% for nitrogen, 38% for phosphorus, and 80% for COD. These results fall short of those reported in the referenced study, which achieved removal efficiencies of 95% for nitrogen, 99% for phosphorus, and 99% for COD.

Together, these results reinforce the importance of tightly managing aerobic and anoxic redox dynamics to support complete nitrification–denitrification cycles in SBR–NDN systems, particularly under varying climatic conditions such as those in the high-altitude province of Ávila.

5. Conclusions

Future research should explore the integration of advanced sensor technologies, such as in situ dissolved oxygen probes within the SBR and atmospheric gas sensors in pre-treatment areas, to enable real-time process monitoring and dynamic control. Additionally, implementing algal photobioreactors as a post-treatment step offers the potential for further nutrient polishing and improved effluent quality—approaches that have shown promise in related pilot studies [42]. These strategies will be critical to enhancing the resilience and environmental performance of treatment systems operating under variable and sometimes extreme climatic conditions, such as those in the province of Ávila.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/nitrogen6030068/s1>, Figure S1: Correlation matrix for COD reduction efficiency; Figure S2: Correlation matrix for NH₄ reduction efficiency; Figure S3: Correlation matrix for NO₂ reduction efficiency; Figure S4: Correlation matrix for total nitrogen reduction efficiency; Figure S5: Correlation matrix for P₂O₅ reduction efficiency; Figure S6: Correlation matrix for redox slope on Phase 1; Figure S7: Correlation matrix for redox slope on Phase 3; Figure S8: Correlation matrix for redox slope on Phase 2; Figure S9: Correlation matrix for redox slope on Phase 4.

Author Contributions: Conceptualization, L.E.-C. and F.J.S.J.; methodology, D.P.-H.; software, D.P.-H.; validation, M.d.P.P.Á.-C. and B.R.; formal analysis, A.J.-S.; investigation, L.E.-C.; resources, A.J.-S.; data curation, D.P.-H.; writing—original draft preparation, L.E.-C.; writing—review and editing, F.J.S.J.; visualization, D.P.-H.; supervision, D.P.-H.; project administration, B.R. and R.M.; funding acquisition, M.d.P.P.Á.-C. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The corresponding author will make the data and materials available on reasonable request.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

TreatVol	Treated Volume
EqTank1_MixTime	Mixing Duration—Equalization Tank 1
EqTank2_MixTime	Mixing Duration—Equalization Tank 2
AerTime	Aeration Duration
MixTime	Mixing Duration
ORP Slope Ph 1	ORP Slope Phase 1
ORP Slope Ph 2	ORP Slope Phase 2
ORP Slope Ph 3	ORP Slope Phase 3
ORP Slope Ph 4	ORP Slope Phase 4
Temp_Avg	Average Temperature
WindSpeed_Avg	Average Wind Speed
RH_Avg	Average Relative Humidity
SlurryTemp1_Avg	Average Slurry Temperature—Sensor 1
Temp_Diff	Temperature Difference
TbH_Avg	Average Wet-bulb Temperature
Corr.	Correlation

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