

Isotopic assessment of nitrate removal in constructed wetlands: comparison between continuous and intermittent flow operation under whey-mediated biostimulation

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Abstract

Nitrate (NO_3^-) is a widespread pollutant in agricultural runoff. Constructed wetlands (CWs) remove NO_3^- via plant uptake and microbial denitrification, often limited by organic carbon availability and hydraulic regime. This study evaluates the denitrification efficiency of a CW in the Lerma gully basin (Zaragoza, Spain), which receives nitrate-polluted runoff (avg. 140 mg/L NO_3^-). A biostimulation strategy using whey, an agro-industrial by-product, was tested under different hydraulic regimes. Continuous flow with daily whey injections achieved up to 20% denitrification efficiency. Under weekly intermittent open–close cycles, unstimulated conditions removed 35% of nitrate, while whey injections led to complete denitrification at the outlet. However, hydraulic limitations reduced the effective removal. Results indicate that hydraulic regime, water renewal efficiency, and electron donor dosing strongly control denitrification performance. Whey represents a feasible and scalable electron donor to enhance nitrate removal in CWs treating agricultural runoff.

Keywords

Biostimulation, Constructed Wetland, Denitrification, Isotopic fractionation, Nitrate pollution, Whey

INTRODUCTION

Nitrate (NO_3^-) is a widespread pollutant in agricultural runoff derived from intensive fertilizer use. Constructed wetlands (CWs) are cost-effective and low-energy systems designed to remove NO_3^- mainly through plant uptake and microbial denitrification. This process converts NO_3^- into N_2 in the presence of an electron donor, typically organic carbon, which often is the limiting factor for denitrification (Rivett et al., 2008). Hydraulic regime also controls performance: longer hydraulic retention times tend to enhance denitrification, whereas high-flow conditions tend to reduce efficiency.

This study, financed by the projects NPP-SOL (PCI2023-143359), ISOTRACE (PID2022-139911OB-C41) and AGROSOSIII (PID2023-147588OB-I00), evaluates NO_3^- removal in a 4000 m² CW located in the Lerma gully basin (Zaragoza, Spain; Fig. 1), treating agricultural runoff (avg. 140 mg NO_3^- /L). The CW combines a 40-cm-deep vegetated surface-flow channel (2130 m²) and a gravel subsurface zone (500 m²). The objective is to compare nitrate removal under two hydraulic regimes during a biostimulation strategy implementation.

MATERIALS AND METHODS

The selected organic carbon source for the biostimulation treatment was whey, an agro-industrial by-product, as previous laboratory assays confirmed its suitability as an electron donor (Margalef-Martí et al., 2019). Additionally, batch experiments were conducted to determine the isotopic fractionation of NO_3^- ($\epsilon^{15}\text{N}-\text{NO}_3^-$ and $\epsilon^{18}\text{O}-\text{NO}_3^-$) during denitrification using the available whey, from a local factory.

Operational strategies tested in the CW consisted of: (i) Strategy 1 (May–December 2024), continuous flow (260 m³/day) with daily whey injections (50 L/day); (ii) Strategy 2 (April–June 2025), intermittent flow with weekly whey injections (350 L/week); and (iii) planned Strategies 3–4 (from May 2026), combining continuous flow (>500 m³/day) and/or open–close cycles ensuring complete water renewal with optimized dosing of whey injections. Control periods without biostimulation were included in all strategies.

Periodic sampling campaigns were conducted during operational stages. Concentrations of NO_3^- , NO_2^- , NH_4^+ , and dissolved organic carbon (DOC) were analyzed, together with the dual isotopic composition of nitrate ($\delta^{15}\text{N}-\text{NO}_3^-$ and $\delta^{18}\text{O}-\text{NO}_3^-$), to quantify denitrification. In addition, NO_3^- concentrations and water flow at the inlet and outlet were continuously monitored using remote sensing probes.

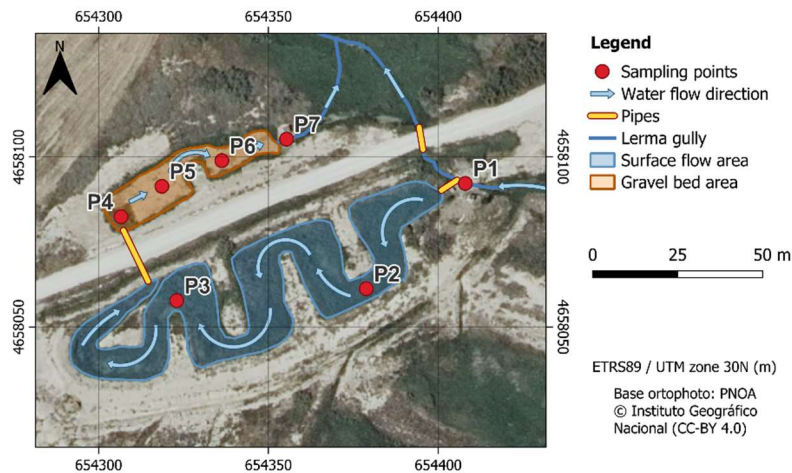


Figure 1. CW in the Lerma gully basin (Zaragoza, Spain). Flow direction from inlet (P1) to outlet (P7).

RESULTS AND DISCUSSION

During Strategy 1, prior to biostimulation, intrinsic denitrification was negligible. With daily whey injections, maximum isotopic enrichment was observed in September 2024, with a denitrification efficiency of 20%, corresponding to the removal of 134 kg NO_3^- that month. During Strategy 2, control weekly cycles resulted in 20-35% NO_3^- removal (35 kg NO_3^- over 3 weeks). In subsequent cycles with biostimulation, outlet NO_3^- concentration fell below detection limits, indicating complete denitrification. However, sediment clogging in distribution pipes along the CW limited hydraulic renewal during intermittent flow, resulting in an estimated total removal of 60 kg NO_3^- over 4 weeks. During both strategies NO_2^- and NH_4^+ remained negligible at the outlet (<1.5 and <0.4 mg/L), and DOC remained below inlet concentrations (<15 mg/l), reinforcing the safety of the treatment from an environmental and human health perspective. Biostimulation strategies planned for implementation from May 2026 aim to increase the system's denitrification capacity.

CONCLUSIONS

Hydraulic regime from influent water, renewal efficiency, electron donor injection dose and frequency, and intrinsic organic carbon availability are key factors for optimizing whey-driven denitrification in the studied CW. Whey represents a feasible electron donor for the biostimulation of denitrifying microorganisms and therefore a scalable solution for enhancing denitrification in large-scale CWs treating agricultural runoff. Intermittent cycles with pulsed injections showed higher potential than continuous flow with daily injections but were constrained by operational flow limitations. Whey enhanced denitrification beyond plant uptake (*Typha* spp. and *Phragmites* spp.), estimated at 18 kg NO_3^- per growth month.

REFERENCES

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