

Denitrification Stimulation Improves Agricultural Runoff Water Treatment in a Constructed Wetland as Revealed by Isotopic Analysis: comparison between two electron-donors.

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Abstract

A constructed wetland (CW) has been operating for nearly ten years in the Lerma Gully basin (Spain) to mitigate nitrate (NO_3^-) pollution from agricultural runoff. However, it shows limited capacity to remove NO_3^- when operated at water flows above 2.5 L/s. This study evaluated biostimulation strategies to enhance NO_3^- removal in the CW. Two field strategies were implemented: 2 punctual applications of corn stubble (2017–2018), and continuous whey injections (2024–2026). Corn stubble application produced high but short-term denitrification, removing up to 2100 kg of NO_3^- within three months. Whey injections achieved lower removal (403 kg of NO_3^- over seven months) but required significantly less maintenance, making them more suitable for long-term operation. Both treatments produced negligible harmful by-products that could decrease water quality, confirming the safety of the approach.

Keywords

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

INTRODUCTION

Nitrate (NO_3^-) is a common pollutant in agricultural runoff water due to the excessive use of fertilizers. Constructed wetlands (CWs) are low cost and easy operation systems for removing NO_3^- and other contaminants. The main pathways that contribute to NO_3^- mitigation in CWs are assimilation by plants and denitrification, a microbial enzymatic process that transforms NO_3^- into innocuous nitrogen gas when an electron donor is available. Therefore, denitrification is an authentic reactive nitrogen sink from water compared to biomass sequestration. If electron donors in the CW are insufficient for effective denitrification, external sources, such as organic carbon compounds, can be added.

A CW (≈ 40 cm depth, ≈ 4000 m²) has been operating for nearly ten years in the Lerma Gully basin (Zaragoza, Spain) to mitigate NO_3^- pollution from agricultural runoff. The system predominantly consists of a vegetated surface flow CW, while 20% of the area is covered with gravel leading to subsurface flow. However, it shows a limited capacity to remove NO_3^- when operated at water flows above 2.5 L/s during the warm months, and an even more limited capacity during the cold months. The aim of the present study was to enhance NO_3^- reduction in the Lerma CW through the addition of organic carbon-rich residues from the agri-food industry and to evaluate the biostimulation treatment efficiency.

METHODS

Prior to the biostimulation implementation at field scale in the CW, we conducted laboratory experiments to evaluate the suitability of using solid and liquid residues of the agrifood industry as external electron donors to enhance denitrification. Corn stubble and whey from nearby producers were selected based on their performance. In 2017–2018 we implemented a biostimulation strategy consisting of two punctual applications of corn stubble (7 tons each, autumn vs. spring). In 2024–2026 we have implemented a new biostimulation strategy consisting of daily/weekly injections of whey (spring). In both cases the CW have been operated in continuous flow rates of up to 16 L/s, resulting in the treatment of up to 9700 m³ of water each week.

Along the study, NO_3^- concentration has been monitored at the inlet and the outlet of the CW using remote sensing probes. This allows to control NO_3^- evolution but it does not reveal the specific processes involved in NO_3^- attenuation (e.g., dilution due to rainfall or denitrification), making it challenging to focus on improvement strategies. Instead, stable isotope analyses can provide information on transformation pathways (e.g., denitrification induces isotopic fractionation in ^{15}N and ^{18}O from NO_3^-). Therefore, periodic sampling campaigns have also been conducted for chemical and isotopic characterization of the samples.

RESULTS

The CW inlet NO_3^- concentration range between 30-200 mg/L, depending on the agricultural practices. The results of the biostimulation strategy with corn stubble were published in Margalef-Marti et al., 2019, and are compared here to those of the biostimulation strategy with whey. Briefly, the punctual application of corn stubble induced efficient denitrification with 10 to 100% NO_3^- removal during 3 months after stubble application in spring (less efficient in autumn). This was equivalent to 2100 Kg of NO_3^- removed in 3 months. After that period denitrification became negligible.

For the recent biostimulation with whey, the NO_3^- isotopic composition indicated that at least 20% denitrification efficiency was achieved after 2 months from the beginning of injections and until they were stopped 7 months later (results from a representative sampling campaign are shown in Figure 1). This is equivalent to 403 Kg of NO_3^- removed in 7 months, which is comparatively 10 times lower than that achieved with stubble. Nevertheless, the whey injection strategy involves much less maintenance works compared to the previous, making it more suitable for long-term application. We are currently adjusting the dose and periodicity of whey injection as well as the CW water flow aiming to treat a higher amount of water circulating through the Lerma ravine to decrease downstream rivers contamination.

For both treatments, harmful denitrification intermediate products with lower thresholds for consumption compared to nitrate such as nitrite or ammonium were negligible at the outlet, reinforcing the treatment safety. Also, in addition to denitrification, in the CW up to 18 Kg of NO_3^- can be removed per month by plant assimilation during the vegetation (*Typha* and *Phragmites*) growth period.

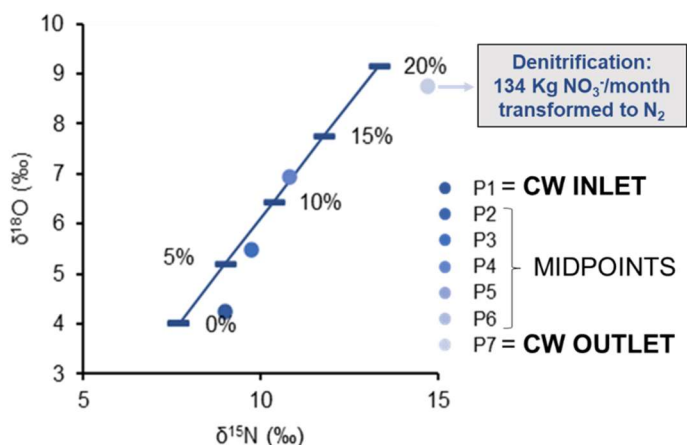


Figure 1. Isotopic results of CW biostimulation with whey conducted in 2024 from a sampling campaign conducted in September. The shown % are calculated from isotopic fractionation values (ϵ) determined in laboratory batch experiments.

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REFERENCES

Margalef-Marti, R., Carrey, R., Merchán, D., Soler, A., Causapé, J., Otero, N. 2019. Feasibility of using rural waste products to increase the denitrification efficiency in a surface flow constructed wetland, *J. Hydrol.* 578, 124035.