This project is part of the PRIMA programme supported by the European Union

PRIMA-SECTION 2-2022

"Modelling and Technological Tools to Prevent Surface and Ground-Water Bodies from Agricultural Non-Point Source Pollution Under Mediterranean Conditions"

NPP-SOL

Report on the validation of AD in the buffer strip of Nahalal stream, Israel Case Study

Deliverable number: D3.3

This project is part of the PRIMA programme supported by the European Union

Non-Point Pollution SOLutions (NPP-SOL)

Deliverable number: D3.3

Report on the validation of AD in the buffer strip of Nahalal stream, Israel Case Study

Authors	Roy Posmanik (Technion – Israel Institute of Technology) Roey Egozi and Elazar Volk (Ministry of Agriculture and Food Security)
Approved by reviewers	Antonio Coppola and Shawkat BM. Hassan (University of Cagliari)
Date of approval	21/10/2026
Approved by project coordinator	Antonio Coppola
Date of approval	21/10/2026
Call	PRIMA Section 2 2022 – Multi-topic Topic 2.1.1 RIA Prevent and reduce land and water salinization and pollution due to agri-food activities
Project URL	https://npp-sol.iamm.ciheam.org/

1. Scope and objective

The Israeli D3.3 activity evaluated an integrated anaerobic digestion-constructed wetland (AD-CW) treatment train for dairy-farm manure management. Anaerobic digestion converts wet dairy manure into biogas, but also produces a liquid digestate containing residual organic matter, nutrients and dissolved minerals. The main validation activity therefore focused on the constructed wetland as an on-farm post-treatment step for the liquid digestate, with the objective of reducing the residual pollutant load before potential reuse, discharge or environmental recovery.

2. Experimental system and operating strategy

The field system consisted of a farm-scale anaerobic digester connected to a free-water-surface constructed wetland **(Fig. 1)**. The AD unit treated wet dairy manure from the calf-rearing unit, had a maximum capacity of approximately 50 kg manure d^{-1}, and produced biogas that could be used for heat and power. Microbial acclimation and the subsequent increase in biogas production required approximately six months of continuous feeding with water-diluted manure. The liquid digestate generated by the AD unit was then directed to the CW for secondary treatment.

Two 10 m^3 metal tanks were established as free-water-surface CW units using volcanic lightweight aggregate and a diverse macrophyte community. After a nine-month establishment period, one CW received AD digestate and the second was maintained with tap water as a control. Digestate was applied in 50-L batches. The loading experiment consisted of four consecutive two-week phases, increasing from 200 to 500 L digestate per phase, corresponding to organic carbon loadings of approximately 320, 480, 640 and 800 g C m^{-3}.

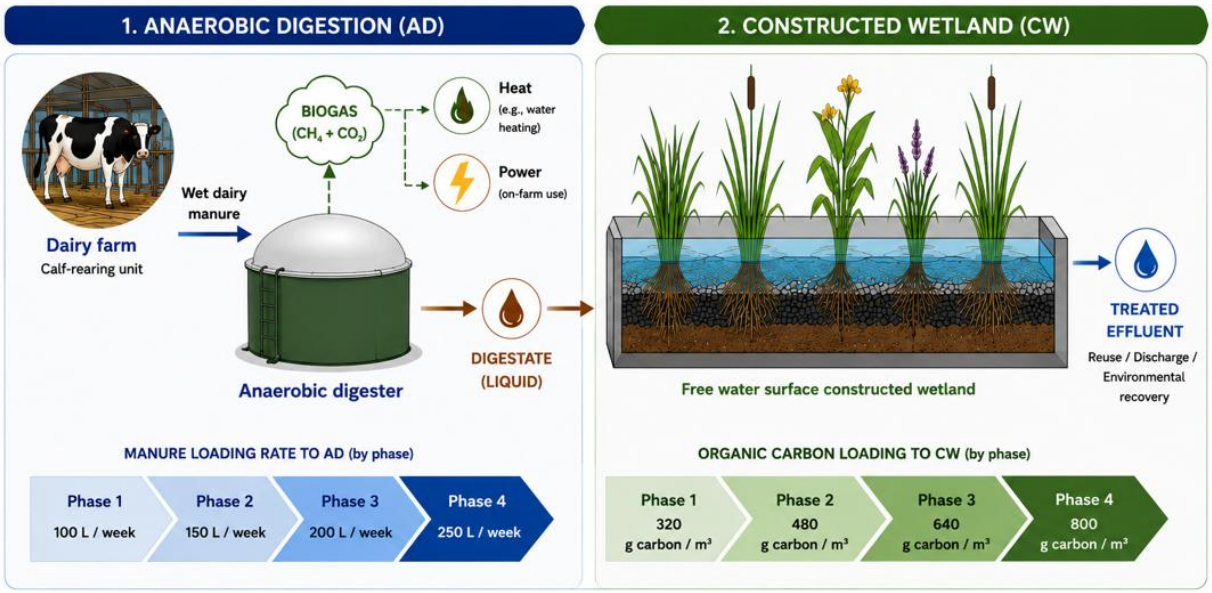


Figure 1. Experimental configuration of the integrated anaerobic digestion-constructed wetland (AD-CW) treatment system.

3. Digestate characteristics and CW monitoring

The liquid digestate represented a concentrated secondary wastewater stream. Reported characterization included pH 9.2, electrical conductivity of 17.4 dS m^{-1}, dissolved organic carbon of 16.0 ± 1.0 g L^{-1} and total dissolved nitrogen of 8.0 ± 0.4 g L^{-1} (n=6). The CW effluent was monitored for pH, electrical conductivity, dissolved organic carbon, total dissolved nitrogen, inorganic nitrogen species, major cations and total phosphorus. In parallel, rhizosphere/filter-bed samples were collected to evaluate microbial community responses using 16S rRNA gene sequencing.

4. Treatment performance under increasing digestate loading

The CW was deliberately challenged with progressively increasing digestate loading to determine whether treatment performance could be maintained as the pollutant load increased. Across the experiment, influent TOC, TN and TP concentrations increased from approximately 160, 110 and 63 mg L^{-1} to 250, 150 and 75 mg L^{-1}, respectively. Despite this increase, effluent concentrations remained below 150 mg L^{-1} TOC, 27 mg L^{-1} TN and 8.5 mg L^{-1} TP. The wetland vegetation also remained visually healthy, with no observable morphological symptoms of stress.

The stable effluent quality despite progressively increasing loading indicates that the imposed load did not exceed the treatment capacity of the mature CW within the tested range. This is the principal

engineering outcome of the D3.3 validation: the nature-based polishing step remained operationally resilient while treating increasingly concentrated AD-derived wastewater.

5. Microbial community response

Microbial analysis was used to determine whether stable treatment performance was associated with a stable community or with biological adaptation. Principal-coordinate analysis based on unweighted UniFrac distances showed a clear separation between the control and digestate-treated CWs **(Fig. 2)**. PCoA axis 1 explained 35.5% of the variation, and PERMANOVA confirmed a significant treatment effect (pseudo-F = 40.6, P = 0.001). In contrast, no distinct clustering according to the increasing organic carbon loading was observed within the digestate-treated CW.

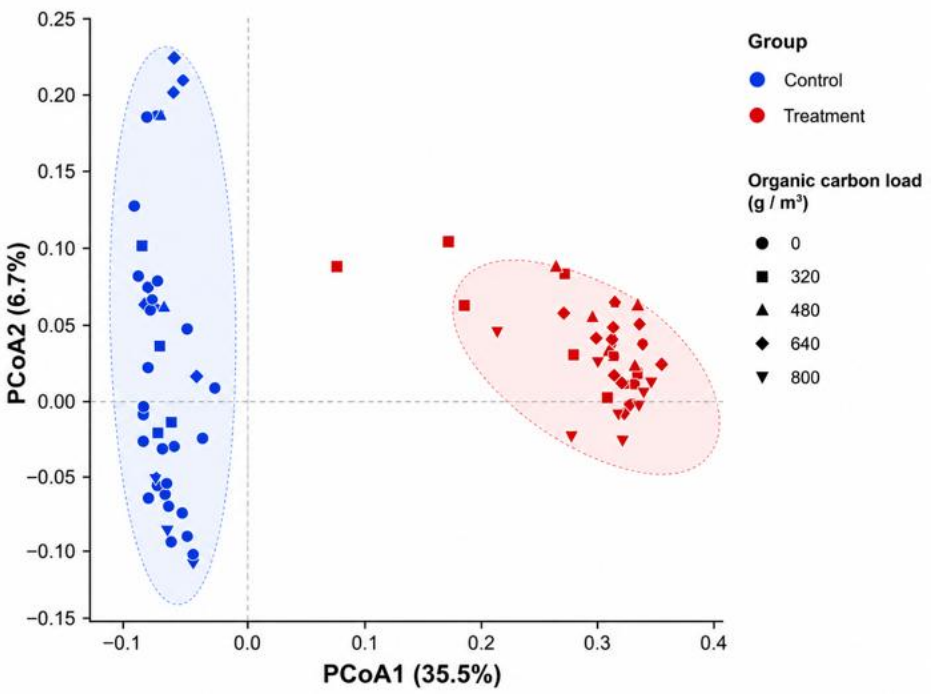


Figure 2. Effect of digestate application on rhizosphere bacterial community composition. PCoA based on unweighted UniFrac distances shows a clear separation between control and digestate-treated CWs. The treatment effect was significant (PERMANOVA pseudo-F = 40.6, P = 0.001), whereas the increasing organic carbon loading produced comparatively limited additional separation within the treated CW.

Alpha-diversity analysis further showed that digestate exposure significantly altered the internal structure of the bacterial community. Shannon diversity was reduced (P = 0.027) and Simpson's dominance index was significantly altered (P = 0.041), consistent with selection of a community increasingly dominated by taxa adapted to the digestate-fed environment.

6. Microbial adaptation across the loading gradient

Taxonomic analysis showed a consistent response to digestate application. Proteobacteria remained dominant, but digestate treatment was associated with increased relative abundance of Chloroflexi and Firmicutes and reduced relative abundance of Proteobacteria and Planctomycetota. At the order level **(Fig. 3)**, several anaerobic and fermentative groups - including Synergistales, Halanaerobiales, Erysipelotrichales, Desulfotomaculales and Peptostreptococcales-Tissierellales - remained enriched across the loading gradient. Several sulfur-oxidizing, metal-reducing and oligotrophic groups were consistently depleted.

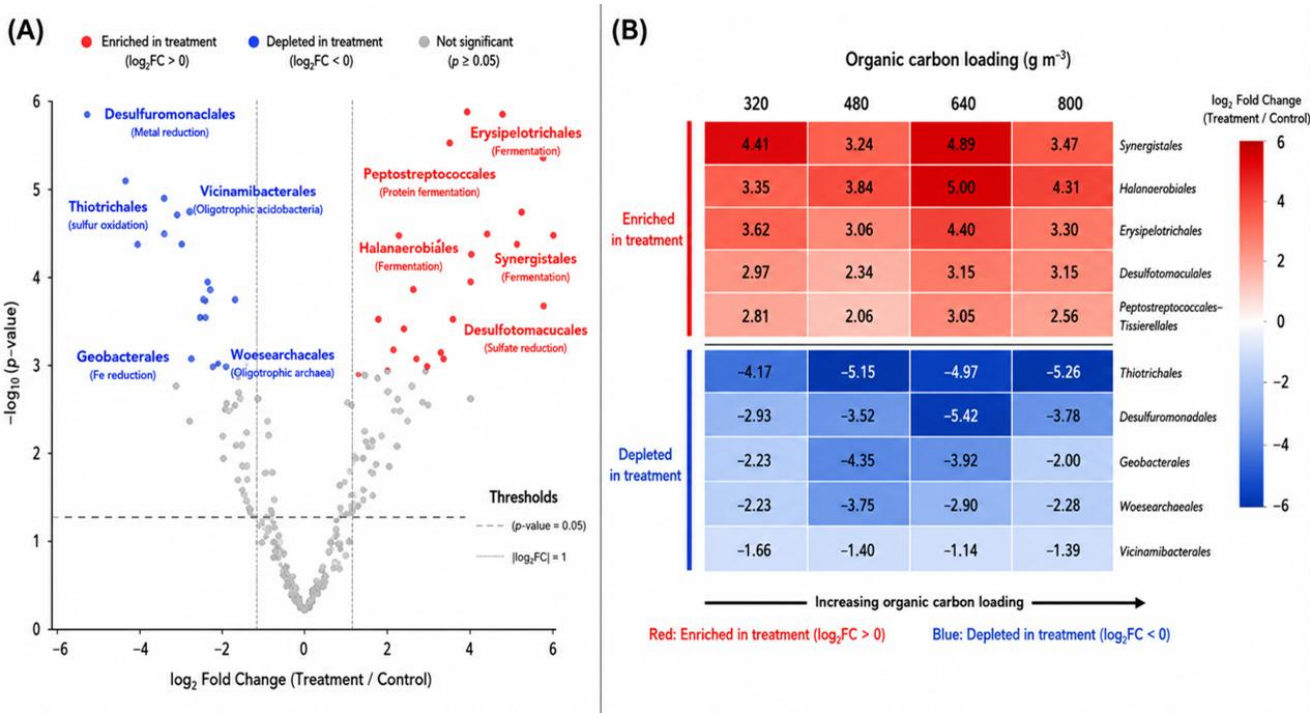


Figure 3. Differential responses of rhizosphere bacterial taxa to digestate application. (A) Volcano plot showing bacterial orders enriched or depleted in the digestate-treated CW during the initial loading phase (320 g C m^{-3}). (B) Heatmap showing taxa that remained significantly enriched or depleted across the four organic carbon loading rates (320-800 g C m^{-3}).

The microbiome changed substantially even though treatment performance remained stable **(Fig. 3)**. This decoupling between community composition and ecosystem function is consistent with functional resilience: the CW community reorganized in response to digestate while retaining the biological functions required for treatment. Continuous digestate application may also act as a biological input by repeatedly introducing manure- and AD-associated microorganisms into the wetland. It should be noted that functional assignments to individual microbial taxa are based on 16S

rRNA taxonomic affiliation and should therefore be regarded as ecological inference rather than direct measurements of metabolic activity.

7. Main outcomes and relevance to D3.3

- The Israeli activity demonstrated an integrated AD-CW treatment train in which dairy manure is first converted to biogas and the resulting liquid digestate is subsequently polished using a nature-based treatment system.
- The CW tolerated progressively increasing digestate loading up to 500 L per 14-day phase (approximately 800 g C m^{-3}) while maintaining low effluent TOC, TN and TP concentrations within the tested operating range.
- Stable treatment performance occurred despite pronounced restructuring of the rhizosphere microbiome, demonstrating biological adaptation and functional resilience of the mature CW.
- The results support constructed wetlands as a post-treatment step downstream of farm-scale anaerobic digestion, addressing residual organic matter and nutrient loads that remain in the liquid digestate after energy recovery.
- The activity extends anaerobic digestion from a stand-alone waste-to-energy process toward an integrated treatment train combining resource recovery through biogas production with nature-based mitigation of residual agricultural pollution.

8. Source note

This report is based on the NPP-SOL manuscript:

Buium, H., Meromi, O., Zohar, M., Iasur Kruh, L., Posmanik, R. System resilience in constructed wetlands under increasing digestate loading: stable nutrient removal despite microbial community restructuring, *Ecohydrology* (Under Review).