Restoring Nitrogen Cycling Integrity in Eutrophied Temperate Wetlands Through Targeted Denitrifying Microbial Consortium Inoculation and Emergent Macrophyte Replanting

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Abstract. Chronic anthropogenic nitrogen loading in temperate freshwater wetlands disrupts biogeochemical cycling, accelerates eutrophication, and undermines ecosystem provisioning services. This study evaluates a dual-intervention restoration protocol combining targeted inoculation of denitrifying microbial consortia (Paracoccus denitrificans and Pseudomonas stutzeri assemblages) with emergent macrophyte replanting (Phragmites australis and Typha latifolia) across six degraded wetland plots in the Rhine-Meuse delta over 24 months. Nitrate removal efficiency, sediment redox potential, dissolved oxygen gradients, and aboveground biomass accrual were monitored at bimonthly intervals. Results demonstrate a 74.3% reduction in interstitial nitrate concentration and a statistically significant recovery of macroinvertebrate assemblage richness ($p < 0.001$). Microbial community profiling via 16S rRNA amplicon sequencing confirmed stable denitrifier establishment. The combined protocol substantially outperforms single-intervention controls, offering a scalable, cost-effective remediation strategy for nitrogen-impaired wetland ecosystems.

Keywords: eutrophication remediation, denitrifying microbial consortia, wetland nitrogen cycling, emergent macrophyte restoration, biogeochemical nitrogen removal, sediment redox dynamics, macroinvertebrate community recovery, 16S rRNA amplicon sequencing, freshwater ecosystem services

Introduction

Freshwater wetlands represent among the most biogeochemically active ecosystems on Earth, functioning as critical buffers between terrestrial nutrient sources and downstream aquatic environments. Their capacity to attenuate nitrogen fluxes through coupled nitrification–denitrification, plant assimilation, and sediment burial has been extensively documented. Yet, by 2025, an estimated 35% of temperate freshwater wetlands in Europe and North America are classified as nitrogen-saturated, a condition characterized by the collapse of autochthonous denitrifying communities, hyperalgal proliferation, hypolimnetic anoxia, and the progressive exclusion of sensitive macrofaunal guilds. These trajectories are overwhelmingly attributable to sustained agricultural runoff, atmospheric nitrogen deposition, and poorly treated municipal wastewater discharge — pressures that continue to intensify under current land-use and climate trajectories. The ecological and socioeconomic consequences of wetland nitrogen saturation are profound. Degraded nitrogen cycling undermines water purification, flood attenuation, and carbon sequestration services, with estimated annual economic losses from impaired wetland provisioning services in the European Union alone exceeding €4.2 billion. Despite the growing body of literature on constructed wetlands and passive bioremediation, practical, field-validated protocols that simultaneously target microbial functional recovery and macrophyte structural restoration within natural wetland matrices remain underrepresented in the primary literature. Existing single-pathway interventions — whether microbial augmentation alone or vegetation replanting without concurrent rhizosphere microbiome management — consistently underperform against remediation benchmarks, particularly in heavily impacted deltaic and floodplain systems characterized by dynamic hydrological regimes and fluctuating redox conditions. This gap is especially critical given the regulatory context established by the EU Water Framework Directive (2000/60/EC) and its 2022 revisions, which mandate measurable ecological status improvements in all surface water bodies by 2027. Field-applicable, evidence-based dual-intervention restoration frameworks are urgently needed to assist wetland managers, conservation agencies, and municipal water authorities in achieving statutory nitrogen reduction targets without the prohibitive costs associated with engineered treatment infrastructure. The present study directly addresses this practical challenge by designing, implementing, and rigorously evaluating a combined denitrifying microbial consortium inoculation and emergent macrophyte replanting protocol within degraded temperate wetlands of the Rhine-Meuse delta. The paper is structured as follows: Section 2 details the study site characteristics, experimental design, and analytical methodologies employed; Section 3 presents quantitative results from nitrate removal assays, sediment redox profiling, 16S rRNA community analyses, and macroinvertebrate biodiversity assessments; Section 4 discusses mechanistic interpretations, scalability considerations, and management implications; and Section 5 synthesizes the principal findings and outlines priority directions for future restoration research in nitrogen-saturated wetland systems.

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