

Soil Organic Carbon Depletion and Microbial Community Restructuring Under Chronic Nitrogen Deposition in Temperate Deciduous Forest Ecosystems: Evidence from a 12-Year Mesocosm Experiment

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Abstract. Chronic atmospheric nitrogen (N) deposition exerts profound yet heterogeneous effects on soil organic carbon (SOC) dynamics and belowground microbial assemblages in temperate deciduous forests. This study integrates 12 years of manipulative mesocosm data from a mixed *Quercus*–*Fagus* stand to quantify SOC mineralization rates, fungal-to-bacterial biomass ratios, and extracellular enzyme stoichiometry under simulated N loading gradients (0–60 kg N ha⁻¹ yr⁻¹). Phospholipid fatty acid (PLFA) profiling and metagenomic sequencing revealed a significant suppression of ectomycorrhizal fungal guilds and a concomitant shift toward copiotrophic bacterial dominance at deposition rates exceeding 30 kg N ha⁻¹ yr⁻¹. SOC stocks declined by 18.4% in high-N treatments, driven primarily by enhanced oxidative enzyme activity. These findings underscore critical thresholds for N-mediated destabilization of forest carbon sequestration and inform deposition-sensitive ecosystem management frameworks.

Keywords: nitrogen deposition, soil organic carbon mineralization, microbial community composition, ectomycorrhizal fungi, extracellular enzyme stoichiometry, temperate deciduous forest, PLFA profiling, carbon sequestration thresholds, belowground biogeochemistry

Introduction

Atmospheric reactive nitrogen (Nr) deposition has escalated dramatically over the past century as a direct consequence of fossil fuel combustion, intensive agricultural fertilization, and industrial livestock production, emerging as one of the most pervasive and structurally destabilizing drivers of terrestrial ecosystem change globally. By 2024, cumulative Nr deposition in densely industrialized regions of Central Europe, East Asia, and the northeastern United States routinely exceeds 40–50 kg N ha⁻¹ yr⁻¹, surpassing

critical load thresholds established by the Convention on Long-Range Transboundary Air Pollution (CLRTAP) for the majority of sensitive forest biomes. The consequences for belowground ecosystem functioning are both multifaceted and deeply consequential: excess inorganic nitrogen fundamentally alters soil pH buffering capacity, disrupts plant–mycorrhizal symbiotic networks, and reconfigures the stoichiometric balance between carbon, nitrogen, and phosphorus that governs microbial metabolic activity and organic matter decomposition cascades. Temperate deciduous forests represent disproportionately significant global carbon sinks, storing an estimated 1.0–1.5 Pg C annually in mineral soil horizons and organic surface layers. However, the long-term stability of this carbon reservoir under escalating N deposition remains critically underresolved. Empirical evidence accumulated through short-term incubation studies and cross-sectional chronosequence surveys has produced contradictory findings: some investigations report net SOC accumulation under moderate N enrichment due to suppressed lignolytic fungal activity, while others document accelerated SOC turnover attributable to priming-effect interactions between labile N inputs and native soil organic matter pools. These contradictions reflect, in part, methodological inconsistencies in experimental design, deposition rate calibration, and the temporal resolution of sampling regimes. The mechanistic link between N-induced microbial community restructuring and SOC fate remains particularly contentious. Ectomycorrhizal fungal networks, which mediate a substantial fraction of soil carbon inputs via hyphal turnover and exudate flux, are acutely sensitive to elevated inorganic N availability, exhibiting reduced colonization intensity and guild diversity across documented deposition gradients. Concurrently, the proliferation of copiotrophic bacterial taxa under N enrichment has been associated with elevated leucine aminopeptidase and β -glucosidase activities, suggesting enhanced enzymatic access to previously occluded organic substrates. Resolving the relative contributions of these competing mechanisms to net SOC balance demands controlled, long-duration experimental platforms with sufficient replication to disentangle treatment effects from interannual climate variability. The present study addresses these critical knowledge gaps by reporting on a 12-year manipulative mesocosm experiment conducted within a mature mixed *Quercus robur*–*Fagus sylvatica* forest ecosystem, implementing five discrete N deposition treatment levels (0, 15, 30, 45, and 60 kg N ha⁻¹ yr⁻¹) replicated across contrasting soil textural classes. The paper is structured as follows: Section 2 details the experimental site characteristics, mesocosm instrumentation, and analytical protocols for SOC fractionation, PLFA profiling, and metagenomic library construction; Section 3 presents longitudinal trajectories of SOC stocks, microbial biomass indices, and enzyme activity profiles across treatment levels; Section 4 discusses mechanistic interpretations within the framework of microbial carbon use efficiency (CUE) and stoichiometric decomposition theory; and Section 5 synthesizes policy-relevant implications for critical load recalibration and adaptive forest management under projected 2030–2050 N emission scenarios.

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