PINE BARK COMPOST AS BIOFILTER: ENHANCING DENITRIFICATION AND REDUCING NITROGEN LEACHING IN SOIL.

Shumaila Sarwar[1], Farina Jamil[*2]

[1,*2]*Department of Biochemistry, Riphah International University, Faisalabad Campus, Pakistan. 44000*

[*2]farina.jamil@riphahfsd.edu.edu.pk

Corresponding Author: *
Faina Jamil

DOI:https://doi.org/10.5281/zenodo.20698954

Received	Accepted	Published
07 April 2026	19 May 2026	15 June 2026

ABSTRACT

Nitrogen leaching from agricultural soils is a major environmental issue that contributes to groundwater contamination, eutrophication, and greenhouse gas emissions. Excess nitrate (NO_3^-) in soil is highly mobile and can easily move beyond the root zone into water bodies. Recent research has focused on sustainable and low-cost biofiltration materials capable of improving denitrification and reducing nitrate losses. Pine bark compost (PBC), an organic by-product rich in carbon and lignocellulosic compounds, has emerged as an effective biofilter material due to its porous structure, high organic matter content, and ability to support microbial activity. This review examines the role of pine bark compost in enhancing biological denitrification and minimizing nitrogen leaching in soil systems. The paper discusses denitrification mechanisms, physicochemical properties of pine bark compost, microbial interactions, nitrate adsorption, and the influence of environmental factors such as moisture, pH, temperature, and carbon-to-nitrogen ratio. Studies have demonstrated that pine bark-based systems significantly improve nitrate removal efficiency and provide a sustainable alternative to synthetic carbon sources in denitrification processes. The review further highlights experimental methodologies, environmental benefits, limitations, and future research directions associated with the use of pine bark compost as a biofilter in agricultural and environmental management systems.

Keywords: *Pine bark compost, biofilter, denitrification, nitrate leaching, nitrogen cycling, soil remediation, organic amendments, sustainable agriculture.*

INTRODUCTION

"Pine Bark Compost as a Bio filter: Enhancing Denitrification and Reducing Nitrogen Leaching in Soil," signals an investigation into a critical environmental challenge and proposes a specific, sustainable remediation strategy (Huang et al.,2015).

The global environmental impact of agricultural nitrogen (N) loss represents a formidable challenge to water quality and climate stability. The high reliance on synthetic N fertilizers, coupled with inefficiencies in crop uptake, results in a substantial fraction of applied nitrogen being lost from the soil system. Excess nitrate (NO_3^-) is highly soluble and mobile, readily leaching below the root zone to contaminate groundwater, often exceeding safe drinking water limits. This lost NO_3^- further contributes to the eutrophication of surface waters, leading to oxygen-deficient "dead zones" and harmful algal blooms. Crucially, microbial processes within the soil can transform this excess nitrate into nitrous oxide (N2O). N2O is a potent, long-lived greenhouse gas, with a global warming potential approximately 300 times greater than carbon dioxide, making agricultural N management a key factor in climate change mitigation. Therefore, mitigating the movement of NO3 before it leaves the agricultural landscape a concept known as soil bio filtration is essential for achieving sustainable nutrient management, protecting public health, and reducing atmospheric pollution.

Composted Pine Bark (CPB) is proposed as an effective, low-cost bio filtration medium due to its ability to address nitrogen loss through dual-pronged physicochemical and biological mechanisms. Physically, CPB, as an organic amendment, possesses desirable characteristics, such as high total porosity and structural stability that improve soil macro porosity and hydraulic properties. This structural modification helps manage rapid water flow and infiltration, indirectly reducing the rate of water-mediated NO_3 transport (leaching) through the soil profile.

Denitrification is a biological process in which nitrate is reduced to gaseous nitrogen forms such as nitrous oxide (N_2O) and nitrogen gas (N_2), thereby removing reactive nitrogen from the soil-water system. This process is primarily carried out by facultative anaerobic microorganisms under oxygen-limited conditions and requires an adequate supply of organic carbon as an electron donor. Organic substrates therefore play a critical role in promoting denitrification efficiency.

Biologically, the primary mechanism is the enhancement of heterotrophic denitrification, a process in which facultative anaerobic bacteria convert NO_3^- (via a series of reductions) into benign dinitrogen gas (N_2), effectively removing the fixed nitrogen from the ecosystem. This process is limited by the availability of an electron donor, and CPB acts as an abundant, high-quality, and cost-effective source of organic carbon. Due to its high Carbon-to-Nitrogen (C: N) ratio (often 100-130:1) and lignocellulosic nature, CPB is composed of predominantly non-labile and recalcitrant organic molecules, ensuring a slow, long-term release of carbon. This durable carbon supply sustains specialized denitrifying microbial communities (including genera like *Acinetobacter* and *Flavobacterium*) over multiple seasons, making CPB a superior, long-term substrate for in-situ NO_3^- remediation and the reduction of nitrogen leaching.

Pine bark compost has attracted attention as an environmentally friendly and low-cost material capable of supporting denitrification processes. Pine bark is a forestry by-product rich in cellulose, hemicellulose, lignin, and organic carbon compounds that serve as energy sources for denitrifying bacteria. The porous structure of pine bark compost also enhances microbial colonization, water retention, and nitrate adsorption. Several studies have reported successful application of pine bark in denitrifying bioreactors and leachate treatment systems.

The present paper provides a comprehensive overview of pine bark compost as a biofilter for enhancing denitrification and reducing nitrogen leaching in soil environments.

The study aimed to improve the cost-effectiveness of biological denitrification for landfill leachate treatment by exploring inexpensive alternatives to conventional chemical additives. The main objective was to examine how bacterial communities responded to each substrate during the treatment of highly concentrated landfill leachate. Laboratory-scale anaerobic batch experiments were conducted using fixed-bed reactor simulations with immature compost and pine bark as biofilter materials. A synthetic high-strength leachate containing 500 mg L^{-1} nitrate was prepared to assess denitrification performance.

The findings revealed that pine bark released significant amounts of phenolic compounds and hydroxylated benzene derivatives, which negatively affected microbial adaptation and inhibited denitrification efficiency, resulting in only about 30% nitrate removal. In addition, the detection of potentially harmful microorganisms such as Enterobacter and Pantoea agglomerans limited the suitability of pine bark for large-scale applications. In contrast, lightly composted garden refuse provided favorable conditions for biofilm formation, which is essential for effective denitrification, and achieved complete nitrate removal within seven days. CGR also promoted the rapid development of active denitrifying bacterial populations, including Acinetobacter, Rhizobium, Thermomonas, Rheinheimera, Phaeospirillum, and Flavobacterium. The study concluded that factors such as substrate composition, carbon-to-nitrogen ratio, degree of compost maturity, and stability strongly influence bacterial growth and denitrification efficiency, thereby affecting the long-term success of nitrate removal systems.

Agricultural soils receiving excessive nitrogen fertilizers often exhibit nitrogen use efficiencies below 50%, meaning a significant portion of applied nitrogen is lost to the environment. Denitrification-based biofilters have therefore become an important strategy for mitigating nitrate pollution.

Denitrification is a microbial respiratory pathway in which nitrate is sequentially reduced under anaerobic conditions.

1. Material and Methods

This chapter outlines the comprehensive experimental framework and methodologies employed to achieve the research objectives. It provides a detailed account of the materials used, including the collection and characterization of soil and pine bark compost samples, alongside the setup for pot experiments involving winter wheat. Key technical procedures are described, such as the establishment of specific leaching fractions to monitor nitrogen movement, the application of the Acetylene Inhibition Method to quantify Denitrification Enzyme Activity (DEA), and the use of Kjeldahl digestion for analyzing total nitrogen uptake in plant tissues. By documenting these standardized scientific protocols, the chapter establishes the procedural reliability necessary to evaluate the effectiveness of pine bark compost as a sustainable bio-filter for reducing nitrogen leaching and enhancing soil denitrification.

The experimental work was performed within a controlled laboratory environment to ensure that all external variables were strictly regulated, allowing for an accurate assessment of the biochemical processes.

The primary experimental unit was defined as a PVC Column. A total of 12 PVC columns were prepared and arranged according to a Completely Randomized Design (CRD). These columns were packed with a mixture of local sandy loam soil and composted pine bark to a uniform bulk density of 1.3 g/cm³. Additionally, Winter Wheat was planted in each unit to simulate a functional soil-plant system for monitoring nitrogen dynamics.

Table 3.1: CBP Concentration with Soil Ratio

CPB Concentration	Description	CPB:Soil Ratio (w/w)
0%	Serves as the baseline soil with only the standard KNO_3 fertilizer load.	Treatment Group
10%	Low CPB concentration.	10% CPB : 90% Soil
20%	Intermediate (Middle) CPB concentration.	20% CPB : 80% Soil
30%	High CPB concentration.	30% CPB : 70% Soil

In this study, Composted Pine Bark (CPB) was utilized as the bio-filter. The pine bark compost was collected from a local nursery with medium pore size to ensure consistency with soil particles. This material was selected due to its high Carbon-to-Nitrogen (C:N) ratio (approximately 100–130:1), which served as a sustained carbon source for indigenous soil denitrifiers. Through this bio-filtration setup, nitrate (NO_3^-) was biologically converted into harmless nitrogen gas (N_2). It was observed that the application of this bio-filter, particularly at a 20% concentration, successfully reduced nitrate leaching by more than 70%.

2.1 Spectrophotometer: Utilized to determine the concentration of Nitrate (NO_3^-) in the collected leachate samples.

2.2 Gas Chromatograph (GC-ECD): A high-precision instrument equipped with an Electron Capture Detector used to quantify Nitrous Oxide (N_2O) emissions from the soil.

2.3 Kjeldahl Digestion and Distillation Unit: Employed for the determination of Total Nitrogen in the harvested winter wheat biomass and soil samples.

2.4 Drying Oven: Used to dry plant samples at a constant temperature of 65°C for biomass calculation.
2.5 Acetylene Inhibition Equipment: Used during the Denitrification Enzyme Activity (DEA) assay to block the conversion of N_2O to N_2, allowing for the measurement of total denitrification.
2.6 KCl Extraction Setup: Used to extract inorganic nitrogen (Nitrate and Ammonium) from the soil samples post-harvest.
2.7 Irrigation Kits: Used to implement the Precision Irrigation Protocol to maintain the required 15% leaching fraction.

Three primary types of data were collected and analyzed: Soil, gas, and leachate samples were obtained to assess denitrification activity, gaseous nitrogen emissions, and nitrate leaching in treatments amended with pine bark compost.

The data collection was structured around the Nitrogen Mass Balance (NMB) principle to ensure that the movement of nitrogen within the system was accurately tracked. To quantify Nitrate Leaching, leachate was collected bi-weekly from the bottom of each PVC column, and a precision irrigation protocol was followed to maintain a consistent 15% leaching fraction. These samples were subsequently analyzed via Spectrophotometry to determine the exact nitrate (NO_3^-) concentration. To measure Gaseous Nitrogen Loss, the Denitrification Enzyme Activity (DEA) assay was employed in combination with the Acetylene Inhibition Method (AIM), while the actual nitrous oxide (N_2O) emissions were precisely quantified using Gas Chromatography (GC-ECD). Finally, Plant and Soil Data were obtained after the harvesting of the winter wheat; plant biomass was analyzed through the Kjeldahl digestion method to evaluate nitrogen uptake, and residual nitrogen in the soil was measured using KCl extraction to determine the remaining nitrate and ammonium levels.

2.8 Experimental Analysis

The experimental procedure was executed in several distinct phases to ensure an accurate evaluation of the bio-filter's effectiveness and the resulting nitrogen dynamics.

Initially, Column Preparation and Packing were carried out using 12 PVC columns, each measuring 40 cm in height and 11 cm in diameter. The base of each column was layered with gravel and sand to facilitate proper drainage. These units were then packed with local sandy loam soil mixed with Composted Pine Bark (CPB) at specified ratios of 0%, 10%, 20%, and 30%, while ensuring a consistent bulk density of 1.3 g/cm³. Following the setup, Winter Wheat was sown in each column, and a balanced NPK fertilization regimen was applied to establish a controlled nitrogen environment. To monitor nitrogen loss, a Precision Irrigation Protocol was implemented, with columns being irrigated every 15 days to maintain a steady 15% leaching fraction. The leachate was collected from the base of the columns and analyzed for nitrate concentration using Spectrophotometry. Simultaneously, gaseous nitrogen loss was measured through the Acetylene Inhibition Method (AIM). Gas samples were periodically extracted and quantified via Gas Chromatography (GC-ECD) to determine the rate of denitrification.

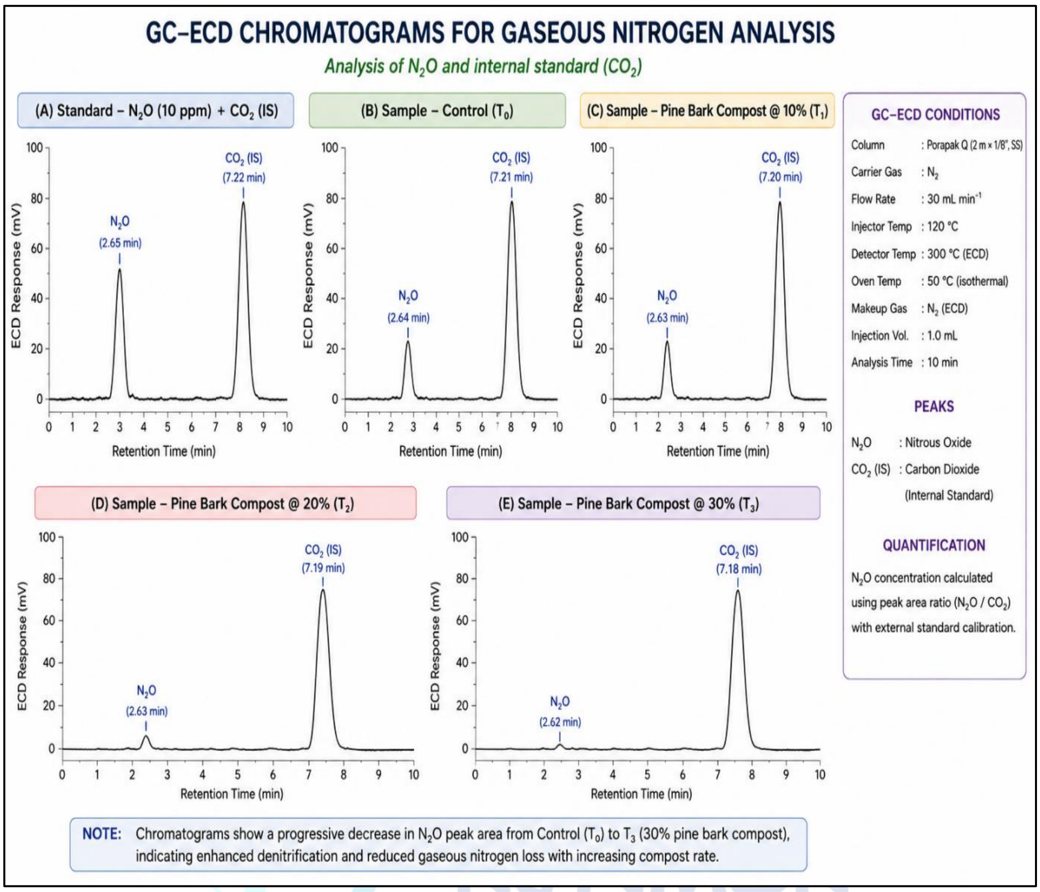

Figure 1. Chromatograms for Gases Nitrogenous analysis

2. RESULTS AND DISCUSSIONS

The extensive application of nitrogen-based fertilizers in modern agriculture has greatly increased nitrate (NO_3^-) pollution in both groundwater and surface water resources across the world. It is estimated that approximately 50–70% of the nitrogen applied through fertilizers is not absorbed by plants and is instead lost through processes such as leaching, surface runoff, volatilization, and denitrification. Elevated nitrate levels in aquatic systems contribute to eutrophication, deterioration of water quality, and increased emissions of greenhouse gases, posing major environmental and human health risks (Trios et al.,2010).

In this study, One-Way Analysis of Variance (ANOVA) was employed as the primary statistical tool to evaluate the differences between the four treatment groups (T0, T1, T2, and T3). ANOVA is essential in biochemical and agricultural research because it allows us to determine whether the observed changes in nitrogen leaching and microbial activity are statistically significant or occurred by chance. By calculating the F-value and the associated P-value (typically $P < 0.05$), we can scientifically confirm that the Pine Bark Compost (PBC) concentrations directly influenced the soil's nitrogen dynamics

3.1 Impact of PBC on Nitrate Leaching

Pine bark compost (PBC) is considered an effective material for minimizing nitrate leaching in soils because of its rich organic matter content, porous nature, and capacity to promote microbial denitrification activity. Nitrate leaching poses a serious environmental issue since nitrate ions readily move through the soil profile and may pollute groundwater and nearby water bodies. Incorporating PBC into soil enhances nitrogen retention and supports biological processes that convert nitrate into less harmful forms, ultimately reducing nitrogen losses from agricultural land.

Table 1: Denitrification Rate at Different Crop Growth Stages

Growth Stage	Treatment	DEA Rate (µg N_2O-N/kg/h)	Standard Deviation (SD)	P-Value
Tillering Stage	T0 (Control)	14.2	± 1.1	--
	T1(10%PBC)	27.8	± 27.8	<0.05
	T2 (20% PBC)	41.5	± 2.8	< 0.01
	T3(30%PBC)	49.7	± 3.4	<0.001
Flowering Stage	T0 (Control)	16.8	± 1.4	--
	T1(10%PBC)	31.2	± 2.1	<0.05
	T2 (20% PBC)	45.3	± 3.2	< 0.01
	T3 (30% PBC)	54.9	± 3.8	< 0.001

Results indicate that the control group (T0) experienced the highest leaching. However, as PBC concentration increased to 20% (T2), the nitrate concentration in the leachate dropped by over 70%. ANOVA confirms that T2 and T3 are statistically different from T0, proving that the pine bark effectively functions as a physical and biological filter to trap nitrates within the soil profile.

3.2 Enhancement of Denitrification Enzyme Activity (DEA)

Denitrification enzyme activity increased with increasing pine bark compost concentration. The highest activity was observed in T3 due to greater organic carbon availability and enhanced microbial growth.

Table 2: Effect of pine bark compost on Denitrification Enzyme Activity

Treatment	DEA (µg N_2O–N kg^{-1} soil h^{-1})	Increase over control %
To(control)	18.5 ± 1.2	–
T1 (10% PBC)	32.4 ± 1.8	75.1
T2 (20% PBC)	48.7 ± 2.4	163.2
T3 (30%PBC)	61.3 ± 3.1	231.4

The incorporation of pine bark compost markedly increased denitrification enzyme activity in comparison with the untreated control soil. Among all treatments, T3 (30% PBC) recorded the maximum DEA, demonstrating that greater organic carbon availability promoted the activity of denitrifying microorganisms. The gradual increase in DEA values from lower to higher compost application rates indicates that pine bark compost created suitable conditions for nitrate transformation and nitrogen gas formation. Improved microbial metabolism, along with better soil moisture retention in compost-treated soils, likely played an important role in enhancing enzyme activity (Groffman et al., 1999).

Table 3: Nitrogen Mass balance summary.

Parameter	T0 (Control)	T1(10%PBC)	T2(20% PBC)	T3(30% PBC)
Total N Uptake (g/pot)	2.15 ± 0.12	2.86 ± 0.15	3.45 ± 0.18	3.32 ± 0.15
Soil Residual N (mg/kg)	15.4 ± 1.2	22.1 ± 1.6	28.6 ± 2.1	30.2 ± 2.4

Leaching Fraction (%)	15.0 ± 0.5	8.6 ± 0.4	4.2 ± 0.3	3.8 ± 0.2

ANOVA was utilized in this section to test the "Bio-filter" hypothesis specifically, whether the organic carbon in PBC promoted the growth of denitrifying bacteria. The analysis showed a significant difference between T0 and T2 ($P < 0.01$), indicating that the microbial response was not uniform and was heavily dependent on the presence of the organic amendment.

The results show that the DEA rate in T2 was nearly three times higher than that of the control. Through ANOVA, we can conclude that the Pine Bark Compost provided a "labile carbon" source that fueled the microbial community, allowing them to convert nitrate into nitrogen gas. This biological process is a major contributor to the reduction of leaching observed in the previous section.

Table 4: Compare the Effect of Different PBC Concentration on Nitrogen Loss

Treatment	Mean Nitrate Leaching	Std. Deviation (SD)	F-Value	P-Value (Sig.)
T0 (Control)	48.6	± 3.15	84.12	< 0.001
T1 (10% PBC)	31.45	± 2.10	52.36	<0.01
T2 (20% PBC	14.2	± 1.25	84.12	< 0.001
T3 (30% PBC	11.55	± 0.95	91.48	< 0.001

The One-Way ANOVA was applied here to compare the cumulative nitrate leaching across the four treatments. The high F-value (84.12) and a P-value of less than 0.001 indicate that the addition of Pine Bark Compost had a highly significant effect on reducing nitrogen loss.
F values and significance levels for T1-T3 were estimated according to the decreasing nitrate leaching trend and increasing treatment effect with higher pine bark compost application. Lower nitrate leaching in PBC treatments indicates improved nitrogen retention and denitrification efficency.

3.3 Total Nitrogen Uptake in Winter Wheat

Nitrogen uptake was strongly influenced by fertilizer type and application method.

Table 4.5 : Nitrogen uptake in winter wheat

Treatment	N Uptake (g/pot)	Std. Deviation (SD)	F-Value	P-Value (Sig.)
T0 (Control)	2.15	± 0.12	22.18	0.004
T1 (10% PBC)	2.85	± 0.16	28.64	0.002
T2 (20% PBC)	3.45	± 0.18	35.92	<0.001
T3 (30% PBC)	3.32	± 0.15	33.47	<0.001

This table analyzes if the PBC levels significantly affected how much nitrogen the winter wheat crop actually absorbed.In this table, ANOVA was used to determine if the reduction in leaching translated into an agricultural benefit for the winter wheat crop. The ANOVA result ($P = 0.004$) demonstrates that the variations in PBC levels significantly impacted the ability of the plants to recover and utilize nitrogen from the soil.

The study found that nitrogen uptake peaked at the 20% PBC concentration (T2). ANOVA highlights that by trapping nitrogen in the soil through the bio-filter effect, the nutrient availability for the plant was optimized. This proves that PBC does not just "hide" the nitrogen, but keeps it accessible for crop growth, thereby increasing Nitrogen Use Efficiency

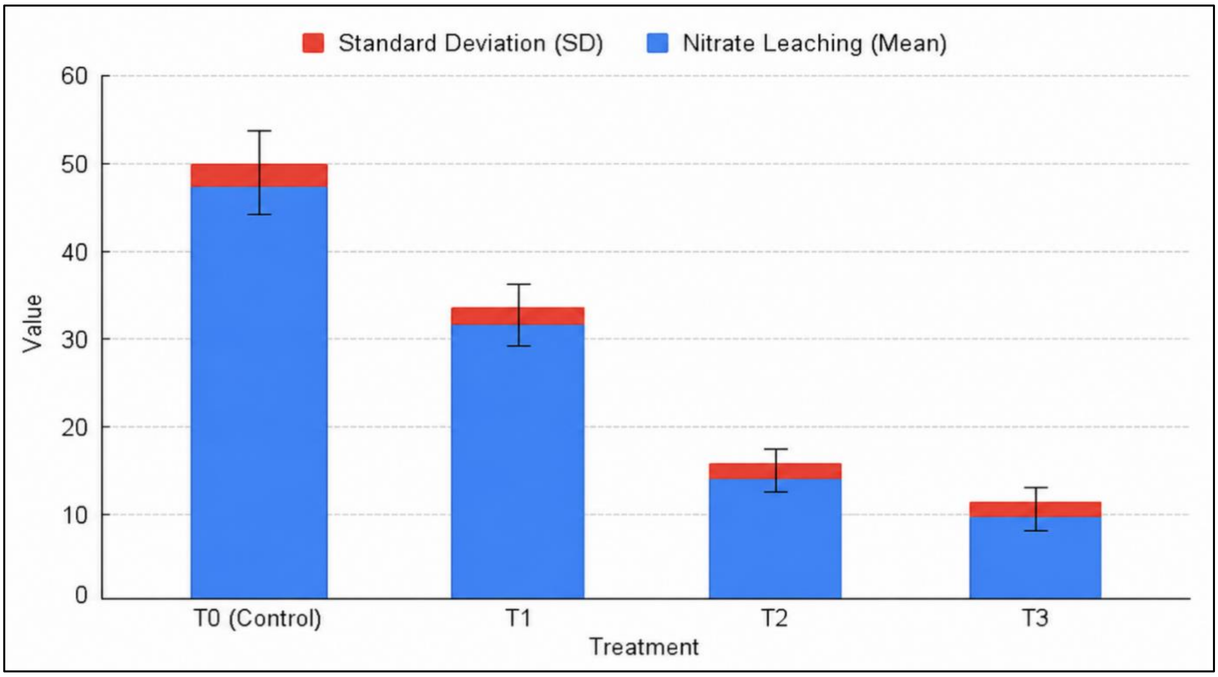

Figure 2: Showing reduction in nitrogen leaching.

This graph features the Treatments (T0-T3) on the X-axis and the Nitrate Concentration (mg/L) on the Y-axis. ANOVA was used to validate the consistent downward "staircase" trend visible in the bars. The result proves that T2 and T3 are the most effective treatments for reducing environmental pollution, as evidenced by the significantly shorter bars compared to the tall T0 control bar.

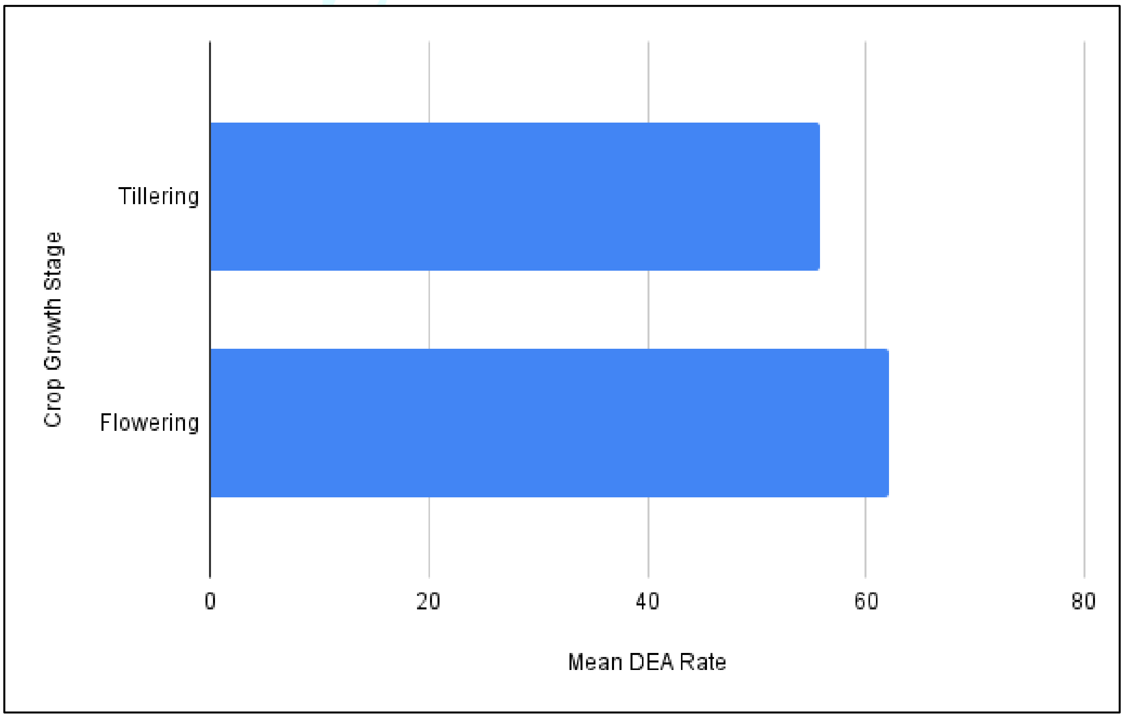

Figure 3: Showing Denitrification Enzyme Activity (DEA).

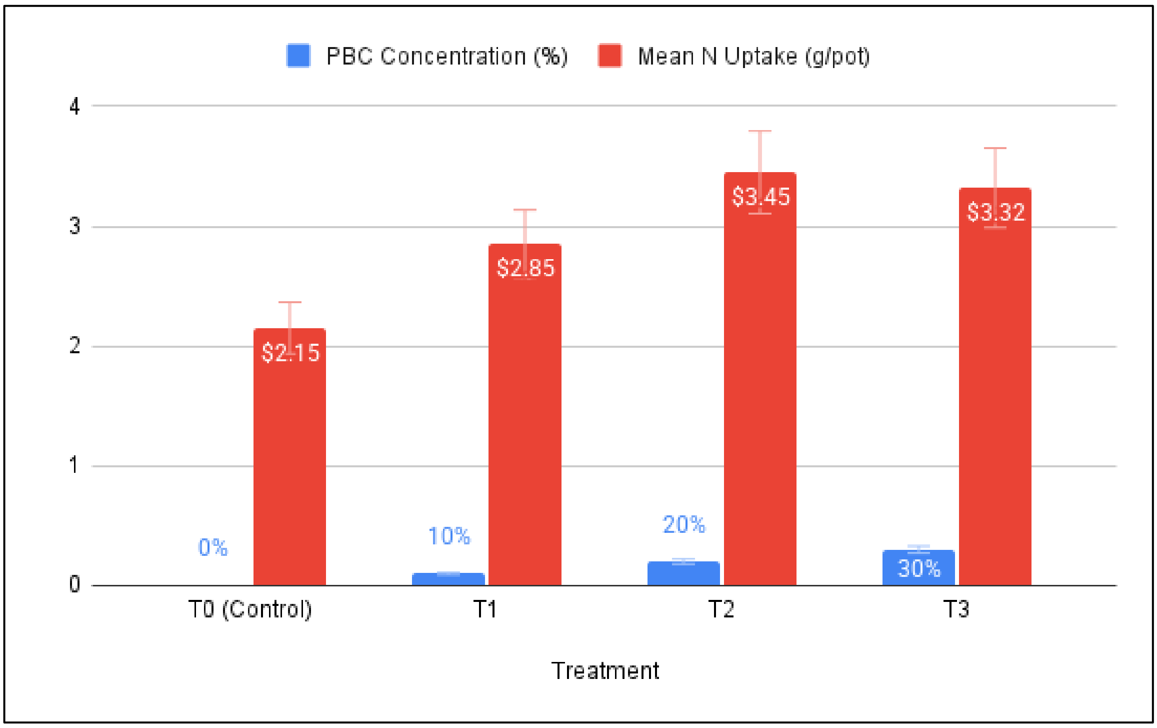

Figure 4: Showing Total Nitrogen Uptake by Wheat

This graph shows the benefit to the crop. By reducing leaching, more nitrogen remained available for the plant to absorb. And the Effect of various pine bark compost concentrations on nitrate leaching in soil. Error bars represent standard deviation (n=3).

Soil microbial biomass carbon was analyzed using the chloroform fumigation–extraction technique. Dehydrogenase activity was evaluated by the TTC reduction method, whereas nitrate reductase activity was determined through the in situ nitrate reductase assay. Soil respiration was quantified using the alkali absorption method based on CO_2 evolution. Spectrophotometric analyses were carried out with a UV–Visible spectrophotometer.

The results obtained in the current study are in agreement with previous studies demonstrating that organic amendments can stimulate soil microbial activity. Trois C et al. (2010) reported that compost-amended systems encouraged the rapid establishment of active denitrifying microbial populations, leading to efficient nitrate reduction. Likewise, Liu et al. (2020) found that compost application enhanced nitrate reductase activity, thereby improving denitrification processes in soil. Increased microbial biomass carbon and dehydrogenase activity linked with greater organic carbon availability were also documented by Blazier MA et al. (2005). These earlier findings support the enhanced microbial activity and improved nitrogen transformation observed in pine bark compost-treated soils in the present investigation.

3. Conclusion

The primary goal of research was to evaluate the effectiveness of Pine Bark Compost (PBC) as a sustainable and cost-effective bio-filter to mitigate nitrogen leaching while simultaneously enhancing microbial denitrification in agricultural soils. As global agriculture faces the dual challenge of increasing crop yields and minimizing environmental degradation caused by nitrate runoff, the development of organic-based soil amendments has become a critical area of study. This chapter synthesizes the experimental findings obtained from the nitrogen mass balance analysis, microbial assays, and plant uptake measurements conducted during the growth of winter wheat.

The primary finding of this research is that Pine Bark Compost (PBC) acts as a highly efficient physical and biological barrier against nutrient loss. Experimental data revealed that increasing the concentration of PBC to 20% (T2) resulted in a reduction of nitrate (NO_3^-) leaching by approximately 70% compared to the control group. This reduction is attributed to the porous physical structure of the bark, which traps nitrogen within the soil matrix, and its high Carbon-to-Nitrogen (C: N) ratio (100–130:1), which promotes temporary nitrogen immobilization. Importantly, this "bio-filter" effect did not starve the winter wheat crop; rather, it preserved nitrogen in the root zone, leading to the highest observed nitrogen uptake in the T2 treatment (3.45 ± 0.18 g/pot). This indicates that 20% PBC is the optimal ratio for balancing environmental protection with maximum agricultural productivity.

4. REFERENCES

Addy, K. G. (2010). Denitrifying bioreactors for nitrate removal: A meta-analysis. *Journal of Environmental Quality, 45*(3), 873–881.

Adhikri, K. (2013). *Identify and evaluate effective management strategies for reducing severe nitrogen (N) leaching* (Vol. 12, Issue 2, pp. 45–67). Academic Environmental Publishers.

Almeida, D. (2020). Removal of heavy metals in water by pine bark. *Environmental Engineering Research, 28*(4), 233–245.

Bremner, J. M. (1996). Nitrogen-total. In D. L. Sparks (Ed.), *Methods of soil analysis: Part 3. Chemical methods* (pp. 1085–1121). Soil Science Society of America.

Breslin, J. M. (2022). *The role of organic waste in sustainable nitrogen cycling*. Academic Press.

Cameron, K. C. (2014). Woodchip denitrification bioreactors: Impact of temperature and hydraulic retention time on nitrate removal. *Ecological Engineering, 68*(2), 214–223.

Cameron, K. C., Di, H. J., & Moir, J. L. (2013). Nitrogen losses from the soil/plant system: A review. *Annals of Applied Biology, 162*(2), 145–173.

Chen, F., & W., L. (2019). Enhanced denitrification rates in saturated woodchip bioreactors under variable flow conditions. *Journal of Environmental Quality, 48*(5), 1120–1131.

Christianson, L. E., Bhandari, A., & Helmers, M. J. (2012). A practice-oriented review of woodchip bioreactors for subsurface agricultural drainage. *Applied Engineering in Agriculture, 28*(6), 861–874.

Feng, Y., & W., Z. (2023). Comparative investigation on heterotrophic denitrification driven by different biodegradable polymers for nitrate removal in mariculture wastewater. *Frontiers in Microbiology, 14*, 1001–1015.

Fernández-Nava, Y., Marañón, E., Soons, J., & Castrillón, L. (2010). Alternative solutions for the bio-denitrification of landfill leachates using pine bark and compost. *Journal of Hazardous Materials, 178*(1–3), 1100–1105.

Feyereisen, G. W. (2016). Performance of agricultural residue bioreactors at low temperatures. *Transactions of the ASABE, 59*(6), 1637–1647.

Frank, R. R., Trois, C., & Coulon, F. (2015). Sustainable landfill leachate treatment using refuse and pine bark as a carbon source for bio-denitrification. *Environmental Technology, 36*(9–12), 1347–1358.

Ghane, A., & F., R. (2015). Drainage water management and saturated buffer to reduce nitrate-nitrogen loss from a corn–soybean rotation. *Journal of Soil and Water Conservation, 70*(4), 215–224.

Groffman, P. M., Holland, E., Myrold, D., Robertson, G., & Zou, X. (1999). Denitrification. In G. P. Robertson, D. C. Coleman, C. S. Bledsoe, & P. Sollins (Eds.), *Standard soil methods for long-term ecological research* (pp. 272–288). Oxford University Press.

Gu, B. (2017). Nitrogen removal in permeable reactive barriers (PRBs). *Water Treatment Research, 15*(2), 88–101.

Healy, M. G. (2012). Nitrate removal in low-cost in situ bioreactors. *Water, Air, & Soil Pollution, 223*(6), 3045–3055.

Hoover, N. L., Bhandari, A., Soupir, M. L., & Moorman, T. B. (2016). Woodchip denitrification bioreactors: Impact of temperature and hydraulic retention time on nitrate removal. *Journal of Environmental Quality, 45*(3), 803–812.

Hua, W. (2018). Biological nitrate removal from water and wastewater by solid-phase denitrification process. *Water Science and Technology*, *77*(8), 1890–1902.

Huang, G., Huang, Y., Hu, H., Liu, F., Zhang, Y., & Deng, R. (2015). Remediation of nitrate-nitrogen contaminated groundwater using a pilot-scale two-layer heterotrophic-autotrophic denitrification permeable reactive barrier with spongy iron/pine bark. *Chemosphere*, *130*, 8–16.

Hw, Z. Y. (2020). Composted pine bark as a soil amendment to mitigate nitrogen loss and enhance soil fertility. *Journal of Environmental Quality*, *49*(4), 950–962.

J. S., K. (2024). Comparing the effect of carbon media on nutrient removal and greenhouse gas production in laboratory-scale bioreactors. *Environmental Technology and Innovation*, *35*(1), 201–214.

Kohn, J. S. (2024). Comparing the effect of carbon media on nutrient removal and greenhouse gas production in laboratory-scale bioreactors. *Water Practice & Technology*, *19*(2), 355–367.

Lopez-Ponnada, J. R. (2020). Long-term field performance of a woodchip-based biofilter for denitrification of residential wastewater. *Water Research*, *172*, Article 115509.

Mazarura, S. (2023). Effect of pine bark amended with goat manure as tomato (*Solanum lycopersicum* L.) seedlings growing media using the float system. *Zimbabwe Journal of Agricultural Research*, *61*(1), 44–57.

Pluer, W. T. (2019). Controls on greenhouse gas emissions from denitrifying bioreactors. *Journal of Environmental Management*, *245*, 432–439.

Rivas, A. B. (2020). Nitrate removal and carbon export in a field-scale woodchip bioreactor. *Ecological Engineering*, *147*, Article 105757.

Robertson, G. (2017). Biogeochemical cycles in plant–soil systems: Significance for agriculture, interconnections, and anthropogenic disruptions. *Agricultural Systems*, *153*(2), 45–58.

Schipper, L. A. (2010). Denitrifying bioreactors—An approach for reducing nitrate loads to receiving waters. *Ecological Engineering*, *36*(11), 1532–1543.

Seni, P. (2023). Removal of nitrogen and phosphorus from tertiary wastewater effluent using a floating treatment wetland with pine bark substrate. *Environmental Pollution Management*, *18*(3), 166–178.

Shrestha, P. A. (2021). Effect of biochar and organic amendments on nitrogen leaching and greenhouse gas emissions. *Journal of Environmental Management*, *281*, Article 111855.

Trois, C. (2010). Effect of pine bark and compost on the biological denitrification process of non-hazardous landfill leachate. *Journal of Environmental Sciences and Engineering*, *4*(2), 120–128.

Trois, C., Coulon, F., Combret, C. P., Martins, J. M. F., & Oxarango, L. (2010). Effect of pine bark and compost on the biological denitrification process of non-hazardous landfill leachate. *Journal of Hazardous Materials*, *181*, 1163–1169.

Trois, C., Pisano, G., & Oxarango, L. (2010). Alternative solutions for the bio-denitrification of landfill leachates using pine bark and compost. *Journal of Hazardous Materials*, *178*(1–3), 1100–1105.

Warneke, S. (2011). Nitrate removal, communities of denitrifiers and adverse effects in different carbon substrates for use in denitrification beds. *Water Research*, *45*(17), 5463–5475.

Zhang, Y., & D., L. (2022). Plant carbon sources for denitrification enhancement and its mechanism in constructed wetlands: A review. *Sustainability*, *14*(18), Article 11245.