Fungal diversity and functional insights in fish waste-amended soils:

A Review on isolation and identification approaches

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Highlights

- **Pioneering Integration**: This review uniquely explores the intersection of fishery waste valorization and soil fungal ecology, presenting a sustainable pathway for nutrient cycling and soil rehabilitation.
- **Functional Fungal Profiling**: It compiles and analyzes the roles of key fungal genera (*Trichoderma*, *Aspergillus*, *Glomus*) in biodegradation, nutrient mobilization, plant growth promotion, and disease suppression within fish waste-amended soils.
- **Bioeconomy Alignment**: The study contextualizes fungal-mediated fish waste management within the circular bioeconomy framework, emphasizing waste-to-resource transformation in agriculture.
- **Research and Policy Roadmap**: It identifies critical research gaps (e.g., fungal diversity, multi-omics, field scalability) and advocates for policy interventions to promote adoption of microbial fish waste technologies.

Abstract

Background: The increasing application of fishery waste as an organic soil amendment has drawn attention to the role of soil fungi in its biodegradation and nutrient recycling. However, comprehensive insights into the fungal communities associated with fish waste-treated soils, their identification techniques, and their ecological functions remain fragmented.

Objectives: This review aims to consolidate current knowledge on (i) fungal species isolated from fish waste-amended soils, (ii) the methodologies used for their isolation and identification, and (iii) their contributions to soil health, nutrient mobilization, and potential biotechnological applications.

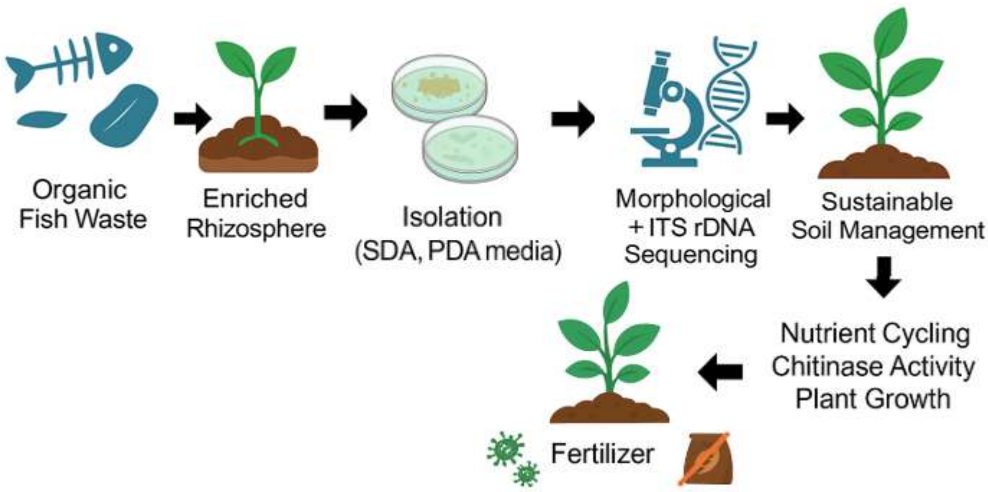
Methods: An extensive literature search was conducted using databases such as Scopus, Web of Science, and Google Scholar. A total of 90 peer-reviewed research articles were reviewed, focusing on both morphological and molecular (ITS-based) identification of fungi in fish waste-integrated soils. Particular attention was given to experimental protocols, diversity indices, and functional assays.

Results: A wide array of fungal taxa, notably *Trichoderma*, *Aspergillus*, *Geotrichum,* and arbuscular mycorrhizal fungi (AMF), have been identified in soils amended with fish waste. Morphological methods remain foundational, but DNA barcoding, especially via ITS region sequencing, is increasingly preferred for accurate taxonomic resolution. These fungi contribute significantly to organic matter decomposition, chitinase production, and enhanced plant growth through improved nutrient uptake.

Conclusion: The integration of fishery waste with beneficial soil fungi offers promising avenues for sustainable agriculture and bioremediation. Advancements in molecular diagnostics are reshaping fungal identification, but standardization of methods is still needed. Future research should emphasize high-throughput sequencing, metagenomics, and global data sharing to map fungal biodiversity more precisely in such environments.

Keywords: *Fish waste, Soil fungi, Isolation techniques, ITS sequencing, Trichoderma, Arbuscular mycorrhizal fungi, Organic amendments, Sustainable agriculture*

GRAPHICAL ABSTRACT



I. INTRODUCTION

Fisheries and aquaculture industries generate a significant amount of organic waste globally, including scales, viscera, bones, and processing residues [1]. Improper disposal of this fishery waste not only contributes to environmental pollution but also leads to the loss of valuable bio-resources [2]. In recent years, fish waste has been increasingly recognized as a potent organic amendment that can be repurposed to enrich agricultural soils due to its rich content of nitrogen, chitin, proteins, and micronutrients [3]. These organic components make fish waste particularly suitable for use as a biofertilizer, which supports sustainable farming practices and reduces dependency on chemical inputs [4].

One of the key biological agents involved in the transformation and mineralization of fish waste in soil is soil fungi [5]**.** These microbial decomposers, including *Trichoderma spp.*, *Aspergillus spp.*, and Arbuscular Mycorrhizal (AM) fungi, play crucial roles in breaking down complex organic matter such as chitin into plant-available nutrients [6] [7]. Besides contributing to nutrient cycling, these fungi enhance soil structure, promote plant root colonization, and offer biocontrol against pathogens [8] [9]. Understanding the diversity, ecological roles, and identification techniques of fungi in fish-waste amended soils is essential to optimize their application in agriculture and environmental remediation [10] [11].

The sequential process involved in utilizing fish waste is to enrich soil and enhance fungal activity [12]. Beginning with organic fish waste, the waste is incorporated into the soil, enriching the rhizosphere—the biologically active zone surrounding plant roots. Fungi are then isolated using culture-based methods such as Sabouraud Dextrose Agar (SDA) and Potato Dextrose Agar (PDA) [13]. These isolates are further characterized through morphological assessments and Internal Transcribed Spacer (ITS) rDNA sequencing, enabling precise identification of fungal taxa [14]. Ultimately, these fungi contribute to sustainable soil management by enhancing nutrient cycling, promoting plant growth, and reducing the need for synthetic fertilizers [15]. Traditional identification of soil fungi relied on macroscopic and microscopic characteristics of colonies and spores [16]. However, this approach has limitations in accuracy and species resolution. The integration of molecular tools—especially ITS region sequencing—has become the gold standard for fungal taxonomy [17]. ITS sequencing offers a high-resolution method for distinguishing closely related fungal species and is now widely used in microbial ecology studies [18]. Moreover, advancements in metagenomics and next-generation sequencing (NGS) technologies promise deeper insights into unculturable and rare fungal communities in fish waste-amended soils [19].

Despite increasing research in this area, standardized protocols for fungal isolation, identification, and functional evaluation in fish waste-treated soils are still lacking [20]. There is also a need to consolidate scattered findings on the ecological benefits and risks of using fish waste as a soil amendment. This review aims to fill this gap by summarizing the current literature on fungal diversity in fish waste-amended soils, evaluating isolation and identification methodologies, and highlighting the functional potential of these fungi in sustainable agriculture and biowaste management.

1.1 Contributions

The novel contributions of this study are:

1 Provides the first integrated synthesis of fungal diversity specifically in fish waste-amended soils, linking taxonomy with ecological function.

2 Introduces a graphical workflow bridging organic fish waste conversion, fungal isolation, and ITS-based identification for sustainable soil management.

3 Highlights underexplored roles of chitinolytic fungi like *Trichoderma* and AMF in nutrient cycling and organic matter mineralization from fishery residues.

4 Identifies critical methodological gaps and proposes a roadmap for standardizing fungal isolation and molecular identification in marine-waste treated soils.

1.2 Graphical Abstract Integration

- To enhance visual communication, a graphical abstract was developed summarizing the methodological and ecological relationships between fishery waste and fungal responses. It illustrates the key stages: fish waste generation → soil amendment → fungal colonization and transformation → agronomic benefits. Acronyms like PDA (Potato Dextrose Agar), ITS (Internal Transcribed Spacer), and AMF (Arbuscular Mycorrhizal Fungi) are fully expanded within the review for clarity.
- Fig 1 is the word cloud for Section 2, visually representing key terms and thematic focus areas such as "fishery waste," "soil fungi," "ITS sequencing," and "circular bioeconomy.

Fig 1: Keyword Cloud: Fungal Ecology in Fishery Waste-Amended Soils

2. Fisheries Waste as a Soil Amendment

2.1 Composition and Characteristics of Fishery Waste

Fisheries and aquaculture industries generate vast quantities of organic waste, encompassing fish viscera, skin, bones, scales, heads, and processing residues. This waste stream, often considered environmentally burdensome, is now recognized as a valuable bioresource for soil fertility enhancement due to its complex biochemical composition. Fishery waste is notably rich in macronutrients such as nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg), alongside organic compounds including amino acids, fatty acids, proteins, and **chitin**—a nitrogenous polysaccharide abundantly found in fish scales and crustacean exoskeletons [22].

The nitrogen content in fish waste ranges between 6–8% (dry weight basis), making it a potent organic nitrogen source for soil microbial communities. Moreover, its high lipid and protein content serves as an energy-rich substrate for heterotrophic organisms, particularly fungi [23]. The C/N ratio of fish waste, though variable depending on processing and species, often falls within an optimal range for microbial decomposition and mineralization. Importantly, the presence of chitin renders fish waste functionally distinct from other organic amendments like cow manure or compost, stimulating chitinolytic microbial activity and triggering shifts in soil microbial ecology [24].

2.2 Agronomic Benefits and Soil Health Enhancement

The application of fish waste to agricultural soils has been associated with a range of agronomic and ecological benefits. Upon incorporation, fish waste undergoes microbial degradation, releasing nutrients gradually and improving soil fertility without the leaching risks associated with synthetic fertilizers [25]. Empirical studies have demonstrated increased cation exchange capacity (CEC), improved soil aggregation, enhanced water holding capacity, and elevated microbial biomass carbon in fish-waste amended soils [26].

In nutrient-deficient or degraded soils, fish waste serves as a biological primer, reactivating dormant microbial communities and supporting enzymatic processes critical for nutrient cycling. Crops such as *Allium cepa* (onion), *Solanum lycopersicum* (tomato), and *Abelmoschus esculentus* (okra) have exhibited enhanced vegetative growth, improved chlorophyll content, and higher marketable yield when cultivated on fish-waste-treated plots [27]. These improvements are attributed to the sustained release of nutrients and the stimulation of beneficial soil microbiota.

Moreover, fish waste acts as a substrate for microbial secondary metabolites and exoenzymes that promote plant growth indirectly. For instance, increased activity of phosphatases and dehydrogenases has been recorded in fish waste-amended soils, indicating elevated metabolic functioning of soil biota [28]. This enzymatic stimulation translates into better root proliferation and nutrient assimilation in host plants.

2.3 Role of Chitin as a Functional Input

A distinguishing feature of fishery waste is its high chitin content, particularly from fish scales, exoskeletal fragments, and shrimp processing residues. Chitin, a β-1,4-linked polymer of N-acetylglucosamine, is the second most abundant natural polysaccharide after cellulose. Its degradation in soil is primarily mediated by fungal taxa capable of producing chitinolytic enzymes, including exochitinases, endochitinases, and chitobiosidases [29].

The incorporation of chitin-rich fish waste into soils selectively enhances the proliferation of chitin-degrading fungi, such as *Trichoderma spp.*, *Aspergillus spp.*, and Arbuscular Mycorrhizal Fungi (AMF) [30]. These fungi not only accelerate organic matter decomposition but also release bioavailable nitrogen, amino sugars, and micronutrients, contributing to soil nutrient pools and enhancing microbial interactions in the rhizosphere.

In addition, the hydrolysis of chitin serves as a biocontrol mechanism, suppressing pathogenic fungi through competitive exclusion or antagonistic enzyme production. This dual role of chitin—as a nutrient source and a plant protection agent—positions fish waste as a multifunctional soil amendment with both nutritional and protective value [31].

2.4 Influence on Soil Microbial and Fungal Ecology

The introduction of fish waste significantly alters the soil's microbial and fungal community structure. Studies employing next-generation sequencing have revealed increased fungal richness and functional diversity, with marked shifts toward saprotrophic and symbiotic guilds [32]. The abundance of organic carbon, amino acids, and chitin provides an energy-dense niche, favoring copiotrophic fungi over oligotrophic taxa. Particularly, the stimulation of Arbuscular Mycorrhizal (AM) fungi and *Trichoderma* species has been well-documented in fish-waste treated soils [33]. These fungi form beneficial associations with plant roots, enhancing phosphorus uptake, improving drought tolerance, and activating plant defense pathways. Moreover, AM fungi contribute to soil aggregation through the production of glomalin-related soil proteins (GRSPs), which bind soil particles and protect hyphal networks. The increased microbial turnover in fish waste-amended environments also leads to elevated soil respiration rates, indicative of heightened metabolic activity. While this may raise concerns regarding CO_2 emissions, it simultaneously reflects accelerated nutrient cycling.

2.5 Challenges and Considerations in Field Application

While the utilization of fishery waste as a soil amendment presents numerous agronomic and environmental benefits, its practical application in open-field conditions is associated with several challenges that require strategic management and scientific scrutiny. A major issue is the heterogeneity in fish waste composition, which is influenced by fish species, processing methods, seasonal variations, and geographical origin. For instance, waste from marine fish typically contains higher levels of salts and lipids compared to freshwater species, potentially impacting soil salinity and microbial balance if applied excessively or without treatment [34]. High fat content may also delay decomposition and promote anaerobic conditions in poorly aerated soils, leading to phytotoxicity or microbial imbalance. Odor emission is another significant constraint, particularly when using raw or unprocessed fish waste. The decomposition of proteins and lipids under ambient conditions can release volatile amines, sulfides, and fatty acids, which may attract scavengers and create a public nuisance. Such effects limit its applicability in peri-urban and horticultural systems unless pre-treatment methods—such as anaerobic fermentation, composting, or enzymatic hydrolysis—are employed to stabilize the material before land application [35]. A more critical concern lies in the potential accumulation of toxic heavy metals and organic pollutants, especially in fish waste derived from polluted marine ecosystems. Elements such as mercury (Hg), cadmium (Cd), arsenic (As), and lead (Pb) can bioaccumulate in fish tissues and subsequently enter agricultural soils upon amendment [36]. Over time, these contaminants can be taken up by crops and infiltrate food chains, posing long-term risks to human and animal health. This necessitates routine quality assessment and adherence to environmental safety standards before fish waste is incorporated into the soil. Additionally, logistical, and operational constraints exist. Fish waste is highly perishable and requires proper storage, handling, and timely transportation to avoid microbial spoilage and nutrient loss. The absence of structured supply chains and infrastructure in many regions hampers its efficient collection and conversion into value-added soil inputs [37]. Moreover, the lack of standardized field application protocols, including dosage guidelines, amendment ratios, and frequency of application, creates uncertainty for farmers and may lead to inconsistent field performance. Addressing these challenges requires a multidisciplinary approach involving microbiologists, soil scientists, waste management experts, and policy-makers. Establishing pilot-scale demonstration sites, advancing safe pre-treatment technologies, and promoting local processing units can help scale the sustainable use of fishery waste in agriculture while mitigating its associated risks.

2.6 Relevance to Circular Bioeconomy and Sustainable Agriculture

The application of fishery waste as a soil amendment aligns strongly with the core principles of the circular bioeconomy, a regenerative economic model that emphasizes resource efficiency, waste valorization, and the sustainable production of food, materials, and energy [38]. Rather than discarding fish waste into landfills, incineration facilities, or water bodies—where it may contribute to eutrophication or greenhouse gas emissions—its reuse in agricultural systems represents a closed-loop solution that recovers nutrients and organic matter for productive use.

From an environmental perspective, converting fish waste into biofertilizers contributes to reducing synthetic fertilizer dependence, thereby minimizing the ecological footprint of farming. Chemical fertilizers, particularly nitrogen-based variants, are associated with high energy input, soil acidification, and nitrous oxide emissions. By contrast, fish waste provides a slow-release, organic nutrient source that fosters microbial biodiversity and enhances natural soil fertility [39].

The chitinous and protein-rich nature of fish waste further supports the development of biostimulants, which improve plant vigor, stress tolerance, and disease resistance. Moreover, its application complements integrated nutrient management (INM) strategies and can be combined with other renewable soil inputs such as green manure, crop residues, and microbial inoculants to optimize resource use efficiency [40].

Economically, valorizing fish waste into value-added soil amendments opens new income streams for coastal communities, fish processors, and entrepreneurs engaged in waste-to-value enterprises. This reduces disposal costs, improves livelihood opportunities, and strengthens rural bio-based economies. Moreover, its integration into sustainable agriculture supports multiple Sustainable Development Goals (SDGs), including SDG 2 (Zero Hunger), SDG 12 (Responsible Consumption and Production), and SDG 13 (Climate Action) [41].

Scientifically, the study of fish waste transformation in soil presents rich opportunities for innovation in areas such as microbial ecology, enzymology, organic chemistry, and agricultural engineering. Continued research into its interaction with soil microbial communities, carbon sequestration potential, and long-term effects on soil quality can significantly advance the science of organic waste management.

In conclusion, the strategic reuse of fishery waste in soil systems exemplifies the transition from a linear "take-make-dispose" model to a circular, regenerative framework. When governed by science-based practices and regulatory oversight, fish waste offers a powerful tool for enhancing soil health, reducing environmental burdens, and moving agriculture toward a more resilient and sustainable future.

3. Soil Fungi in Fish Waste-Amended Environments

3.1 Introduction to Soil Fungal Communities

Soil fungi represent one of the most functionally diverse microbial groups in terrestrial ecosystems, contributing significantly to organic matter decomposition, nutrient mineralization, plant health, and soil structure stabilization. In fish waste-amended soils, the presence of nutrient-rich, chitinous substrates creates an ideal ecological niche for a variety of fungal taxa to proliferate [42]. These fungi not only aid in the biotransformation of complex organic residues but also influence rhizospheric dynamics and nutrient uptake efficiency in plants.

3.2 Common Fungal Genera Associated with Fish Waste

Numerous studies have documented the presence of specific fungal genera in fish waste-enriched environments. Among the most frequently reported are Trichoderma spp., known for their cellulolytic and chitinolytic capabilities and strong antagonistic properties against soil-borne pathogens [43]. *Aspergillus spp.* and *Penicillium spp.* also commonly colonize fish waste residues due to their robust enzymatic systems capable of degrading proteins, lipids, and polysaccharides [44]. Moreover, *Geotrichum*, *Pestalotiopsis*, and *Cladosporium* have been isolated in marine organic matter degradation contexts, suggesting their adaptability to nutrient-dense, chitin-rich substrates [45].

Arbuscular Mycorrhizal Fungi (AMF), belonging to the phylum *Glomeromycota*, have also shown symbiotic proliferation in fish-waste-treated soils. These fungi colonize plant roots and significantly enhance phosphorus and micronutrient acquisition [46]. The synergistic effect of AMF with fish waste is particularly evident in improving the photosynthetic rate, root development, and tolerance of plants to abiotic stress.

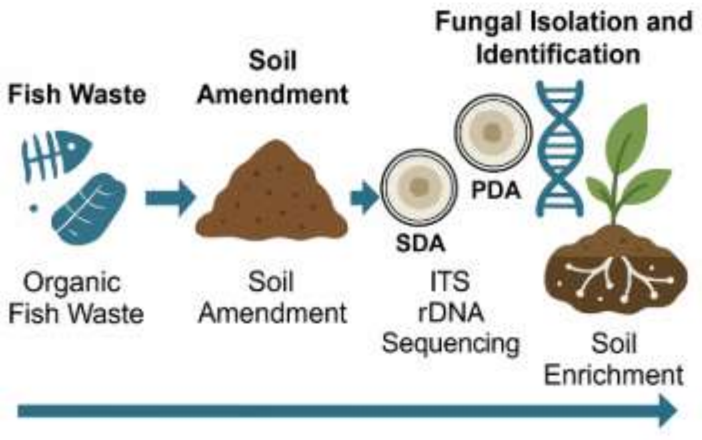


Fig 2: Workflow for fungal isolation and identification from fish waste-amended soils

In Fig 2, fish waste is applied as a soil amendment, promoting microbial activity. Fungi are isolated using SDA and PDA culture media and identified using ITS rDNA sequencing. Resulting fungal populations contribute to soil enrichment through nutrient mobilization and organic matter transformation.

3.3 Functional Roles of Fungi in Organic Waste Biodegradation

Fungi isolated from fish waste-amended soils serve as primary agents of chitin degradation, a key biochemical pathway in converting fish waste into plant-available nutrients [47]. Chitin, a polymer of N-acetylglucosamine, is enzymatically hydrolyzed by fungal chitinases, including exochitinases, endochitinases, and chitobiosidases. Fungi such as *Trichoderma harzianum*, *T. virens*, and *T. citrinoviride* are notable for their production of these enzymes, which are also employed in biotechnological applications [48].

Apart from nutrient release, these fungi contribute to soil aggregation through secretion of glomalin-related soil proteins (GRSP) by AMF, which bind soil particles and enhance aggregate stability [49]. This improves water retention and reduces erosion in sandy or degraded soils. Furthermore, fungal colonization enhances microbial biomass and enzymatic activity, leading to long-term improvements in soil health and fertility.

3.4 Fungal–Plant Symbiosis and Growth Promotion

The presence of beneficial fungi in fish waste-treated soils promotes a range of plant growth-promoting (PGP) traits. These include the synthesis of auxins, cytokinins, and gibberellins, which stimulate root elongation and lateral root formation [50]. Fungi such as *Trichoderma spp.* and AMF also facilitate the mobilization of otherwise inaccessible nutrients like Zn, Fe, and Mn, supporting enhanced chlorophyll synthesis and overall plant vigor [51]. The tripartite interaction between fish waste, fungi, and plant roots presents a model for sustainable, low-input agriculture.

In addition, the induced systemic resistance (ISR) effect has been reported in several crops following inoculation with *Trichoderma* and AMF in fish waste-amended conditions. This enhances the plant's defense mechanisms against pathogens, reducing the need for chemical fungicides [52]. The cumulative effect is improved crop resilience, nutrient use efficiency, and yield performance.

3.5 Ecological and Biotechnological Implications

The dynamic fungal communities in fish waste-amended soils not only benefit agriculture but also offer significant biotechnological value. The production of extracellular enzymes, such as proteases, lipases, and ligninases, has potential applications in bioremediation, enzyme industries, and biofertilizer formulation [53]. Additionally, exploring the secondary metabolites of these fungi could uncover new antimicrobial or antifungal compounds relevant to pharmaceuticals and biocontrol. From an ecological standpoint, these fungi contribute to carbon and nitrogen cycling, playing essential roles in greenhouse gas regulation and soil ecosystem services. Their resilience and adaptability to chitinous substrates also make them important models for understanding microbial succession and functional guild dynamics in amended soils.

4. Isolation and Characterization Techniques of Soil Fungi from Fish Waste-Amended Soils

4.1 Overview of Fungal Isolation in Amended Soils

Isolation of indigenous fungi from fish waste-treated soils is critical for understanding the ecological dynamics of microbial succession, evaluating beneficial strains, and exploring enzymatic or biotechnological potential. Owing to the nutrient richness and presence of chitin in fishery waste, such environments selectively promote the growth of chitinolytic, cellulolytic, and proteolytic fungi**,** offering a distinct mycobiome for exploration [54].

The choice of fungal isolation technique, including sampling strategy, culture media, and incubation conditions, significantly impacts the diversity and types of fungi recovered. Modern approaches increasingly combine traditional culturing methods with molecular tools to ensure accurate identification and functional characterization.

4.2 Soil Sampling Strategy and Pre-Treatment

Effective fungal isolation begins with a representative soil sampling protocol. Soil is typically collected from the rhizosphere and bulk zones at varying depths (0–20 cm), particularly from plots with repeated fish waste application [55]. Sampling during early decomposition stages or post-harvest periods enhances the probability of isolating active decomposer fungi. Samples are sieved (usually 2 mm mesh), stored at 4 °C, and processed within 24–48 hours to maintain microbial viability.

In some studies, enrichment pre-treatments such as baiting with chitin flakes or crustacean shells are used to selectively promote chitin-degraders prior to culturing, enhancing isolation efficiency.

4.3 Culture-Dependent Isolation Methods

Traditional serial dilution and plating techniques remain the cornerstone of fungal isolation. One gram of soil is typically suspended in sterile distilled water (1:10 dilution series) and spread onto selective and non-selective media. Commonly used media include:

- Potato Dextrose Agar (PDA) – general purpose medium supporting broad fungal growth [56]
- Sabouraud Dextrose Agar (SDA) – for yeasts and filamentous fungi [57]
- Chitin Agar / Colloidal Chitin Agar (CCA) – selective for chitinolytic fungi [58]
- Czapek Dox Agar (CDA) – favors Aspergillus and Penicillium species [59]

Antibiotics (e.g., chloramphenicol, streptomycin) are often added to suppress bacterial interference. Plates are incubated at 25–28°C for 5–7 days, after which colonies are sub-cultured to obtain pure isolates. Morphological features such as spore arrangement, pigmentation, and colony texture provide preliminary identification cues.

4.4 Molecular Characterization and Phylogenetic Analysis

Although culture-based methods are widely employed, they may fail to detect unculturable or slow-growing fungi, thus limiting ecological insight. Therefore, molecular techniques such as polymerase chain reaction (PCR) and internal transcribed spacer (ITS) sequencing have become the gold standard for accurate identification.

The ITS region of ribosomal DNA, particularly ITS1-5.8S-ITS2, is the most used barcode for fungal identification. DNA is extracted using commercial kits (e.g., CTAB-based extraction), amplified using universal primers (ITS1 and ITS4), and sequenced using Sanger or next-generation platforms [60]. The sequences are aligned against fungal databases such as NCBI GenBank or UNITE to determine taxonomic affiliation.

For community-level analysis, metagenomic approaches using high-throughput sequencing (e.g., Illumina MiSeq) allow for the profiling of total fungal diversity, including rare and non-culturable species. This facilitates the detection of functional guilds (e.g., saprotrophs, mycorrhizae, pathogens) and their response to fish waste amendments [61].

4.5 Enzymatic and Functional Assays

To evaluate the functional roles of isolated fungi, a series of enzyme activity assays are conducted:

- Chitinase activity: detected by clearing zones on colloidal chitin agar or quantified using p-nitrophenyl-N-acetylglucosaminide substrates [62]
- Cellulase and xylanase activity: measured using Congo red staining or DNS assay [63]
- Protease activity: evaluated on gelatin or casein agar plates [64]
- Phosphate solubilization: assayed on Pikovskaya's agar [65]

Additionally, fungal isolates are screened for plant growth-promoting traits (PGPTs), such as indole-3-acetic acid (IAA) production, siderophore secretion, ammonia production, and tolerance to abiotic stress (pH, salinity, temperature).

4.6 Microscopy and Imaging Techniques

Microscopic examination remains essential for confirming spore structures and identifying genera based on morphological criteria. Light microscopy with lactophenol cotton blue staining is used for observing hyphal and spore characteristics. For detailed ultrastructural studies, scanning electron microscopy (SEM) may be used to visualize hyphal morphology, spore ornamentation, and surface interactions with chitin or soil particles [66].

Fungal colonization of plant roots, especially by AMF, is evaluated using Trypan Blue staining and quantified through the gridline intersect method under a compound microscope.

4.7 Relevance

The combined use of culture-dependent and culture-independent techniques provides a holistic understanding of the fungal populations thriving in fish waste-amended soils. These techniques not only enable the identification of dominant fungal taxa but also provide insights into their ecological functions, enzymatic potential, and roles in sustainable soil management.

Standardizing isolation protocols and advancing molecular and functional characterization frameworks can significantly enhance our ability to harness soil fungi for waste bioconversion, biofertilizer development, and environmental resilience.

5. Functional Roles of Soil Fungi in Fish Waste-Amended Environments

Soil fungi play indispensable roles in the decomposition of organic residues, nutrient cycling, pathogen suppression, and overall enhancement of soil fertility. In the context of fish waste-amended soils, the nutrient-rich and chitin-laden organic inputs promote the proliferation of specific fungal taxa that are functionally adapted to degrade complex biomolecules and establish symbiotic relationships with plants. This section highlights the major ecological functions of fungi influenced by the application of fishery waste.

5.1 Decomposition and Organic Matter Mineralization

The primary role of fungi in fish waste-amended soils lies in the biodegradation of complex organic compounds, particularly proteins, lipids, and chitin. Saprophytic fungi such as *Aspergillus*, *Penicillium*, and *Fusarium* produce extracellular hydrolytic enzymes, including proteases, lipases, chitinases, and cellulases, which catalyze the breakdown of fish residues into assimilable forms [67].

Chitin, a key component of fish exoskeletons and scales, serves as a selective substrate for chitinolytic fungi like *Trichoderma harzianum*, *Myrothecium verrucaria*, and *Verticillium lecanii*, which produce chitinases that break β-1,4-linkages in N-acetylglucosamine polymers [68]. These fungi facilitate the release of nitrogen, carbon, and micronutrients into the rhizosphere, promoting microbial succession and soil nutrient enrichment.

5.2 Enhancement of Soil Nutrient Availability

Fungal metabolism of fish waste results in the mobilization of key nutrients, including nitrogen (N), phosphorus (P), potassium (K), and calcium (Ca). Phosphate-solubilizing fungi such as *Aspergillus niger* and *Penicillium citrinum* produce organic acids (e.g., citric, oxalic, and gluconic acids) that convert insoluble phosphates into plant-available forms [69]. Similarly, the mineralization of proteinaceous fish waste contributes to ammonification and nitrate formation via fungal pathways.

Furthermore, arbuscular mycorrhizal fungi (AMF), including *Glomus* and *Rhizophagus* species, colonize plant roots in amended soils and extend extraradical hyphae into the soil matrix. This enhances the acquisition of immobile nutrients (especially P and Zn) while improving root absorptive surface area and water uptake [70].

5.3 Disease Suppression and Biocontrol

Several fungi isolated from fish waste-treated soils exhibit antagonistic activity against soilborne pathogens, thus functioning as natural biocontrol agents. *Trichoderma spp.*, widely enriched in such soils, suppress phytopathogens like *Rhizoctonia solani*, *Pythium ultimum*, and *Sclerotium rolfsii* through mechanisms such as:

- Mycoparasitism [71]
- Competition for nutrients and space [72]
- Secretion of antifungal metabolites (e.g., gliotoxin, viridin) [73]
- Induction of systemic resistance in host plants [74]

These properties reduce reliance on chemical fungicides and promote biologically based integrated pest management (IPM) strategies.

5.4 Stimulation of Plant Growth and Yield

Fungal populations in fish waste-amended soils often include plant growth-promoting fungi (PGPFs), which enhance root development, seedling vigor, and overall biomass. These effects are mediated by:

- Production of phytohormones (e.g., IAA, gibberellins) [75]
- Improved nitrogen fixation (via associative diazotrophs) [76]
- Ammonia and siderophore production that boosts nutrient availability and iron uptake [77]

Experimental studies have shown that crops grown on fish waste-treated plots with fungal inoculation demonstrate enhanced yield performance, improved nutrient uptake, and greater tolerance to abiotic stressors such as drought or salinity.

5.5 Role in Soil Aggregation and Carbon Sequestration

Fungi contribute to soil structural stability by producing hyphal networks and secreting extracellular polysaccharides (EPS), which bind soil particles and enhance aggregate formation. Fungal hyphae also entrap organic residues from fish waste, promoting their incorporation into soil organic matter (SOM) pools [78].

Through their active role in carbon stabilization, fungi mediate the transformation of labile organic carbon into more recalcitrant forms, thus playing a pivotal role in long-term soil carbon sequestration and mitigating greenhouse gas emissions.

5.6 Interactions with Soil Microbiota

Fungi do not act in isolation; their presence in fish waste-amended soils influences the structure and function of broader soil microbial communities. They interact synergistically or competitively with bacteria, actinomycetes, and archaea in the rhizosphere, often leading to enhanced microbial diversity and activity [79].

Co-occurrence network analysis has revealed that fungal keystone taxa can shape microbial assembly through niche differentiation, metabolite exchange, and quorum sensing, creating a dynamic soil ecosystem responsive to organic inputs such as fish waste.

5.7 Future Potential

The multifunctional roles of fungi in fish waste-enriched soils extend beyond decomposition. From biocatalysis and nutrient mobilization to disease resistance and soil health restoration, these organisms are essential agents of biological transformation in agroecosystems. Unlocking their full potential requires integrative studies combining microbiomics, functional assays, and field validation trials to scale their application in climate-resilient agriculture.

6. Applications and Implications in Sustainable Agriculture

The utilization of fishery waste as a soil amendment not only valorizes an abundant organic byproduct but also enhances soil health and agricultural productivity through microbial mediation—particularly by fungi. The beneficial interactions of fungi with fish waste components unlock new frontiers in low-input, biologically integrated farming systems. This section highlights real-world applications, potential impacts, and considerations for deploying fish waste-fungi systems in sustainable agriculture.

6.1 Fish Waste as a Biofertilizer Source

When incorporated into soil, fish waste serves as a biofertilizer, providing essential macro- and micronutrients such as nitrogen (N), phosphorus (P), calcium (Ca), and trace elements. The synergistic action of indigenous soil fungi accelerates the mineralization of these nutrients, transforming them into plant-available forms. Fungi such as *Trichoderma* and *Penicillium* act as decomposers and nutrient solubilizers, enabling sustained nutrient release across the cropping cycle.
Numerous field trials have reported improvements in:

- Soil organic matter (SOM) content [80]
- Cation exchange capacity (CEC) [81]
- Root biomass and shoot vigor [82]
- Chlorophyll content and yield indices [80]

These outcomes reduce the dependency on synthetic fertilizers, thereby cutting costs and environmental burdens associated with agrochemical use.

6.2 Enhancing Soil Microbial Diversity and Resilience

The integration of fish waste into cropping systems fosters the development of a diverse and functionally active soil fungal community [83]. This biological enrichment improves soil structure, porosity, and water-holding capacity while enhancing the soil’s resistance to degradation and climate variability.
Long-term use contributes to:

- Enhanced fungal-to-bacterial ratios
- Recruitment of beneficial mycorrhizal and saprotrophic fungi
- Suppression of soilborne pathogens via microbial competition

Such systems promote ecological balance in the rhizosphere, ensuring sustainable crop production with minimal ecological disruption.

6.3 Integrated Pest and Disease Management (IPDM)

The enrichment of biocontrol fungi like *Trichoderma spp.* and *Verticillium spp.* through fish waste application contributes to natural disease suppression. These fungi outcompete pathogenic organisms for nutrients and space, produce antifungal metabolites, and trigger plant immune responses.
Incorporating fish waste in IPDM strategies allows farmers to:

- Lower their reliance on chemical fungicides
- Protect crops from root rot, damping-off, and wilt diseases
- Improve root zone health and reduce pathogen load in soil

This aligns with the principles of organic farming and residue-free agriculture, fulfilling consumer demand for safe food.

6.4 Waste Valorization and Circular Economy Integration

Fish waste is one of the most underutilized by-products in the seafood industry. Its use in agriculture transforms waste into value-added products, preventing environmental contamination from improper disposal practices such as dumping into water bodies or landfills.

Incorporating fungal biotransformation of fish waste supports:

- Circular bioeconomy models where nutrients are recycled within ecosystems [84]
- Integration of aquaculture and agriculture (aquaponics-inspired systems) [85]
- Zero-waste strategies that minimize ecological footprints [86]

Thus, this approach bridges marine and terrestrial sustainability efforts.

6.5 Field-Scale Applications and Case Studies

Several case studies across Asia and Europe have documented the successful field application of fish waste-fungal systems. For example:

- In Kerala, India, fish waste compost combined with *Trichoderma viride* increased rice yield by 22% over control [87].
- In Norway, integrated use of fish sludge and AMF enhanced barley phosphorus uptake and improved soil aggregation [88].
- In Ghana, fermented fish waste biofertilizer with *Aspergillus* species promoted maize growth and early flowering under rainfed conditions [89].

These studies underscore the replicability and scalability of fungal-mediated fish waste amendments in diverse agroecological zones.

6.6 Challenges and Practical Considerations

Despite the benefits, there are certain barriers to widespread adoption:

- Odor and handling issues with raw fish waste
- Variability in nutrient content due to species-specific composition
- Risk of pathogen transmission if not properly processed
- Need for standardized guidelines for dosage and application timing
- Limited awareness and technical knowledge among smallholder farmers

Addressing these issues through training programs, policy incentives, and demonstration trials is essential for mainstreaming this sustainable practice.

7. Research Gaps and Future Directions

While the integration of fishery waste and soil fungi presents an ecologically viable approach for enhancing soil health and productivity, there remain several unresolved questions and methodological limitations that require focused scientific attention. This section outlines the key research gaps identified from the current literature and provides future research directions to harness the full potential of fungal interactions in fish waste-amended soils.

7.1 Limited Taxonomic and Functional Profiling

Most studies have relied on culture-dependent techniques, which capture only a fraction of the total fungal diversity. High-throughput sequencing (HTS) approaches such as metabarcoding (ITS1/ITS2) and metagenomics are underutilized in evaluating the complex fungal communities enriched by fish waste application [90].

Future direction:

- Employ multi-omics approaches (metatranscriptomics, metabolomics) to link taxonomic data with functional traits.
- Develop and standardize bioinformatics pipelines tailored to fish waste-enriched soil microbiomes.

7.2 Lack of Long-Term and Multi-Seasonal Field Trials

Existing field experiments are often short-term, failing to assess the cumulative effects of repeated fish waste amendments on soil fungal dynamics and crop responses over time.

Future direction:

- Design longitudinal studies across different agro-climatic zones to evaluate persistence, seasonal shifts in fungal populations, and their correlation with soil health indicators and yield stability.

7.3 Inadequate Characterization of Chitinolytic Fungi

Although chitin is a key component of fish waste, only a few chitinolytic fungal species (e.g., *Trichoderma*, *Myrothecium*) have been extensively studied. Many native fungi with potential chitin-degrading abilities remain unexplored.

Future direction:

- Isolate and characterize novel chitin-degrading fungi from fish waste-treated soils using selective media and enzymatic screening.
- Engineer or screen for strains with high-efficiency chitinase activity for use in biofertilizers or biocontrol formulations.

7.4 Interaction with Other Microbial Guilds

Current studies predominantly focus on fungi in isolation. The interactions between fungi and other soil microbes—such as rhizobacteria, archaea, and actinomycetes—remain poorly understood, limiting our ability to optimize microbial consortia.

Future direction:

- Conduct co-occurrence network analyses and functional interaction studies to explore synergistic or antagonistic relationships.
- Investigate microbial consortia design tailored to specific crops or soil types.

7.5 Variability in Fish Waste Composition

Fish waste varies considerably by species, processing method, and storage, which affects its nutrient content and impact on fungal activity. Few studies have standardized waste input for experimental reproducibility.

Future direction:

- Create fish waste classification systems based on origin, processing status, and nutrient profile.
- Establish dose-response guidelines for consistent application in agronomic research.

7.6 Lack of Commercial Scaling Models

Despite promising experimental data, few efforts have been made to commercialize fungal-based fish waste biofertilizers or integrate them into mainstream agricultural practices.

Future direction:

- Develop scalable fermentation technologies for co-formulation of fish waste with beneficial fungi.
- Create value chain models linking fisheries, biofertilizer industries, and farmers under circular economy frameworks.

7.7 Gaps in Policy, Training, and Farmer Awareness

Adoption of fish waste-based fungal technologies is hindered by a lack of awareness, regulatory ambiguity, and limited extension efforts.

Future direction:

- Advocate for policy incentives and subsidies promoting on-farm fish waste recycling and fungal biofertilizer use.

- Develop extension modules and digital platforms for farmer training, particularly in coastal and aquaculture-intensive regions.

Table 1: Key Research Gaps and Future Directions

Sr. No.	Identified Research Gap	Implications	Suggested Future Focus
1	Limited field-scale studies on fungal colonization in fish waste-amended soils	Hinders validation of fungal benefits across diverse agro-climatic regions	Design multi-location trials under controlled and open-field conditions
2	Inconsistent results in fungal diversity due to variability in fish waste composition	Challenges reproducibility and limits generalizability of findings	Characterize fish waste inputs and develop compositional databases
3	Lack of standardized protocols for fungal isolation from fishery substrates	Affects comparability of results and hinders meta-analyses	Establish harmonized microbial isolation and DNA barcoding protocols
4	Underexplored functional roles of non-pathogenic fungi in waste transformation	Potential loss of biotechnologically valuable fungal strains	Investigate enzymatic, biocontrol, and nutrient-cycling potentials
5	Insufficient data on long-term ecological effects of fish waste application	Uncertainty regarding environmental safety and soil health impacts	Monitor soil biochemical markers and biodiversity over extended periods

Table 1 shows Research Gaps summarizing the key challenges and future directions in the study of fungal interactions with fishery waste in soil. Bridging these research gaps will require interdisciplinary collaborations across soil microbiology, agronomy, waste management, biotechnology, and policy science. A systems approach—integrating molecular tools, field ecology, and socio-economic feasibility—is essential to establish fish waste-fungal amendments as a sustainable pillar in regenerative agriculture.

9. Conclusion

The utilization of fishery waste as a soil amendment, coupled with the functional capabilities of soil fungi, represents a promising approach for advancing sustainable agriculture, waste valorization, and microbial-driven soil restoration. This review comprehensively explored the biochemical composition of fish waste, the ecological interactions of soil fungi, their roles in nutrient cycling, disease suppression, and soil structure enhancement, and how these synergies translate into agronomic benefits.

Fungi such as *Trichoderma, Aspergillus, Penicillium*, and mycorrhizal genera like *Glomus* and *Rhizophagus* play pivotal roles in decomposing complex fish waste materials and converting them into bioavailable nutrients, while simultaneously enhancing soil biodiversity and resilience. Molecular techniques such as ITS-based DNA barcoding have improved the accuracy of fungal identification, supporting deeper ecological insights. Furthermore, the application of fish waste aligns seamlessly with circular bioeconomy principles, promoting low-cost, eco-efficient, and regenerative farming systems.

However, despite these advantages, key research gaps remain in areas such as long-term field validation, molecular profiling of fungal communities, chitinolytic strain exploration, and integration into commercial farming practices. Addressing these challenges through transdisciplinary approaches and inclusive policy frameworks can pave the way for mainstreaming this biotechnological solution.

In conclusion, the fish waste–fungus interface offers a bio-integrated, scalable, and environmentally aligned strategy for future-ready agriculture. Its success will depend on continuous innovation, farmer engagement, and collaborative models that bridge marine resources and terrestrial productivity in a sustainable and circular manner.

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