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Exploratory profiling of *Rhizophora apiculata* root-associated fungi and preliminary polycaprolactone surface modification assessed using amplicon sequencing and culture-based approaches

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ABSTRACT

Microplastics are persistent environmental contaminants, and fungi associated with mangrove roots may offer a source of isolates for studying interactions with polymeric materials. This exploratory study profiled fungal DNA in a single pooled sample of *Rhizophora apiculata* roots using ITS amplicon sequencing and examined the FTIR spectra of polycaprolactone (PCL) samples following exposure to five cultured fungal isolates, designated fungal isolates S2–S6. Amplicon sequencing detected 48 fungal taxa, predominantly assigned to *Ascomycota*, with *Aspergillus* as the most abundant genus. The diversity indices suggested moderate diversity and relatively low dominance within the pooled sample. FTIR analysis revealed differences in PCL associated spectral features following exposure to the isolates, while principal component analysis (PCA) showed preliminary spectral differentiation among the analyzed samples. These observations indicate differences in the chemical or surface associated characteristics of the tested PCL samples; they do not demonstrate polymer mass loss, depolymerization, assimilation, or mineralization. The sequencing results represent fungal DNA detected in one pooled biological sample and do not establish the viability or endophytic status of the detected taxa. A correspondence between the cultured isolates and the detected amplicon sequence variants was not established. Quantitative mass-loss measurements, molecular weight and degradation product analyses, and experiments with appropriate controls and biological replication are needed to determine the nature and cause of the observed PCL associated spectral changes.

Keywords: *Rhizophora apiculata*; mangrove root-associated fungi; ITS amplicon sequencing; polycaprolactone; FTIR spectroscopy; preliminary spectral changes.

INTRODUCTION

Global plastic production and consumption continue to rise, resulting in the accumulation of plastic waste across terrestrial, freshwater, and marine environments. Weathering processes such as ultraviolet radiation, mechanical abrasion, oxidation, and biological activity fragment plastics

into microplastics, which persist due to their resistance to natural degradation. These particles have been detected in diverse ecosystems and are capable of adsorbing and transporting contaminants, thereby increasing environmental toxicity and altering microbial community structures. Microplastics are now recognized as emerging pollutants that demand sustainable management and effective remediation strategies (Greenshields et al., 2024; Khurana et al., 2025). Mangrove ecosystems, located at the interface of land and sea, are particularly vulnerable to microplastic accumulation. Plastic debris originating from rivers, urban runoff, fishing activities, and ocean currents can become trapped in mangrove sediments due to low-energy hydrodynamic conditions and the complexity of root systems. High organic matter content further promotes particle retention, making mangroves important sinks for microplastics. Such accumulation may alter sediment physicochemical properties, disrupt biogeochemical cycles, and negatively affect microbial activity and vegetation health (Duan et al., 2021; Y. Wang et al., 2023).

Among Indo-Pacific mangrove species, *Rhizophora apiculata* is notable for its stilt-root system, which effectively traps sediments and suspended particles, including microplastics. The rhizosphere of this species is enriched with root exudates and organic matter, supporting diverse microbial communities. Microplastics within this niche may impose selective pressure, favoring microorganisms capable of adapting to plastic-contaminated environments and transforming complex polymeric compounds (Huertas et al., 2024; Lin et al., 2024). Fungi are recognized as important contributors to plastic biodegradation. Fungi colonize polymer surfaces, form biofilms, and release extracellular enzymes that break down plastics into compounds of lower molecular weight. Enzymes such as laccases, peroxidases, and esterases have been implicated in these processes. Fungi associated with mangrove roots may possess traits relevant to bioremediation, although their capacity to modify or remove polymer pollutants requires direct experimental validation. Although endophytes associated with *R. apiculata* are known to produce oxidative and hydrolytic enzymes, experimental evidence of their ability to degrade microplastics remains limited (Ekanayaka et al., 2025; Ferreira-Filipe et al., 2024). Beyond their role in biodegradation, endophytic fungi establish mutualistic associations with plants, enhancing nutrient uptake, producing growth promoting metabolites, and providing tolerance to abiotic stress. These traits reflect broad metabolic capabilities and further highlight their relevance to bioremediation applications (Li et al., 2022). Nevertheless, the specific contribution of mangrove-associated endophytes to microplastic degradation has not been systematically explored. Traditional culture-dependent methods have been widely used to study endophytic fungi, enabling the recovery of viable isolates for functional evaluation. However, these approaches capture only a fraction of the overall fungal community. Molecular techniques with high sequencing capacity, such as ITS amplicon sequencing, provide broader insights into community composition, including unculturable taxa, but they do not confirm organism viability or biological activity. The integration of culture-based methods and molecular approaches is essential for achieving a comprehensive and reliable characterization of endophytic fungal communities (Shah et al., 2024).

Despite rapid advances in research on microplastics, mangrove microbiomes, and endophytic fungi, these areas have largely been investigated independently. Few studies have examined mangrove endophytic fungi as potential agents of microplastic biodegradation, and experimental evidence linking fungal community composition to biodegradation related functions remains scarce (Sari Lubis et al., 2025). Addressing this gap may improve our understanding of fungi associated with mangrove roots and provide a basis for future controlled studies evaluating their potential for polymer mycoremediation.

This exploratory study aimed to characterize fungal DNA associated with a pooled *Rhizophora apiculata* root sample using ITS amplicon sequencing and to recover fungal isolates through culture-dependent methods. It further assessed whether exposure to selected isolates was associated with differences in the FTIR spectra of polycaprolactone (PCL). The sequencing and culture-based results provide complementary observations, but a direct correspondence between the recovered isolates and the detected ITS ASVs was not established. The FTIR assessment was intended to identify preliminary PCL-associated spectral changes, not to demonstrate polymer biodegradation.

MATERIALS AND METHODS

Sample collection

Root samples of *Rhizophora apiculata* were collected from a mangrove area in Lampulo, Banda Aceh, Indonesia (5.5825960° N, 95.3296880° E), using a 5 × 5 m quadrat. *Rhizophora apiculata* was the only plant species present within the selected quadrat. Five individual trees were sampled: four trees located near the corners of the quadrat and one tree located near its center. Root sections approximately 2 cm in diameter were aseptically collected at a soil depth of approximately 7 cm. Samples from each tree were placed separately in sterile plastic bags and transported to the laboratory in an icebox at approximately 4 °C to minimize contamination and sample deterioration. In the laboratory, approximately equal portions of root tissue from the five trees were pooled to obtain 50 g of composite tissue. The pooled tissue constituted one composite biological sample for DNA extraction and ITS amplicon sequencing and was assigned the sample ID N949-3.

ITS amplicon sequencing

A total of 50 g of surface-sterilized root tissue was submitted to Genetika Science Indonesia for ITS amplicon sequencing. The material comprised 10 g of root tissue from each of five individual Rhizophora apiculata trees within a single 5 × 5 m quadrat: four trees near the corners and one near the center. Tissue from the five trees was pooled before DNA extraction, yielding one composite biological sample, designated N949-3. A single DNA extraction and ITS amplicon sequencing analysis were performed on this pooled sample. Therefore, the five trees did not constitute independent biological replicates for sequencing. The results provide an exploratory profile of fungal DNA detected in the pooled root material at this site and cannot be used to assess variation among individual trees.

PCR conditions

Genomic DNA was extracted using ZymoBIOMICS™ DNA Miniprep in accordance with the standard operating procedure established by PT GSI. The internal transcribed spacer (ITS) region was amplified using the ITS1-1F primer. PCR amplification was performed in a total reaction volume of 25 µL, with a final primer concentration of 0.5 µM. The thermal cycling protocol consisted of 35 amplification cycles, with an annealing temperature of 55 °C.

ASV filtering criteria, sequencing depth and quality

Illumina sequencing generated an average of approximately 45,000 reads per sample, with more than 90% of the bases achieving a Phred quality score of Q30 or higher. Amplicon sequence variants (ASVs) were inferred using the DADA2 pipeline. Quality filtering was performed using a maximum expected error threshold of two (maxEE = 2) and a truncation length of 250 nucleotides (truncLen = 250). Chimeric sequences were subsequently identified and removed using the default chimera-removal settings. These parameters are reported to facilitate the reproducibility of the bioinformatics analysis.

Isolation of root-associated fungi from mangrove

Roots of *Rhizophora apiculata* were initially washed under running tap water to remove adhering soil and debris. The roots were then cut into manageable sections and subjected to surface sterilization by immersion in 80% (v/v) ethanol for 1 min, followed by immersion in 0.25% (v/v) sodium hypochlorite (NaClO) for 1 min. After sterilization, the root sections were rinsed three times with sterile distilled water to remove residual sterilizing agents and then dried aseptically on sterile filter paper inside a laminar airflow cabinet. To evaluate the effectiveness of surface sterilization, an aliquot of the final rinse water was inoculated onto corn meal agar (CMA), and incubated under the same conditions used for fungal isolation. The absence of microbial growth on the control plates was considered an indication that the surface-sterilization procedure had effectively removed cultivable surface-associated microorganisms. The sterilized roots were subsequently cut into segments of approximately 1-1.5 cm and immediately used for fungal isolation. The effectiveness of sterilization was confirmed by inoculating aliquots of the final rinse water onto corn meal agar (CMA). A total of 72 root segments were prepared from the composite root material pooled from five individual *Rhizophora apiculata* trees sampled within the 5 × 5 m quadrat.

The segments were placed on culture medium and incubated at room temperature 28-30 °C for 15 days. Colonies were purified on potato dextrose agar (PDA). Microscopic characterization was performed on slide cultures stained with lactophenol cotton blue to examine hyphal, conidiophore, conidial, and septal morphology (Akhir et al., 2024). Five cultured isolates were selected for subsequent PCL exposure experiments based on their distinct growth patterns and dark hyphal pigmentation. They were assigned the operational isolate codes S2, S3, S4, S5, and S6. These codes identify cultured isolates and do not imply correspondence with the ITS amplicon sequence variants (ASVs) detected in the pooled root sample.

Assessment of PCL-associated spectral changes following fungal exposure

Fungal isolates were screened for growth on agar medium containing polycaprolactone (PCL), following the medium-preparation methods described (Kim et al., 2022; Molitor et al., 2020). Polycaprolactone (product no. 440744; Sigma-Aldrich, St. Louis, MO, USA) was supplied as white to off-white pellets approximately 3 mm in size. The polymer had a number-average molecular weight (M_n) of approximately 80,000 g mol^{-1}, a molecular-weight dispersity (M_v/M_n) of <2, a water content of <0.5%, a melting point of 60 °C, and a density of 1.145 g mL^{-1} at 25 °C. Each of the five fungal isolates (S2–S6) was inoculated separately onto PDA supplemented with 1% (w/v) emulsified PCL using a 4-mm agar plug from a pure culture. Each of the five fungal isolates (S2, S3, S4, S5, and S6) was evaluated separately in triplicate. The plates were incubated at 25 °C for 15 days. Three plates were prepared for each isolate, giving 15 inoculated plates in total. These plates constituted technical replicates for the agar-medium screening and were not independent biological replicates. Growth on PCL-containing medium was recorded as an initial screening observation and was not interpreted as evidence of PCL biodegradation.

Chemical changes in PCL were evaluated using Fourier-transform infrared spectroscopy s(FTIR). Fourier transform infrared (FT-IR) spectra were acquired using a Spectrum Two FT-IR Spectrometer (PerkinElmer) fitted with a lithium tantalate ($LiTaO_3$) detector and a Universal Attenuated Total Reflectance (UATR) accessory. Each sample was placed directly onto the diamond ATR crystal and compressed with the pressure arm to maintain consistent contact with the crystal surface. Spectral measurements were performed in transmittance mode (%T) over the wavenumber range of 4000–400 cm^{-1}, using a spectral resolution of 4 cm^{-1} and a data spacing of 1 cm^{-1}. A background spectrum was collected with the clean ATR crystal before each series of sample measurements. Each experimental sample was measured four times using the same sample material, resulting in four technical replicates per sample. These repeated measurements were intended to assess measurement repeatability and within-sample spectral consistency; therefore, they should not be interpreted as independent biological replicates. A background spectrum was collected before sample measurement, and the ATR crystal was cleaned between successive measurements. The resulting spectra were subsequently subjected to baseline correction, normalization, and smoothing before comparative analysis and principal component analysis (PCA).

Data analysis

Statistical analyses were conducted using one-way ANOVA followed by Duncan's new multiple range test (DNMRT) at $p \leq 0.05$ in SPSS version 23. FTIR spectral data were further analyzed using principal component analysis (PCA) in SIMCA to differentiate untreated PCL controls from PCL samples exposed to fungal isolates.

RESULTS AND DISCUSSION

Amplicon sequencing of roots

Amplicon sequencing of the fungal community associated with *R. apiculata* roots revealed moderate diversity with relatively low dominance. Alpha diversity indices (Figure 1) showed an observed richness of 48 taxa, indicating considerable taxonomic wealth. The Shannon index (2.40) reflected an even distribution of abundance, while the Simpson index (0.84) indicated limited dominance by a single group. The Inverse Simpson value (6.29) suggested that the community structure was shaped by several dominant taxa supported by numerous rare taxa, a pattern typical of endophytic fungal communities in mangrove root systems (Cassol et al., 2025; Roswell et al., 2021). Together, these indices highlight a

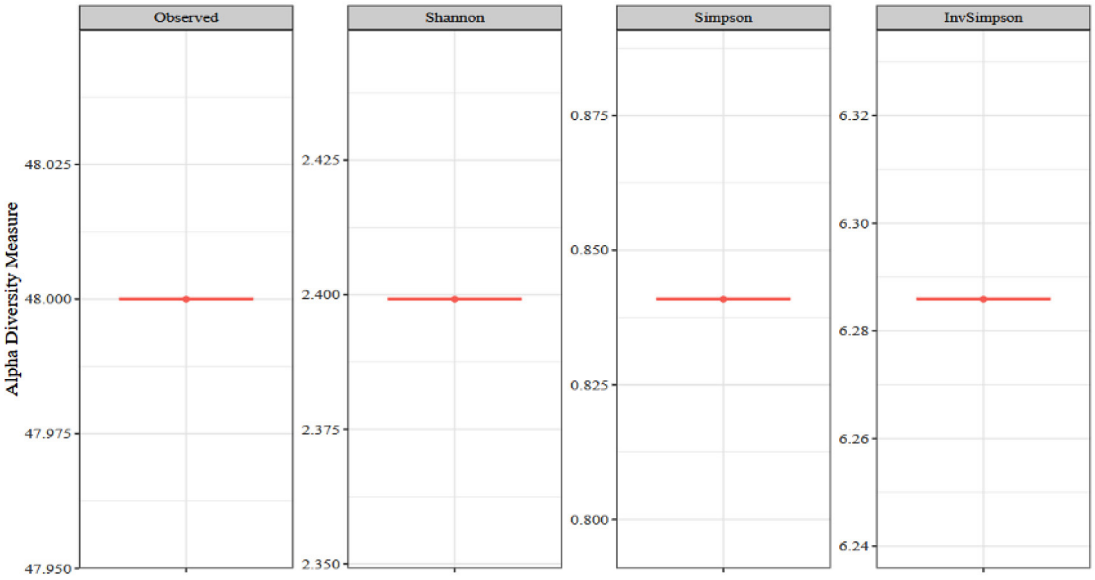

Figure 1. Alpha diversity indices of the fungal community associated with R. apiculata roots

balanced community where richness and evenness are both significant. (Kvålseth, 2024).

Taxonomic analysis (Figure 2A) showed that Ascomycota dominated the community (92%), while *Basidiomycota* and *Rozellomycota* were detected in minor proportions. This dominance of Ascomycota aligns with previous metabarcoding studies reporting its prevalence in mangrove ecosystems due to diverse lifestyles as endophytes, saprotrophs, and decomposers (Zhang et al., 2021; Zhu et al., 2024). Basidiomycota contributed to lignocellulose decomposition, whereas *Rozellomycota* appeared in low abundance with ecological roles that remain poorly understood.

At the class level (Figure 2B), *Sordariomycetes, Eurotiomycetes*, and *Dothideomycetes* were most abundant, recognized for producing extracellular enzymes and adapting to salinity stress (Zhu et al., 2024). At the order level (Figure 2C), *Capnodiales, Eurotiales, Pleosporales,* and *Saccharomycetales* predominated. Capnodiales included pigmented fungi such as *Cladosporium*, which thrive under extreme conditions, while *Pleosporales* encompassed root-associated dematiaceous fungi (Z. Wang et al., 2024).

At the family level (Figure 2D), *Cladosporiaceae* was most abundant, followed by *Meruliaceae, Debaryomycetaceae,* and *Pleosporaceae*. At the genus level (Figure 2E), *Aspergillus* dominated, followed by *Cladosporium*, *Penicillium*, and yeasts such as *Candida* and *Debaryomyces*. The dominance of *Aspergillus* and *Penicillium* is consistent with their high metabolic capacity, including the production of hydrolytic enzymes and secondary metabolites relevant to polymer degradation (Amaeze et al., 2026; Zhu et al., 2024). *Cladosporium* was identified as a tolerant endophyte capable of producing diverse metabolites with potential biotechnological applications (Nazir et al., 2024).

Integrating amplicon sequencing with culture-based characterization provides complementary insights into the fungal community associated with mangrove roots. Amplicon sequencing offers a broad overview of fungal diversity and community composition, whereas culture-based methods enable the phenotypic and functional characterization of viable isolates. In the present study, root-associated fungi fungal isolates recovered from the roots of *R. apiculata* showed the potential to induce alterations in the surface morphology of plastic polymers. These surface changes indicate possible fungal–polymer interactions; however, they do not, by themselves, constitute conclusive evidence of polymer biodegradation. Further investigations incorporating appropriate abiotic and biotic controls, polymer mass-loss measurements, chemical and structural analyses, enzyme-activity assays, and identification of degradation products are required to determine whether these isolates can depolymerize and metabolize plastic polymers. Further controlled experiments are needed to characterize the observed polymer-substrate modifications, investigate the processes responsible for these changes, and assess whether these isolates have potential for microplastic mycoremediation.

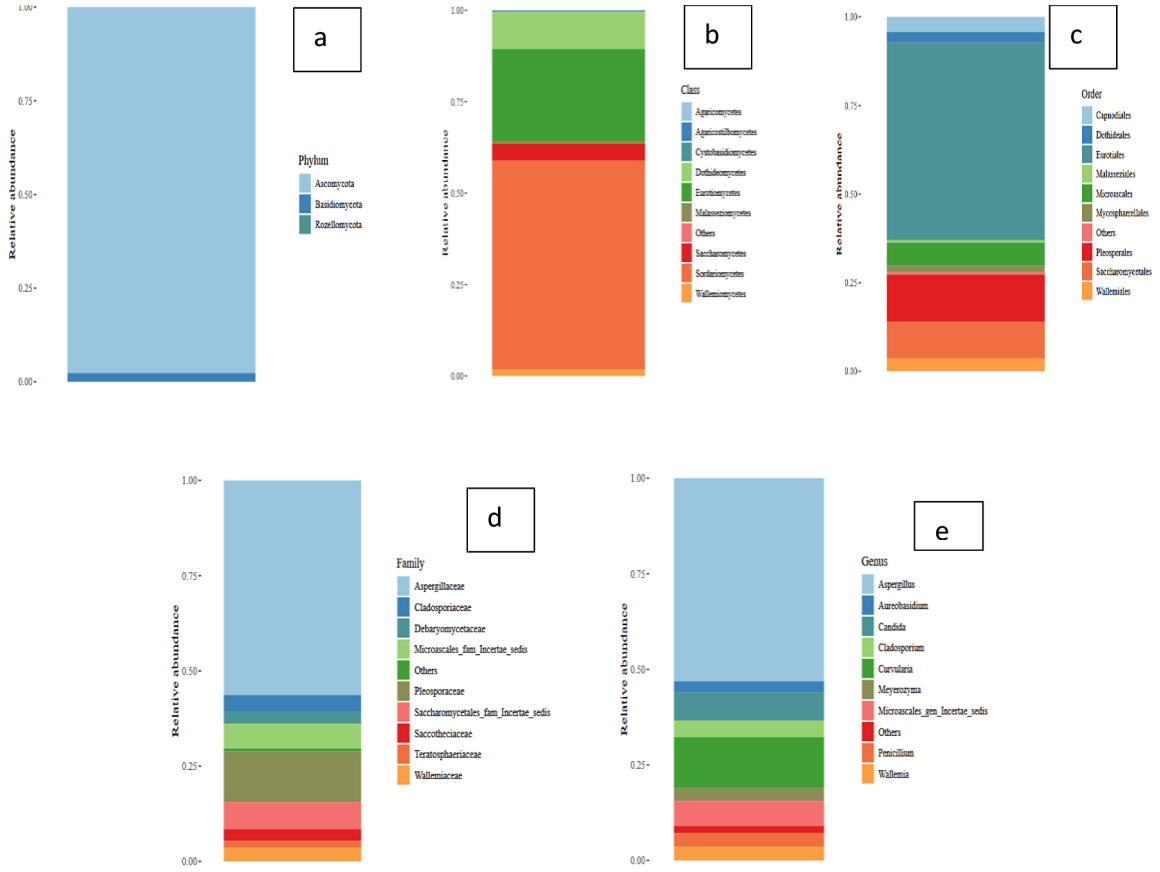

Figure 2. Relative abundance of fungal communities at the phylum (a), class (b), order (c), family (d), and genus (e) levels

Their reported tolerance to challenging environmental conditions and capacity to produce extracellular enzymes warrant further investigation of their potential role in mycoremediation strategies for coastal ecosystems. The findings thus establish a preliminary scientific basis for further investigation of root-associated fungal isolates, including their physiological characterization and evaluation as agents of microplastic biodegradation, aligning directly with the aim of developing innovative and sustainable approaches to mangrove ecosystem remediation (Deng et al., 2024; Kauserud, 2023).

Genus-level composition

Genus-level analysis (Figure 3) confirmed *Aspergillus* as the most dominant genus, accompanied by *Curvularia*, *Candida*, *Cladosporium*, *Wallemia*, *Penicillium*, *Meyerozyma*, *Aureobasidium*, *Hortaea*, *Malassezia*, and *Sterigmatomyces*. This composition reflects a community structured around one highly dominant genus with several genera of lower abundance.

The predominance of *Aspergillus* underscores its role as a principal component of the mangrove mycobiome, frequently isolated as an endophyte and known for producing diverse secondary metabolites and extracellular enzymes. Similar dominance has been reported in *Ceriops tagal* (Revathy et al., 2024). Other genera also play important roles: *Candida* and *Meyerozyma* are yeasts with metabolic flexibility, *Wallemia* is halotolerant and adapted to saline environments, while *Cladosporium* and *Curvularia* produce bioactive metabolites that enhance stress adaptation. These taxa collectively contribute to the resilience and functional diversity of mangrove fungal communities.

ITS amplicon sequencing detected sequences assigned to *Aspergillus, Candida, Penicillium*, and other fungal taxa in the composite surface-sterilized root sample. These findings represent DNA-based records within the analyzed material and should not be interpreted as independent confirmation of either fungal viability or endophytic status. ITS amplicon sequencing cannot distinguish DNA originating from viable organisms from that of non-viable cells or residual environmental

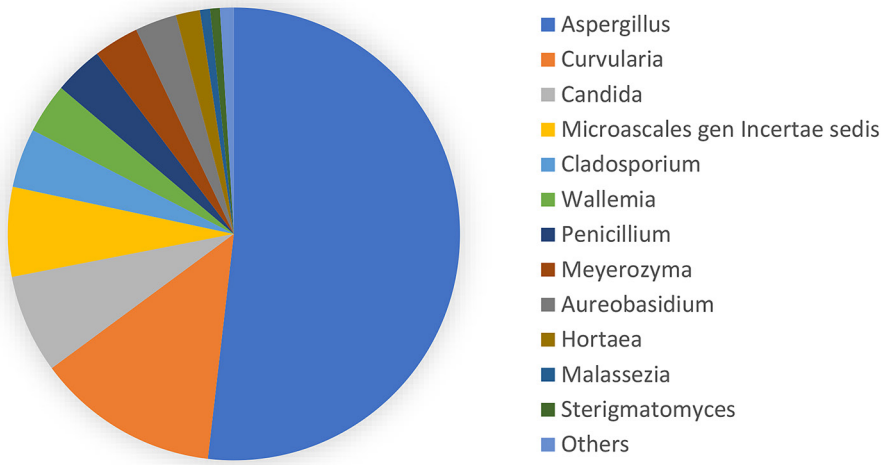

Figure 3. Relative abundance of fungal communities at the genus level

DNA. Furthermore, because surface sterilization was validated using only a final-rinse control, the possibility of residual epiphytic contamination cannot be conclusively excluded. Accordingly, the detected taxa are more appropriately described as root-associated fungal taxa rather than confirmed viable endophytes. Additional validation through culture dependent assessments, tissue-imprinting controls, viability-sensitive molecular approaches, and molecular identification of recovered isolates would be required to confirm their viability and root associated fungi.

The genus-level fungal community associated with *R. apiculata* roots comprised taxa known for their adaptability to coastal environments and diverse metabolic capabilities. These findings provide an ecological basis for the subsequent culture-based isolation and functional characterization of root-associated fungi. However, the functional capabilities of individual isolates, particularly their potential roles in microplastic biodegradation, must be validated through culture-based experiments, including physiological characterization, enzymatic assays, and controlled biodegradation trials.

The culturable isolates recovered in this study may correspond to some of the fungal taxa represented by the ASVs, particularly when both approaches detect the same fungal genera. However, substantial differences between the two datasets are also plausible because culture-dependent and culture-independent approaches recover different fractions of the fungal community. Durán et al., (2021) demonstrated that metabarcoding detected approximately 5.8 times more root associated fungi taxa than conventional culturing. Nevertheless, the most frequently recovered fungus in culture was not detected by metabarcoding. This finding indicates that a fungus may be readily recovered and even dominant under culture conditions while remaining undetected or poorly represented in the amplicon-sequencing dataset. Conversely, metabarcoding may detect numerous low-abundance, slow growing, host-dependent, dormant, or otherwise non culturable taxa that cannot be recovered under the selected culture conditions.

This discrepancy can arise because culture-based recovery is influenced by medium composition, incubation conditions, fungal viability, growth rate, sporulation, and competition among microorganisms. Rapidly growing fungi may therefore become disproportionately represented on artificial media, whereas slow-growing or nutritionally fastidious fungi may be overlooked. Amplicon sequencing, in contrast, provides a broader DNA-based representation of the community but may detect DNA originating from viable organisms, non viable cells, or residual environmental DNA. Its taxonomic profile may also be affected by DNA extraction efficiency, primer–template mismatches, PCR amplification bias, sequencing depth, bioinformatics parameters, the completeness of reference databases, and the taxonomic resolution of the selected ITS region (Tedersoo et al., 2022).

The taxa detected by ITS amplicon sequencing represent DNA-based records from a single pooled root sample; their detection does not, by itself, establish organism viability or endophytic status. A correspondence between the detected ASVs and fungal isolates S2–S6 has not been established. Such correspondence cannot be

inferred from colony morphology, hyphal pigmentation, or recovery from the same pooled root material. It would require ITS sequencing of each pure isolate and direct comparison with representative ASV sequences using predefined sequence-identity and query-coverage criteria, preferably supported by phylogenetic analysis. Assignment of an isolate and an ASV to the same genus would not, on its own, establish that they are identical. Thus, the culture dependent and amplicon-sequencing datasets should be regarded as complementary: culture confirms the recovery of viable fungi and enables functional experimentation, whereas metabarcoding provides a broader assessment of fungal DNA associated with the root sample (Durán et al., 2021; Høyer & Hodkinson, 2021; Tedersoo et al., 2022).

Screening of microplastic biodegradation potential

Based on the preliminary screening results, five fungal isolates S2, S3, S4, S5, and S6 were selected from the cultures obtained from 72 root segments for further investigation. The isolates were selected on the basis of their distinctive growth patterns and dark hyphal pigmentation. When incubated on potato dextrose agar (PDA) supplemented with polycaprolactone (PCL) microplastics, these isolates exhibited morphological responses, including changes in colony pigmentation and irregular growth patterns (Figure 4). These changes suggest physiological responses to PCL, a polyester substrate that is relatively difficult to utilize.

The fungal isolates examined in this study were designated fungal isolates S2–S6 based on differences in their culture characteristics and morphological features. However, molecular identification of these isolates was not performed because the present study was designed as an initial exploratory screening of culturable root associated fungi for their possible interactions with PCL. Therefore, fungal isolates S2–S6 should be regarded as operational isolate codes rather than taxonomically identified fungal species. Morphological observations alone are insufficient for reliable identification, particularly because several fungal taxa may exhibit similar colony and hyphal characteristics. Furthermore, no direct correspondence can currently be established between fungal isolates S2–S6 and the fungal taxa detected through ITS amplicon sequencing of the composite root sample. The amplicon sequencing results represent DNA based community records, whereas the selected isolates were obtained independently through a culture based approach. Consequently, the taxonomic identities of fungal isolates S2–S6 and their relationship to the detected amplicon sequence variants remain undetermined.

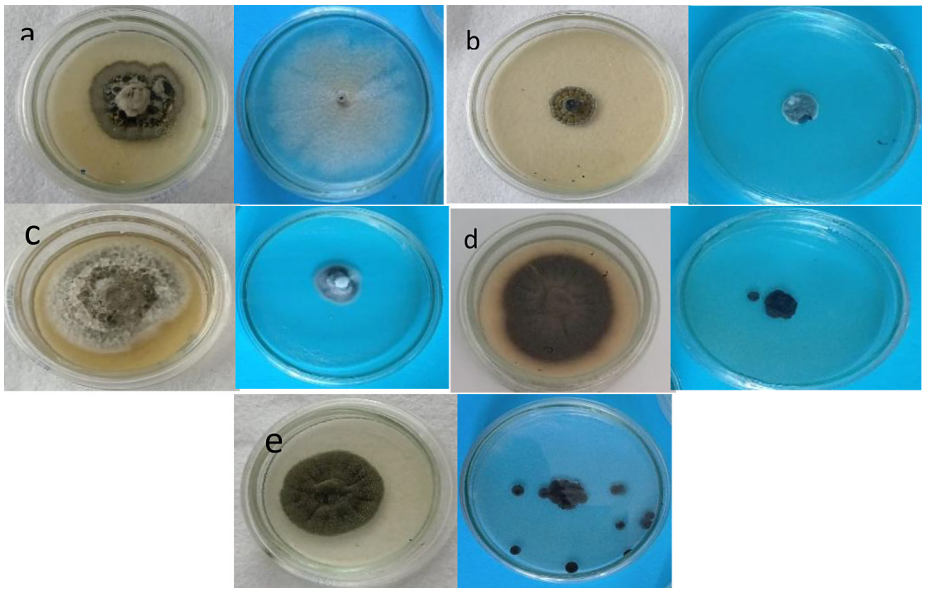

Figure 4. Colony morphology of fungal isolates grown on PDA supplemented with PCL (blue background) and those grown without PCL (white background): (a) S2, the mycelium appeared dispersed and became light gray; (b) S3, the colony exhibited lighter pigmentation; (c) S4, the colony exhibited lighter pigmentation and an elevated surface; (d) S5, the colony showed darker pigmentation and distinct separated sectors; and (e) S6, the colony became darker and developed as several dispersed colonies

The FTIR differences observed in samples exposed to fungal isolates S2–S6 should therefore be interpreted as isolate specific, qualitative indications of possible fungal polymer interactions rather than evidence of PCL modification by particular fungal species. Although S5 and S6 produced more pronounced polymer associated spectral differences than the other isolates, they can only be considered preliminary candidates for further investigation and cannot yet be classified as confirmed PCL degrading fungi or microplastic bioremediation agents. Molecular identification through ITS sequencing of each isolate, followed by phylogenetic analysis and comparison with representative amplicon sequence variants, will be required in subsequent studies to establish their taxonomic identities and evaluate their potential applications in polymer bioremediation. In the present study, PCL was incorporated into the culture medium as an emulsified suspension that became embedded within the solidified agar matrix. As a result, the residual PCL could not be separated and quantitatively recovered from the medium without potential contamination from agar components, fungal biomass, and other medium constituents. Therefore, direct quantitative determination of PCL loss, such as gravimetric massloss measurement, was not technically feasible under the experimental conditions used. Accordingly, the present assessment was limited to qualitative and comparative evaluation of the recovered polymer-associated material using FTIR spectroscopy. The observed spectral differences indicate changes in functional group associated signals following fungal exposure; however, they do not provide quantitative evidence of polymer mass loss, depolymerization, assimilation, or mineralization. Thus, these results should be interpreted only as preliminary evidence of fungal polymer interaction and possible PCL associated surface or chemical modification, rather than conclusive evidence of PCL biodegradation.Future studies should employ recoverable PCL substrates, such as pre-weighed films or pellets, together with appropriate uninoculated, abiotic, and biological controls. This approach would allow quantitative assessment through mass loss measurements and complementary analyses, including molecular-weight determination, degradation-product analysis, and CO_2 evolution assays.

During the initial screening, isolates S2, S3, S4, S5, and S6 grown on PDA supplemented with PCL microplastics exhibited changes in colony morphology, suggesting a potential response to the presence of PCL. According to Lu et al., (2024), fungal morphology may change in response to variations in nutrient availability and environmental conditions during fermentation. Factors influencing the morphology of filamentous fungi include inoculum age and volume, medium composition, exogenous additives such as surfactants, dissolved oxygen concentration, agitation speed and impeller design, pH, temperature, fermenter type and size, and power input. Although no clear zones were observed after 15 days of incubation, potential chemical changes in PCL were qualitatively evaluated using Fourier-transform infrared spectroscopy (FTIR) (Figure 5 and Table 1), followed by principal component analysis (PCA) of the resulting spectra (Figure 6). Each group in the PCA was represented by four technical replicates. These replicates were used to assess the consistency of the FTIR spectral measurements and do not constitute independent biological replicates. Therefore, the separation observed in the PCA should be interpreted as preliminary spectral differentiation among the analyzed samples, rather than evidence of reproducible biological differences or PCL degradation capacity.

The preliminary spectral differentiation chemical or surface-associated changes in the analyzed PCL samples following exposure to the fungal isolates (Fig. 5). However, these spectral differences alone do not demonstrate PCL mass loss, depolymerization, assimilation by the fungi, or mineralization. Accordingly, the findings should be interpreted as preliminary evidence of fungal-associated PCL spectral changes. Quantitative mass-loss measurements, molecular-weight and degradation-product analyses, and appropriate experimental controls are required to determine the nature and extent of the observed changes.

An untreated PCL sample was included as a reference for comparison with the fungal-exposed samples. However, the experimental design did not include an uninoculated medium control, an inactivated fungal biomass control, or a positive biodegradation control. Therefore, the observed FTIR spectral differences cannot be attributed exclusively to fungal metabolic activity. Changes associated with the culture medium, sample recovery and preparation, abiotic processes, or the physical interaction of fungal biomass with the polymer cannot be completely excluded.

Accordingly, the FTIR results should be interpreted as qualitative evidence of differences

Table 1. Characteristic FTIR absorption bands of untreated PCL and PCL exposed to mangrove root-associated fungal isolates

Sample	C=O (carbonyl stretching vibrations) 1750 – 1710		C–H (aliphatic stretching vibrations) 3000 – 2840		C–O–C (symmetric stretching vibrations) 1180 – 1050		C–O–C (asymmetric stretching vibrations) 1300 – 1200	
	cm^{-1}	%T	cm^{-1}	%T	cm^{-1}	%T	cm^{-1}	%T
PCL	1723	26.79	2943	76.24	1164	37.85	1239	50.68
S2 MP	1745	93.13	2923	86.04	1150	81.17	1249	86.37
S3 MP			2926	85.87				
S4 MP			2924	82.95	1152	73.17	1251	77.93
S5 MP	1744	88.12	2925	78.39	1152	69.61	1240	74.15
S6 MP			2924	79.68	1151	68.35	1245	75.52

between untreated and fungal exposed PCL associated samples, rather than definitive evidence of biologically mediated PCL degradation. Although isolates S5 and S6 showed more pronounced spectral differences than fungal isolates S2–S4, these differences indicate possible fungal polymer interactions or preliminary surface-associated chemical modification and do not establish polymer depolymerization, assimilation, or mineralization.

The absence of the necessary experimental controls is acknowledged as an important limitation of this exploratory study. Future experiments should include PCL incubated in uninoculated sterile medium as an abiotic control, PCL exposed to inactivated fungal biomass to distinguish passive physical interactions from metabolic activity, and a positive control containing a microorganism or enzyme with established PCL degrading activity. These controls, together with quantitative mass loss measurements, molecular weight analysis, degradation-product detection, and CO_2 evolution assays, will be required to determine whether the observed changes result from genuine fungal mediated PCL biodegradation.

Following incubation, the ability of each fungal isolate associated with mangrove roots to induce changes in the polymer was evaluated using Fourier-transform infrared (FTIR) spectroscopy. The FTIR spectrum of untreated PCL exhibited several characteristic absorption bands that served as reference markers for the polymer. The bands observed at approximately 2940–2949 cm^{-1} and 2850–2870 cm^{-1} were attributed to the asymmetric and symmetric stretching vibrations of CH_2 groups, respectively, representing the aliphatic chains of PCL. The prominent absorption

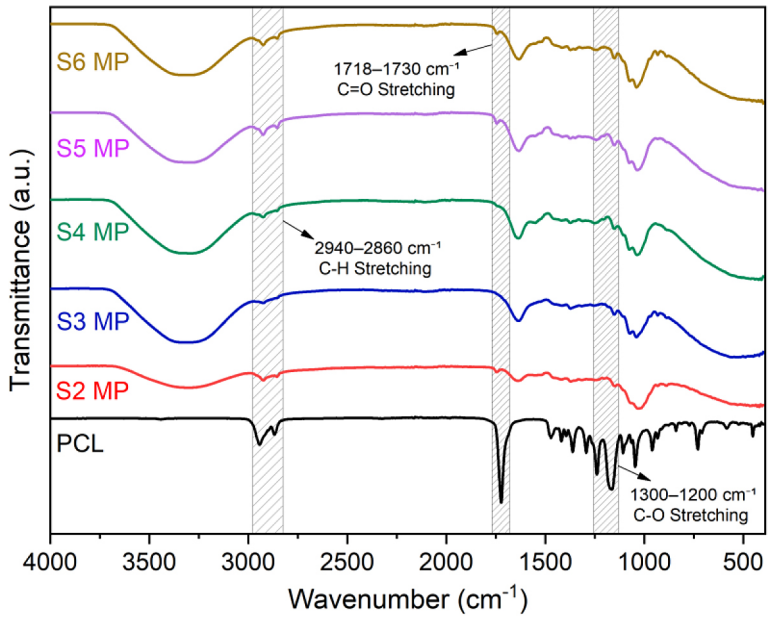

Figure 5. FTIR spectra of untreated and fungal-treated PCL samples

band at approximately 1718–1730 cm^{-1} was assigned to the carbonyl (C=O) stretching vibration of the ester group and represented one of the principal spectral markers of the PCL structure. Furthermore, the bands occurring at approximately 1293–1295 cm^{-1}, 1240–1249 cm^{-1}, and 1166–1174 cm^{-1} were associated with C–O, C–C, and C–O–C vibrations within the ester linkages and polymer backbone (Elzein et al., 2004; Fakhr et al., 2025; Phillipson et al., 2014; Richert et al., 2023; Stewart et al., 2020)

FTIR spectra revealed characteristic absorption bands of PCL, including C–H stretching (2940–2860 cm^{-1}), C=O stretching (1718–1730 cm^{-1}), and C–O ester stretching (1470–1173 cm^{-1}). In untreated controls, these peaks appeared sharp and well-defined, reflecting the intact chemical structure of PCL (Table 1). In contrast, fungal treatments (fungal isolates S2-S6) produced changes in peak intensity and shape, particularly in the carbonyl and ester regions, suggesting limited chemical modifications at the polymer surface. These alterations are consistent with early hydrolytic or enzymatic cleavage of ester bonds, processes commonly mediated by microbial lipases, esterases, and depolymerases (Krumov et al., 2025).

The morphological and spectroscopic alterations observed following exposure to the mangrove root associated fungal isolates provide preliminary evidence of fungal polymer interactions and possible surface modification of PCL. Changes in surface morphology and FTIR absorption profiles may indicate erotion, hydrolysis, or modification of functional groups within the polymer. However, these observations alone are insufficient to demonstrate complete biodegradation because surface deterioration does not necessarily confirm polymer chain cleavage, assimilation of polymer derived carbon by fungal cells, or its subsequent mineralization. Furthermore, these changes should be compared with uninoculated abiotic controls to distinguish biologically mediated effects from spontaneous hydrolysis or other non biological alterations occurring during incubation (Obrador-Viel et al., 2024).

Further validation should therefore integrate complementary analytical approaches. Changes in number average and weight average molecular weight and molecular eight distribution measured using gel permeation chromatography or size exclusion chromatography (GPC/SEC) would provide evidence of polymer chain scission and depolymerization. Analysis of soluble degradations products would help identify oligomers, monomers, or other low molecular weight compound released from the PCL matrix. Nevertheless, depolymerization should be distinguished from ultimate biodegradation because the formation of smaller molecules dose not necessarily demonstrate their uptake and metabolism by fungal cells. Controlled CO_2 evolution assays are therefore required to determine whether polymer derived carbon is ultimately mineralized, preferably alongside uninoculated polymer controls, inoculated controls without PCL, and appropriate positive control(Chamas et al., 2020; Rose et al., 2020). Accordingly, the present findings should be interpreted as evidence of potential surface

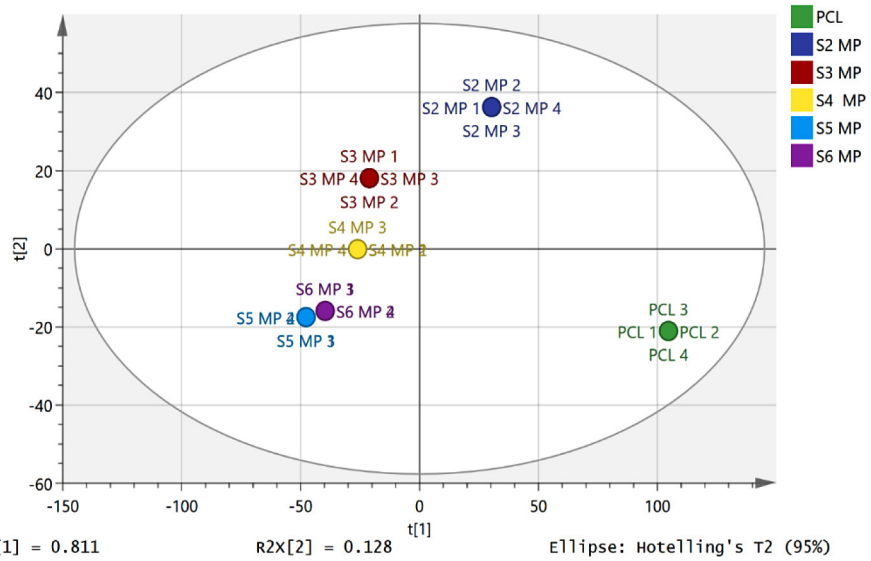

Figure 6. PCA score plot of FTIR spectra showing separation between control and fungal-treated PCL samples

modification or preliminary polymer deterioration rather than definitive PCL biodegradation .

Prior to multivariate analysis using Principal Component Analysis (PCA), the ATR-FTIR spectra (range 4000–600 cm^{-1}, were scaled using the Unit Variance (UV) method in SIMCA 14 software (Sartorius, Malmö, Sweden) (Figure 6); in this process, each spectral variable was mean-centered and divided by its standard deviation to assign equal analytical weight to all wavenumbers, regardless of their absolute intensities. The optimal number of principal components was determined automatically via cross validation (using the Autofit function).

The PCA results are interpreted as an exploratory analysis. PC1 and PC2 explained 81.1% and 12.8% of the total spectral variance, respectively (cumulatively 93.9%), and the score plot revealed a clear spatial separation between treatment groups along the PC1 axis. The PCL control samples are located at positive PC1 values (t[1] = +100), clearly separated from all fungal treated samples. Among the fungal treatments, the S2 MP samples are located at intermediate positive PC1 values (t[1] = +50), whereas S3 MP and S4 MP occupy the central regio (t[1] = +7). The S5 MP and S6 MP samples are located at negative PC1 values (t[1] = −50), the position furthest from the PCL control along the PC1 axis. Technical replicates (n = 4 measurements per treatment) showed perfect overlap in PCA space, confirming the high instrumental reproducibility of the FTIR measurements, and all 24 observations fell within the 95% Hotelling's T^2 confidence ellipse, indicating the absence of spectral outliers. Isolates S5 and S6 formed a distinct group, reflecting more pronounced chemical changes, whereas S2 exhibited weaker spectral shifts. Based on the PCA clustering pattern, the extent of polymer-substrate modification was categorized as low for isolates S2, S3, and S4 and moderate for S5 and S6. Principal Component Analysis (PCA) of FTIR spectra provided preliminary spectral differentiation of chemical modifications in polycaprolactone (PCL) after fungal treatment. All samples clustered within the Hotelling's T^2 95% confidence ellipse, confirming the absence of extreme outliers and supporting the reliability of the dataset (Conterosito et al., 2025; Derksen et al., 2023; Ivleva, 2021; Tarhan & Kestek, 2024).

The PCA score plot further confirmed separation between control and treated samples, with isolates S3 and S4 clustering closely, indicating similar spectral profiles. Isolates S5 and S6 formed a distinct group, reflecting more pronounced and consistent chemical changes in PCL. In contrast, isolate S2 showed weaker spectral shifts, suggesting only initial stages of polymer modification. The preliminary spectral differentiation of the isolates was therefore categorized as weak for fungal isolates S2, S3, S4, and moderate for S5 and S6.

Ecological and applied implications

The observed modifications in PCL structure highlight the potential role of mangrove-associated fungi, particularly root-associated fungi, in microplastic biodegradation. While complete degradation was not achieved, the chemical changes detected by FTIR and PCA provide preliminary spectral differentiation of initial bio-deterioration processes or surface-associated modification of PCL, but do not establish polymer degradation.. This is ecologically significant, as endophytic fungi are already known to enhance plant tolerance to abiotic stress through root colonization and melanized hyphae formation (Gaber et al., 2023; Grünig et al., 2011; Lukács et al., 2025). The dual functionality of these isolates as plant growth-promoting endophytes and potential polymer degraders suggests that they could be harnessed in integrated mycoremediation strategies. By simultaneously supporting mangrove resilience and contributing to the breakdown of synthetic polymers, endophytic fungi offer a sustainable approach to mitigating microplastic pollution in coastal ecosystems. Importantly, this study provides preliminary evidence of surface modification of PCL microplastics by culturable fungi associated with *Rhizophora apiculata* roots, thereby contributing initial insights into their potential role in polymer biodeterioration. Further investigations incorporating quantitative mass-loss measurements, appropriate experimental controls, molecular identification of the fungal isolates, and enzymatic analyses are required to confirm their preliminary spectral differentiation and evaluate their potential application in environmental biotechnology.

The interpretation of the present findings is based on the primary data generated within the scope of this exploratory study. To improve transparency, the available primary data has been compiled in the Supplementary File, including the FTIR spectra obtained from four technical replications for each analyzed sample, the corresponding

preprocessed spectral data, the relative intensities of the principal FTIR bands, and the data matrix used for principal component analysis. Relevant sequencing quality information and fungal community data generated through ITS amplicon sequencing are also provided where available.

Nevertheless, the dataset remains limited by the exploratory experimental design. The four FTIR measurements represent technical replications of the same experimental sample and therefore cannot be used to estimate biological variability. Moreover, quantitative PCL mass loss data, molecular weight measurements, degradation product measurements, and molecular identification data for fungal isolates S2–S6 were not generated in the present study. These data are accordingly not included in the Supplementary File and should not be inferred from the qualitative FTIR findings.

Accordingly, the available primary data supports only a qualitative and comparative assessment of spectral differences between untreated and fungal exposed PCL associated samples. They do not provide conclusive evidence of PCL depolymerization, assimilation, mineralization, or biodegradation. A more complete primary dataset based on independent biological replicates, appropriate experimental controls, molecular identification of the isolates, and direct quantitative measurements of polymer degradation will be required in subsequent studies.

This exploratory study has several limitations. First, PCL mass loss was not quantified, and neither polymer molecular weight nor potential degradation products were analyzed. Consequently, the observed FTIR spectral differences cannot establish whether polymer mass loss or depolymerization occurred. Second, the PCL exposure experiment lacked an uninoculated or abiotic control, an inactivated-biomass control, and a positive biodegradation control. Without these controls, the spectral differences cannot be attributed specifically to fungal activity or distinguished reliably from changes arising from the medium, incubation conditions, or sample handling. Third, molecular identification of the cultured fungal isolates S2–S6 was not established in the data presented here, and no direct sequence comparison was performed between these isolates and the ITS amplicon sequence variants (ASVs). Therefore, the isolates cannot be assigned to specific taxa or linked to particular ASVs on the basis of the present results. Finally, ITS amplicon sequencing was performed on a single pooled biological sample comprising root tissue from five trees within one quadrat. This design does not provide independent biological replication or allow variation among trees or sampling sites to be assessed. Taken together, these limitations restrict the findings to a site-specific fungal DNA profile and preliminary PCL-associated spectral changes following exposure to cultured fungal isolates. Controlled experiments incorporating biological replicates, appropriate controls, quantitative mass-loss measurements, molecular-weight analysis, and degradation-product analysis are needed to test the proposed fungal contribution to polymer modification.

CONCLUSIONS

ITS amplicon sequencing detected 48 fungal taxa in a single pooled sample of surface-sterilized *Rhizophora apiculata* roots. The detected assemblage showed moderate diversity and relatively low dominance. Ascomycota predominated, with taxa assigned to *Sordariomycetes* and *Capnodiales*, while *Aspergillus* was the most abundant genus. Other detected genera included *Candida, Cladosporium*, and *Meyerozyma*. These findings provide a site-specific profile of fungal DNA in the pooled root material. They do not establish the viability or endophytic status of the detected taxa. A correspondence between the detected ITS amplicon sequence variants and cultured isolates fungal isolates S2–S6 was not established.

FTIR analysis identified differences in PCL associated spectral features after the samples were exposed to the cultured isolates, including differences in regions associated with carbonyl and ester groups. The differences observed for S5 and S6 were more pronounced than those observed for fungal isolates S2–S4. PCA of four technical replicates per group showed preliminary spectral differentiation among the analyzed samples. These findings document spectral differences in the tested PCL samples but do not demonstrate polymer mass loss, depolymerization, assimilation, mineralization, or biodegradation. Because adequate experimental controls were not included, the differences cannot be attributed specifically to fungal activity. Technical FTIR replicates do not establish reproducibility across independent biological experiments.

Further studies should repeat the PCL exposure experiment with independent biological replicates and appropriate controls, including an uninoculated

or abiotic control, an inactivated-biomass control, and a positive biodegradation control. Quantitative measurements of PCL mass loss, molecular weight and molecular-weight distribution, and degradation products are required to determine the nature and extent of any polymer alteration. Molecular identification of fungal isolates S2–S6 and direct comparison of their sequences with representative ASV sequences are also needed to investigate whether the cultured isolates correspond to taxa detected in the pooled root sample. Only after these steps can subsequent studies evaluate the processes involved and the potential relevance of the isolates to microplastic mycoremediation.

This study does not establish PCL biodegradation. The observed spectral changes are preliminary; the accompanying raw FTIR and ITS amplicon sequencing data are provided to support their evaluation. Determining whether fungal activity causes PCL biodegradation will require quantitative mass-loss measurements, molecular-weight and degradation-product analyses, and appropriately controlled experiments.

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