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Opinion

Melanized root-associated fungi: key players in plant–soil systems

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Melanized root-associated fungi are a group of fungi that produce melanized structures and form root associations, including different mycorrhizal and endophytic symbioses with plants. They are pervasive across terrestrial ecosystems and play an important role in the prevailing soil carbon (C) and nutrient cycling syndromes through direct and indirect mechanisms, where they may strongly modulate plant–microbe interactions and structure root and soil microbiomes. Furthermore, melanized root-associated fungi can confer on plants an enhanced ability to tolerate abiotic and biotic stressors such as drought, extreme temperatures, heavy metals, and pathogen attacks. We propose that melanized root-associated fungi are a cohesive and ecologically relevant grouping that can be an indicator of plant–soil system functioning, and considering them will advance research on plant–soil interactions.

Highlights

Melanin is an important molecule in fungi that confers stress tolerance and enhanced interactive capacities.

Melanized root-associated fungi produce highly-melanized hyphae and are widespread in terrestrial ecosystems where they may profoundly influence carbon and nutrient cycling regimes, and the stress tolerance of their hosts.

Considering melanized root-associated fungi as an ecological grouping may advance our understanding of plant–soil system functioning.

Root-associated fungi in plant–soil systems

The quest to find important belowground traits that explain ecological and biogeochemical processes across terrestrial ecosystems is constantly evolving and being refined. Biotic interactions between a multitude of organisms in plant–soil systems drive ecosystem processes such as C and nutrient cycling in constant feedback with climatic and **edaphic conditions** (see Glossary). Plants rely heavily on relationships with other organisms; these relationships span a spectrum of **mutualistic**, **commensal**, **competitive**, and **parasitic interactions**. Arguably, the most widespread, integrated, and biologically important interactions in plant–soil systems are between plants and root-associated fungi at the root–soil interface. At this interface most plants form one of several to multiple types of mycorrhizal associations, including **arbuscular mycorrhizal** (**AM**), **ectomycorrhizal** (**EcM**), **ericoid mycorrhizal** (**ErM**), **orchid mycorrhizal** (**OrM**), and mycorrhizal-like **symbioses** in bryophytes [1–3]. Mycorrhizal symbioses play a central role in the cycling of C and nutrients, as well as influencing population and community dynamics and the health and productivity of plants [4–10]. In addition to mycorrhizal fungi, other common, widespread, and often overlooked root-associated fungi may play an important role in plant–soil systems, such as **dark septate endophytes** (**DSEs**) [11,12]. DSEs are a functionally heterogeneous and taxonomically ambiguous group of fungi that extensively colonize living plant roots with melanized **septate hyphae** and sometimes **microsclerotia**, where they potentially act as mutualists, parasites, and latent saprotrophs [11,13,14]. Certain vital traits can be shared between DSEs and mycorrhizal fungi, namely the ability to form root associations and to produce and accumulate **melanin**. Here we propose that all fungi that exhibit these two traits designate the ecological grouping of melanized root-associated fungi (MRF) (Box 1), which appear to greatly influence ecosystem processes in plant–soil systems.

Focusing on ecologically important shared traits rather than differences between guilds of root-associated fungi may facilitate new mechanistic insights into their role in ecosystem processes.

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Box 1. MRF: who are they, and what are their traits?

We suggest that the grouping of MRF includes any fungus that forms highly melanized – brown to black pigmented – structures and a root association with plants, including mycorrhizal and endophytic associations (see Figure 1 in main text). Fungi from all phyla engaging in root associations are theoretically included within the MRF. The ability to produce melanin is phylogenetically widespread, although this capacity varies to some degree between phyla [15]. That is to say, a continuum of melanin production exists, and the genetic capacity for melanin production alone does not designate MRF membership; instead, the phenotypic expression of highly melanized structures as root-associated fungi is their defining feature, meaning that MRF are a dynamic yet observable grouping. Species of the phylum Ascomycota are the most well studied MRF; whether this is a bias or ascomycetes are indeed the dominant MRF taxa is to be resolved. Some of these MRF taxa form an EcM association, such as the widespread, ubiquitous, and cryptic *Cenococcum* complex [16], while many others exhibit much more flexible lifestyles, structurally and functionally blurring the boundaries between EcM, ErM, DSEs, other endophytes, and saprotrophs, such as members of *Hyaloscypha s. str.* [17] and the *Phialocephala fortinii-Acephala applanata* species complex (PAC) [18–20]. These MRF appear to commonly co-colonize woody roots across plants with different mycorrhizal types, forming functionally and structurally different associations depending on host species [20–24]. While EcM and ErM associations are generally limited to forests and shrublands, DSE associations extend from forests and shrublands into croplands, grasslands, drylands, and coastal saline ecosystems much like AM associations [11,25,26]. In grasslands – important ecosystems which cover around 40% of global terrestrial surface area and over two-thirds of global agricultural area [27] – MRF taxa such as species of *Periconia* and *Darksidea* that form DSE associations are some of the most dominant and widespread core root-associated fungal community members [28–31]. Thus, MRF are pervasive and potentially impactful globally across plant phylogenies and terrestrial ecosystems.

Combined with their production of highly melanized structures, the ability of MRF to associate intimately with tree roots as mycorrhizal fungi or asymptomatic endophytes represents one of their key traits (Figure 1). Symbiotically associating with plant roots confers many ecological advantages compared to a lifestyle as a pathogen or a purely free-living soil fungus, including a niche habitat and receiving a potentially steady supply of plant-derived C or other plant by-products without being perceived as antagonistic by the plant's defense response [14].

Thus, we bring attention to the presence and prevalence of MRF in plant–soil systems as being an important grouping beyond mycorrhizal and vegetation types as an indicator of plant–soil interactions and biogeochemical cycling across terrestrial ecosystems (Figure 1). We present a growing

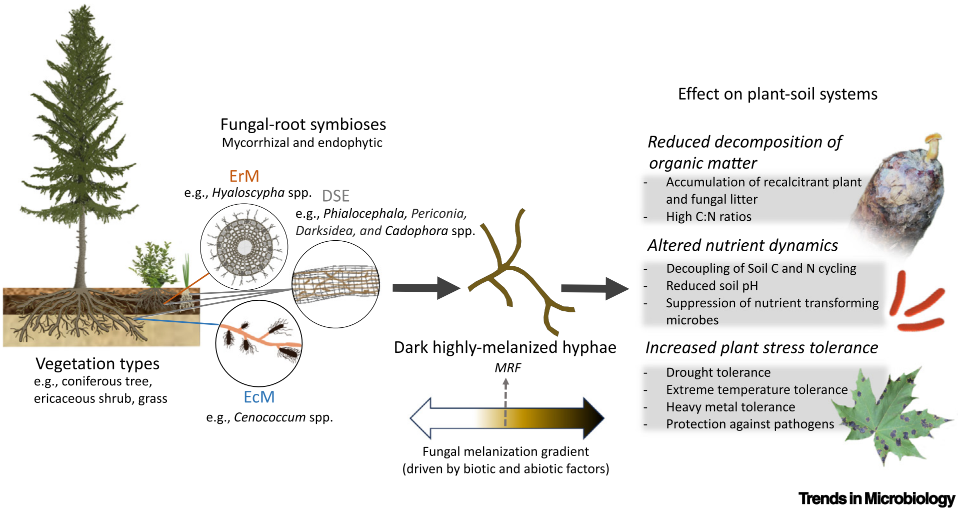

Figure 1. Melanized root-associated fungi (MRF) appear to greatly influence ecosystem processes in plant–soil systems. Symbioses with MRF occur across vegetation types and ecosystems in the form of mycorrhizal symbioses such as ectomycorrhizal (EcM) and ericoid mycorrhizal (ErM) associations with woody plants, and endophytic associations with dark septate endophytes (DSEs), which can encompass woody and non-woody plants such as grasses and herbs. Aside from forming symbiotic root associations, the defining trait of MRF is their ability to produce highly melanized hyphae, which occurs across a gradient that is likely driven by a range of both biotic and abiotic factors. Melanization contributes to the effect of MRF on decomposition and carbon (C) cycling, altered nutrient conditions with decoupled soil C and nitrogen (N) stocks, and reduced activity of nutrient-transforming microbes, and increased plant abiotic and biotic stress tolerance. Furthermore, melanin may even facilitate mutualisms between MRF and their hosts. Elements of this figure have been adapted from [12] under CC BY 4.0 https://creativecommons.org/licenses/by/4.0/.

Glossary

Accessory chromosomes: strain-specific chromosomes that exist in addition to the core chromosomes of a species.
Arbuscular mycorrhizal (AM) symbiosis: a specific symbiosis between plants and members of the fungal phylum Glomeromycota, which involves the formation of arbuscules by the fungus within the root cells of the host plant, and involves the exchange of carbon and nutrients between the fungus and plant.
Carbohydrate-active enzymes (CAZymes): enzymes involved in the synthesis, metabolism, and recognition of complex carbohydrates.
Commensal interactions: when the outcome of the interaction between two different organisms is beneficial for only one yet does not harm the other.
Competitive interactions: when different organisms require the same limited resource such that their interaction may reduce the fitness of the organisms involved.
Dark septate endophytes (DSEs): a group of fungi that colonize root cells with melanized septate hyphae and live as asymptomatic endophytes.
Dihydroxynaphthalene (DHN): an organic compound and precursor to various other molecules.
Ectomycorrhizal (EcM) symbiosis: a specific symbiosis between many different taxa of fungi and mainly woody plants involving the formation of a hartig net by the fungus between root cells and a hyphal mantle ensheathing shortened root tips; it involves the exchange of carbon and nutrients between the fungus and plant.
Edaphic conditions: soil properties, such as soil type and structure, texture, pH, salinity, moisture, temperature, carbon and nutrient content.
Ericoid mycorrhizal (ErM) symbiosis: a specific symbiosis between several fungal taxa and plants from the family Ericaceae, which involves the formation of hyphal coils by the fungus within the hosts root cells, and involves the exchange of carbon and nutrients between the fungus and plant.
Halophyte: a salt-tolerant plant.
Melanin: a group of darkly pigmented biomolecules.
Microsclerotia: small, compact hyphal aggregates formed by fungi that may act as a storage reserve for resources during stressful conditions.

body of evidence showing that, through both direct and indirect mechanisms mainly relating to the production of melanin (Box 2), MRF appear to confer enhanced stress tolerance in plants, shape belowground communities, and influence patterns of C and nutrient cycling as both living organisms and through the legacy of their **necromass**.

MRF may shape patterns of decomposition and associated microbial communities

A growing body of evidence indicates a central role for MRF in soil C cycling through their highly melanized biomass or necromass, leading to the accumulation of soil organic matter, and their capacity to participate in decomposition through the expanded **carbohydrate-active enzyme** (**CAZyme**) arsenal of many MRF taxa. Melanized hyphae have long been posited to be relatively resistant to degradation [42] and were suggested to contribute to soil organic matter formation [43]. In line with this, and building on early observations that abundant structures of the EcM fungus *Cenococcum geophilum* may be persistent after death and contribute to soil organic matter accumulation [44,45], it was shown that the persistence time (multiple years) of *C. geophilum* ectomycorrhizae was up to ten times greater than that of the other studied EcM species [46]. This finding indicates that *C. geophilum* ectomycorrhizae are highly recalcitrant and may profoundly influence soil biogeochemical cycling. A follow-up study linked the persistence or slower decomposition of *C. geophilum* necromass to melanin production, that is, that melanized fungal necromass decomposes more slowly than non-melanized necromass [47]. Furthermore, a long-term litter bag incubation study showed that the initial melanin fraction of EcM and ErM necromass remains intact after 2 years, while other cell components are decomposed within weeks [48]. Similarly, C accumulation as humus in boreal forest soils has been linked with the successional shift to the dominance of stress-tolerant melanized ErM ascomycetes with biomass resistant to decomposition and a decline of EcM basidiomycetes with organic nitrogen (N) mobilization capabilities [22]. The role of melanin in shaping decomposition patterns and associated microbial communities on the necromass of dark root-associated ascomycetes was further expanded by evidence showing that melanized necromass of the ErM/EcM fungus *Hyaloscypha bicolor* (syn. *Meliniomyces bicolor*) decomposed slower than non-melanized necromass and corresponded to a strong restructuring of microbial communities, more so for fungi than bacteria, and with a stronger successional pattern in fungal communities on non-melanized necromass with time, that is, more species turnover [49]. This was followed up by a study tracing labeled C and N stable isotopes on low- and high-melanin-

Box 2. Fungal melanin in root-associated fungi: a brief overview

Melanin production is a key ecological trait in fungi [32]. Melanins are dark brown polymeric pigments that play diverse roles and functions across various organism groups from all kingdoms of life, displaying a variety of forms and structural complexity with unique properties [33]. The functions of melanin in fungi, which typically accumulates in and around the cell wall, range from photo, mechanical, and chemical protection to metal binding, energy harvesting, cell development, antioxidant activity, anti-desiccation, and thermoregulation (reviewed in [34]). Melanin is a complex and energetically expensive molecule to produce and comes at the expense of growth in fungi. Thus, it is generally associated with an **oligotrophic lifestyle** strategy and may confer a growth advantage in harsh conditions [15,35]. This has been shown in two distinct populations of the DSE-forming fungus *Laburnicola rhizohalophila* isolated from a **halophyte** host, where the population associated with strong positive selection for a highly divergent genomic island that included additional genes involved in melanin biosynthesis had stronger growth faced with a salinity treatment and accumulated more melanin compared to the population lacking these additional genes [36]. Fungal melanin has also received attention as a **virulence factor** and is associated with opportunistic **pathogenesis** [37–39]. It has also been shown that increased virulence by pathogenic fungi may be associated with a more rapid production of melanin but not total melanin production [40], suggesting that there exists fast versus slow melanization strategies within fungi related to the expression of different lifestyles. While the role of melanin in nonpathogenic symbiotic associations is less clear, it appears to confer stress tolerance in interacting partners [34], and may even facilitate the transition from a parasitic lifestyle to a more mutualistic one in root-associated fungi. For example, it was demonstrated that the loss of the **accessory chromosome** in the poplar root-associated fungus *Stagonosporopsis rhizophilae* was associated with a shift from a parasitic lifestyle to a host-beneficial DSE lifestyle; in turn, this lifestyle shift was associated with enhanced melanization, indicating that melanin may play an important role in establishing symbioses [41]. The role of melanin in the establishment and/or maintenance of root symbioses requires further attention.

Mutualistic interactions: when two different organisms interact in a way that is mutually beneficial for both.
Necromass: dead organic matter.
Oligotrophic lifestyle: a lifestyle in which an organism can live and thrive in low nutrient conditions.
Orchid mycorrhizal (OrM) symbiosis: a specific symbiosis between several fungal taxa and plants from the family Orchidaceae, which plays a role in seed germination and growth and involves different stages such the protocorm during which the plant requires carbon and nutrients from the fungus, and an adult stage that may involve the exchange of carbon from the plant for nutrients from the fungus via fungal peletons in the root cortex.
Parasitic interactions: when an organism lives on or in another organism and causes it harm.
Pathogenesis: the process in which a disease develops.
Phytohormone: plant hormones that regulate growth, development, reproduction, and death.
Polyketide synthases: a family of enzyme complexes that produce polyketides, a large class of secondary metabolites.
Polyphyletic group: a group of organisms with mixed evolutionary origin.
Secondary metabolites: compounds produced by an organism which are not directly involved in the normal growth, development, or reproduction of that organism.
Septate hyphae: hyphae with defined cellular compartments separated by septa.
Symbiosis: any close, long-term biological interaction between different organisms.
Transposable elements: nucleic acid sequences in DNA that can change their position within a genome.
Virulence factors: structures, molecules, and regulatory systems that enable pathogens to successfully colonize, infect, and live inside their hosts.

containing *H. bicolor* necromass, which found once again that melanized necromass slowed decomposition and also reduced the uptake of C and N resources by a taxonomically and functionally diverse community of bacteria and fungi [50].

Compared with melanized ErM and EcM fungi, the role of DSEs in soil C cycling has not been well explored, although it is plausible that, through the same mechanisms discussed above, they may also play an important role. Some evidence suggests that DSE colonization on a diverse range of plant species is positively correlated with increasing soil organic matter and C:N content [12,51,52]. Outside of forest ecosystems, in agricultural systems and grasslands where ErM and EcM fungi are generally absent, DSE fungi may play a more central role in the prevailing patterns of soil C cycling. Overall, the role of fungal melanin and root-associated fungi in soil C dynamics is not trivial considering that melanized fungal hyphae form a major component of total fungal biomass in soil across a diverse range of ecosystem types [53].

MRF may shape soil nutrient cycling regimes

MRF appear to thrive in soils with low nutrient availability, which they may reinforce through the turnover of their recalcitrant melanized necromass, their potential to access organically bound nutrients through selective decomposition, and their potential for adverse interactions with nutrient-transforming soil microbes. This ability to thrive in nutrient-poor conditions is exemplified by ErM fungi and their ericaceous hosts, which may rely on ErM fungi for access to organically bound nutrients – which is the main nutrient form present, in exchange for photosynthetically fixed C [3,54,55]. These ErM associations may reinforce nutrient-poor conditions by promoting the accumulation of recalcitrant plant litter and fungal necromass and by hampering the activity of certain fungal saprotrophs [56]. When nutrients become enriched in more available forms, it is inferred that plants may shift C allocation away from ErM fungi [57,58]. Ericaceous roots can also be extensively colonized by DSE fungi co-occurring with ErM fungi and forming a morphological continuum [59], and both ErM and DSE fungi appear to have the capacity to decompose cellulose and hemicellulose, yet DSE fungi appear to lack the ability to decompose lignin [60,61]. When basidiomycetes with ligninolytic white-rot capabilities are less abundant, both ErM and DSE fungi, and MRF in general, may reinforce low-nutrient conditions through a closed organic nutrient cycle where soil organic matter accumulates and soil C and N cycling becomes decoupled [56,62]. Root colonization rates by DSE fungi have been negatively associated with the ratio of bacteria to fungi and the diversity and relative abundances of N and phosphorus (P) cycling genes in soil and root microbiomes of widespread tree species across Europe [12], suggesting that MRF may negatively interact with and suppress nutrient-transforming microbes and reinforce slow nutrient cycling and nutrient-poor conditions under which they are perhaps more valuable to plant nutrient acquisition strategies. In line with this, DSE colonization may replace AM colonization when soil pH and N availability decrease and soil organic matter increases [63–65].The value of MRF to plant nutrient acquisition under nutrient-poor conditions may also be related to the accumulation and storage of various cations in the melanized cell wall matrix, which may act as an important nutrient source [66].

The role of *Cenococcum* EcM associations in nutrient decoupling mechanisms, such as those involving ErM and DSE fungi, is less clear; the presence of ErM fungi and ericaceous shrubs can be negatively associated with the relative abundance of *C. geophilum* [56], yet *C. geophilum* can intracellularly colonize ericaceous roots [21], and root-associated ascomycetes, including ErM and DSE-forming species, are common inhabitants of root tips colonized by *Cenococcum* [67], as well as *Cenococcum* sclerotia [68], indicating that melanized fungi may promote other melanized fungi. Furthermore, root colonization by *Cenococcum* is positively correlated with increasing soil organic matter content and the N:P ratio [69], indicating a preference for an organic nutrient cycle with nutrient-limiting conditions, despite being depleted in the enzymatic capacity for decomposition [70]. This preference for low-nutrient conditions is further shown through a long-term forest liming treatment, where an increased soil pH led to a steep decline in the relative abundance of *Cenococcum*, which was previously the dominant EcM fungal guild [71].

While MRF may thrive in soils with low nutrient availability, they may also be relatively insensitive to changes in nutrient availability, including nutrient addition, compared to other root-associated fungi, owing to shifts in the C allocation patterns of host plants. This has at least been shown in nutrient-limited versus nutrient-enriched boreal spruce forests, where trees appeared to shift C allocation from EcM basidiomycetes with high enzymatic capacity for decomposition to more investment in defense processes and greater functional coordination with melanized root-associated ascomycetes, which were relatively insensitive to nutrient enrichment [72]. This hints at the potential buffering role of MRF in ecosystems under nutrient enrichment and also at their importance in plant stress response strategies.

Biotic and abiotic stress tolerance: the main benefit of associating with MRF?

While root-symbiotic fungi are generally considered from a plant nutrient acquisition perspective, one of the main benefits of plants associating with MRF may be a conferred resistance to abiotic and biotic stress (Figure 1). Melanin production appears to be an essential trait for conferring drought tolerance due to its anti-desiccation properties [34,73]. In line with this, ErM, DSE, and *Cenococcum* EcM associations are generally considered to be drought tolerant and abundant in water-stressed habitats, where they can survive for long periods, withstand desiccation, and contribute to the drought tolerance of their hosts [73–78]. In addition, MRF have been shown to provide their hosts with enhanced tolerance to extreme temperatures [79,80] and heavy metals [26,81,82], likely in part due to the production of melanin [34].

Protection against pathogens and disease may also be one of the central benefits driving associations between plants and MRF, with evidence for DSE [12,26,83–85], ErM [86] and *Cenococcum* EcM associations in this respect [87], which have also been shown to have higher root colonization rates in forests infected by *Phytophthora* [88]. The mechanisms of pathogen protection probably include a combination of physical protection [87], direct antagonisms via the secretion of **secondary metabolites** [89], and by locking up nutrients [66]. Several DSE and ErM fungi appear to have genomes enriched with genes encoding for secondary metabolites [60,90,91], as does *Cenococcum* compared to many other EcM fungi [70]. Interestingly, certain MRF appear to have genomic traits similar to those of several plant pathogens, including expanded genomes due to the proliferation of **transposable elements** [70,92,93], and they also produce melanin, which can act as a fungal virulence factor to avoid a plant's defense response [37]. These characteristics suggest that MRF may generally outcompete fungal pathogens in colonizing roots using mechanisms similar to those of pathogens, yet with the added benefit of forming symbioses with perceived benefits for the host plants. Finally, MRF may be less palatable to insects; this has been shown at least for *Cenococcum* EcM structures, which were a less preferred food source for Collembola compared to some other studied EcM species. When Collembola are at high population densities in soil they may disrupt mycorrhizal hyphal networks and plant nutrient acquisition; under such biotic conditions, symbioses with *Cenococcum* may be resistant to Collembola feeding and play a more important role in plant nutrient acquisition [94].

MRF research: looking forward

Research on MRF has greatly advanced in recent years, yet there are critical gaps, particularly in our knowledge of non-mycorrhizal root endophytic fungi such as DSEs. Our ability to disentangle the elusive nature of MRF has been hindered by the reliance on, or difficulties in, culturing them, their **polyphyletic** and cryptic nature making molecular characterization difficult, combined with the general limitations of available databases for identification of fungi and the annotation of their genes, as well as their seemingly transient and flexible lifestyles. Yet a combination of existing methods can be used to further investigate and characterize MRF in ecological studies; these include quantification of root colonization rates of melanized mycorrhizal and endophytic structures, together with culturing and molecular identification and quantification of taxa and genes enriched in heavily colonized roots from controlled experiments and field studies. Concurrently, melanin could be quantified and characterized in roots, fungal tissue, and soil using a variety and combination of spectroscopic, chromatographic, fluorescence, and microscopy techniques, and additionally via pyrolysis and elemental analysis [95]. However, this is difficult in soil due to its complex nature. In addition, we identify some significant topics of investigation that will improve our understanding of the ecology and functioning of MRF (see Outstanding questions).

Ecological patterns and environmental preferences

Although MRF appear to be widespread, they are likely to show strong biogeographic patterns due to their symbiotic associations with plants. Nevertheless, there needs to be more information about the biogeography, community dynamics, root colonization rates, and co-occurrence patterns across time and space of MRF. These questions can be partly answered for known MRF taxa, such as species of *Cenococcum*, *Phialocephala*, *Hyaloscypha*, *Cadophora*, *Periconia*, and *Darksidea* using existing datasets and repositories. An example would be to use a fungal metabarcoding database such as GlobalFungi [96] to study the biogeographic patterns and underlying environmental preferences of such taxa in more detail. Another possibility is to perform metanalyses of root colonization patterns of MRF such as DSEs and ErM similarly to what has been done for AM and EcM colonization [97].

Plant–microbe and microbe–microbe interactions

Several studies show that MRF strongly influence decomposer communities through their melanized necromass, that is, the fungal necrobiome [98]. Yet how they directly interact with other microbes, particularly nonpathogenic microbes, at the root–soil interface remains largely unknown, including the role of melanization in these interactions. Melanin has been shown to have antimicrobial activity, and many melanin precursors are broad-spectrum antibiotics [99]. This suggests that MRF may profoundly influence the activity of microbes in their sphere of influence at the root–soil interface, which has been hinted at in a recent study showing that DSE colonization is an indicator of the structure and function of root and soil microbiomes [12]. Furthermore, ascomycetous MRF likely produce melanin primarily via the **dihydroxynaphthalene** (**DHN**) pathway [15,38], which is catalyzed largely by **polyketide synthases** [100]. Polyketide synthases are multifunctional enzymes involved in the production of many secondary metabolites [101]. Thus, the enhanced melanization of certain MRF fungi is possibly also related to the enrichment of other secondary metabolites [91,102], which are not only an important determinant of microbial communities but also play a central role in interactions with plants and other organisms, and overall plant–soil interactions. This may explain the potential for MRF to parasitize plant parasitic nematodes [103], suppress plant pathogenic microbes [85,89], herbivorous insects [104], modulate root exudates, **phytohormone** production, and secondary metabolites [105,106]. Future studies should aim to further distinguish how an increased prevalence of MRF may shape distinct root and soil microbiomes, including the composition of key nutrient cycling microbes and their functional genes. Unraveling key interactions between MRF, plants, and associated microbes will improve our understanding of these enigmatic fungi.

Melanin production within ecologically relevant conditions

The potential conditions or factors that influence the melanization of MRF within complex ecological communities and heterogeneous environments remain to be uncovered – for example, how persistent are melanized phenotypes in fungal communities over time, and how sensitive is this to environmental conditions and biotic interactions? Within the human opportunistic pathogen *Cryptococcus neoformans*, genetic factors and genotype–environment interactions were found to explain most variation in melanin production; while environment alone explained little variation [107], it is probable that MRF exhibit similar controls over melanin production. Whereas, within pure cultures, melanin production of the MRF *H. bicolor* was successfully manipulated by altering the level of submersion in a liquid growing medium, which was suggested to be due to differences in oxygen exposure [49]. How the effect of oxygen exposure on melanin production translates into the ecologically relevant setting of a root **symbiosis** within the soil environment remains to be explored. Some related questions are: do MRF become more melanized across oxic–annoxic gradients such as in coastal wetland communities, and how do changes in soil water immersion patterns influence melanization? The role of plant–symbiont interactions in influencing melanin production needs to be further explored; as melanin is metabolically expensive to produce, it is likely that access to labile C from plants is a major constraining factor. Additionally, interactions with other microbes in the soil may be an important determinant of melanization. This is supported by a study showing that genes encoding polyketide synthases – which modulate melanin biosynthesis – in the fungus *Aspergillus nidulans* may remain inactive if not triggered by intimate interactions with bacteria [108].

Applications in forestry and agriculture

Given the important yet underappreciated contribution of MRF to fungal necromass, they may offer a range of applications for improving soil C storage and counteracting nutrient enrichment, for example, by enhancing the C:N ratio [50]. Owing to their potential capacity to suppress plant pathogens, MRF may be promoted or managed to improve plant health [109]; this could be through the development and application of MRF bioinoculants. They could also possibly be used to modulate broader plant–microbiome interactions, given their potentially enhanced capacity for producing secondary metabolites [60,90,91]. However, this requires further efforts to culture and manipulate MRF in controlled experiments and field-based trials, which stands to further our understanding of the biology and ecophysiology of MRF and the mechanisms through which they influence ecological processes.

Environmental change response of plant–soil systems and buffering capacity

In light of the traits of MRF we have explored here, they are likely to be key players in the response of plant–soil systems to environmental change. Therefore, there is an important need to further explore whether MRF can contribute to the persistence of microbial and plant communities and their functioning in response to environmental perturbations, that is, by acting as a buffer.

Concluding remarks

Here, we have introduced and given a brief overview of MRF as an ecological grouping and have shed light on their potentially important role in plant–soil interactions. This grouping unifies fungi from different lifestyle guilds, such as mycorrhizal fungi and endophytes, that would otherwise be considered to have potentially contrasting functions despite sharing similar important traits. We consider the defining traits of MRF to include their ability to form root symbioses and their production of highly melanized structures. Additionally, they appear to have a seemingly expanded capacity for secondary metabolite production. Such traits may confer on MRF a central role in plant–soil interactions, including shaping patterns of decomposition and soil C and N cycling, as well as providing plants with enhanced abiotic and biotic stress tolerance. This suggests that MRF may have the capacity to buffer plant–soil systems against environmental changes such as nutrient enrichment, climate change, and pest and disease outbreaks. Most of the evidence presented here is from boreal and temperate northern forests. As the distribution and

Outstanding questions

How does the biogeography, community dynamics, root colonization rates, and co-occurrence patterns of MRF vary across time and space?

How do MRF mediate plant–microbe and microbe–microbe interactions, and shape distinct root and soil microbiomes?

What are the main factors that influence melanin production in MRF within complex ecological communities and heterogeneous environments?

What is the role of melanin in the formation and maintenance of root symbioses?

What are the potential applications for MRF in forestry and agriculture?

Can MRF buffer plant–soil systems under environmental change?

abundance of MRF extend to tropical and southern temperate regions, as well as dryland and coastal ecosystems, the role of these fungi remains to be further resolved, including in non-woody ecosystems. MRF can be quantified by observing mycorrhizal and endophytic structures on and in roots via microscopy, and they may be molecularly characterized using high-throughput sequencing techniques, which is limited not only by existing databases but also by the cryptic nature of these fungi. Furthermore, melanin content can be measured and characterized by various methods, although this is difficult in soil due to its complexity. Thus, there should be an immediate research priority to improve the identification and classification of MRF, and to study their ecology and quantify their production of melanin in complex communities and varied environments. The presence and prevalence of MRF in plant–soil systems appears to be an important ecosystem trait in addition to mycorrhizal and vegetation types, that may be used as an indicator of plant–soil interactions, biogeochemical processes, and ecosystem health.

Author contributions

T.N. and M.B. conceived the idea of the manuscript together, T.N. led the writing of the manuscript and subsequent revisions with contributions from M.B.

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Declaration of interests

No interests are declared.

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