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Diversity, detection and exploitation: linking soil fungi and plant disease

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Plant-associated fungi are incredibly diverse, comprising over a million species of mycorrhiza, endophytes, saprophytes and pathogens worldwide. This diverse fungal community is highly important for plant health. Many fungi are effective biocontrol agents that can kill or suppress fungal pathogens, with pathogen biocontrol found for both individual microorganisms and plant-associated fungal consortia. Meanwhile, increased plant community diversity aboveground corresponds to an increase in below-ground fungal community diversity, which contributes in turn to improved rhizosphere soil health and pathogen suppression. In this review, we discuss the role of fungal diversity in soil health and plant disease suppression and the various mechanisms by which mycorrhizal and endophytic fungi combat plant pathogenic fungi. We also discuss the array of diagnostic tools, both well-established and newly developed, which are revolutionising fungal pathogen detection and rhizosphere community analysis.

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Diversity in the fungal-plant microbiome

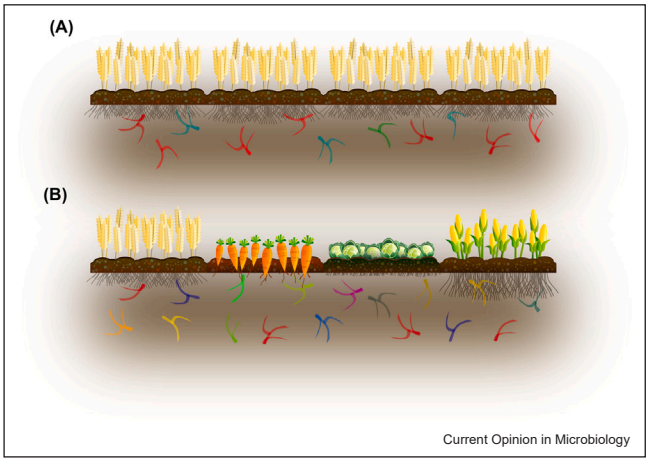
Diversity is vital for effective ecosystem functioning and has been a longstanding part of biodiversity and ecosystem research [•1,2]. The diversity of plant communities impacts other trophic levels, such as the above-ground community structure of herbivorous insects [3–5]. The link between plant community and soil microbial community diversity, however, is not yet fully understood [•1,6,••7] and is therefore increasingly the focus of current research [•1,5,8,9].

Soils harbour the greatest microbial biodiversity on Earth [5,10]. The plant biodiversity and ecosystem functioning hypothesis predicts that high plant community diversity promotes biotic and abiotic stress tolerance of its members [•1]. Recently, it has been shown that increases in plant community diversity are accompanied by increases in below-ground fungal community diversity [6,8,9,11]. As plants and soil microbes are connected through numerous interactions, such as carbon, nitrogen and micronutrient exchange, as well as mutual or antagonistic effects [6,8], increases in soil fungal community diversity therefore, according to the biodiversity and ecosystem functioning hypothesis, could also be beneficial for plant stress tolerance. However, stress-mediating qualities of soil fungal communities on plants, considering both the microbial and plant community diversity, have been rarely tested [11–13].

In a recent study comparing root-associated fungal taxa of plant monocultures and diverse plant communities, Mommer et al. show that in highly diverse plant communities, the number of detected pathogenic taxa was reduced by 57% [11]. This agrees well with the concept of the 'dilution effect', describing a reduction in the risk of infection with increasing community diversity [14]. Similar results, indicating beneficial, reciprocal feedbacks of above- and below-ground diversity, have been obtained studying arbuscular mycorrhiza fungi (AMF). Guzman et al. have shown that in an agricultural setting, higher crop diversity leads to more diverse and richer AMF communities in contrast to farm sites with low-diversity crops and high- intensity farming regimes (i.e. high nutrient input, low crop diversity and high tillage frequency (Figure 1, [••7]).

Both studies highlight the importance of understanding the connection between above-ground plant and below-ground soil fungal diversity. An increase in plant community diversity could promote more diverse soil fungal communities with fewer plant pathogens, or may enrich plant beneficial taxa, resulting in positive effects for plant health through multiple ecosystem services [••7,15]. The integration of biodiversity theory into soil fungal research could therefore be an important step

Figure 1



Variation in soil fungal community is linked with plant diversity **(a)** Monoculture field with low fungal community diversity in the soil. **(b)** Polyculture field with higher fungal community diversity (shown with different colours) in the soil. Higher fungal diversity is likely to positively influence nutrient uptake and stress tolerance in plants.

towards solutions for current agricultural problems, minimising the use of synthetic chemicals and intensive fertilisation while autonomously maintaining plant performance and health.

Mycorrhizal and endophytic fungi in plant protection

The overwhelming majority of plant–fungal interactions in the rhizosphere do not result in pathogenesis. As stated above, plant-associated fungi are highly diverse, with over a million mostly commensal or beneficial endophytic species estimated to exist alongside similarly large numbers of different saprophytes, AMF and other mycorrhiza. AMF are highly widespread symbionts of plants, colonising the roots of approximately 80% of all terrestrial plants. They are major components of soil microbial communities, contributing to healthy soils and providing ecosystem services for agriculture [••7,9,10]. AMF are involved in enhancing plant nutrition as well as plant resistance to biotic (e.g. pathogens) and abiotic stresses (e.g. drought, see below) and are keystone symbionts in agricultural soils [••7,9].

Fungal endophytes meanwhile are ubiquitous, are found in both wild and domesticated plants and have been identified in every plant tissue, with individual plants able to support numerous endophytic species simultaneously. These microbes may remain localised, leading to tissue-specific disease protection, or can spread systemically in herbaceous plants [16]. Many plant-associated fungi confer effective biocontrol and biostimulation characteristics on their hosts and unravelling how these protective microbes interact with fungal pathogens is important for a complete understanding of fungal-plant pathogenesis. Despite this, only a small fraction of the vast array of protective fungal-plant associations have been studied in depth [16,17].

The mechanisms of biocontrol and biostimulation used by plant-associated fungi have much in common with those found in soil and rhizosphere-dwelling bacteria [18]. Firstly, both endophytes and mycorrhizal species produce diverse secreted toxins and specialised metabolites that can directly antagonise microbial pathogens. Conventional extraction and isolation approaches have identified a vast array of different specialised metabolite classes from soil fungi [19]. While identifying roles for these molecules in phytopathogen antagonism can be challenging, there have been some successes. Analysis of the metabolically highly talented saprotrophic fungus *Hypoxylon fendleri* BCC32408 discovered 13 new drimane–phthalide molecules alongside previously identified compounds. Several of these showed strong antifungal effects against the phytopathogenic *Colletotrichum capsica* [20].

Competitive niche exclusion, where commensal microbes compete for key plant niches with pathogens, represents another important mechanism for fungal phytopathogen biocontrol. A fascinating example of this is given by Oliva and co-workers for *Diplodia sapinea* shoot blight in European pine [•21]. *D. sapinea* infection proceeds following a plant stress response to drought or hail, which increases the availability of nutrient-rich metabolites in the plant tissue. By comparing the fungal microbiomes of blight-affected and asymptomatic pines following a hailstorm, the authors identified a community of antagonistic endophytes that showed a strong negative association with *D. sapinea* in the asymptomatic trees. They proposed that rapid niche occupation is

critical for *D. sapinea* to cause disease, but competition with other endophytes for key metabolites could suppress the pathogen and prevent symptom onset [•21].

Endophytes can also enhance resistance to fungal pathogens by inducing systemic defence responses in their host plants. A major defensive strategy is the stimulation of cell-wall deposition to defend against hyphal penetration [22]. Plant-associated fungi have also been shown to prime plant immune defences through transcriptional reprogramming. For example, Tian and co-workers recently showed that the dicotyledon pathogen *Sclerotinia sclerotiorum* grows endophytically in diverse cereals, providing protection against *Fusarium* head blight, stripe rust and rice blast. *S. sclerotiorum* colonisation leads to the expression of genes for disease resistance and increased auxin levels in wheat [23]. *Piriformospora indica* pre-colonisation primes the tomato immune system, enabling the rapid activation of jasmonic acid and ethylene-mediated basal defences upon encountering the pathogen *Alternaria solani* [24]. Colonisation with a consortium of mycorrhiza has been shown to stimulate superoxide dismutase, peroxidase, polyphenol oxidase and catalase activities, and to reduce oxidative damage in olive roots [25] and peanut plants [22]. Fungal colonisation has also been linked to increased photosynthesis gene expression, cell lignification, callose deposition and phytoalexin accumulation [22,23].

Finally, plant-associated fungi can indirectly promote plant health, and hence pathogen defence by bolstering responses to abiotic stressors, including temperature, salinity, toxic compounds and heavy metal contamination, drought and flooding. For example, Su and co-workers recently defined the mechanism of *Piriformospora* alleviation of cadmium stress in tobacco and showed that *P. indica* colonisation systemically enhances Cd tolerance at physiological, cytological and protein levels [26]. Inoculation with *P. indica* markedly improved the Cd tolerance of tobacco, with increased Cd accumulation in the cortex, as opposed to the epidermis, of roots and decreased accumulation in leaves. *P. indica* colonisation apparently alters the subcellular repartition of Cd, with increased Cd accumulation in cell walls and reduced levels in membranes, organelles and soluble fractions of plant cells. *P. indica* further enhanced the content of antioxidant glutathione (GSH) and activity of peroxidase enzyme (POD) and the expression of photosynthesis-related proteins in response to tobacco Cd stress [26].

Dual-culture assays between the fungal endophytic community of a plant and its known pathogens have become a popular approach to identifying potential biocontrol fungal strains and secreted bioactive metabolites [27–29]. A nice example is given by the endophytic isolate *Hypoxylon rubiginosum*, which shows striking activities in dual culture with the Ash dieback pathogen *Hymenoscyphus fraxineus*. This activity was traced to the antifungal phomopsidin [••30], with the production of phomopsidin derivatives in response to *H. fraxineus* also detected in other closely related endophytic species such as *H. guilanense* [31]. Curiously, most of the pathogen-suppressive endophytes isolated in the initial study also caused disease symptoms in axenically cultivated Ash seedlings [••30], highlighting the importance of *in planta* verification when identifying effective biocontrol agents.

Pathogen biocontrol is frequently associated with communities of endophytic or mycorrhizal fungi, as well as with individual species. For example, 348 separate species were recently isolated as part of a screen for protective watermelon endophytes. Of these, *Trichoderma lentiforme* and *T. harzianum* showed significant pathogen inhibition in dual-culture assays, alongside a reduction of plant disease occurrence of around two-thirds. In addition, several other watermelon endophytes, including *Ceratobasidium*, *Epicoccum purpurascens*, *Aureobasidium pullulans* and *Bionectria ochroleuca*, also displayed significant biocontrol activity in vitro, using a combination of the mechanisms describe above. To differing extents, they produced and secreted antifungal-specialised metabolites, outcompeted pathogens for nutrients and space and engaged in parasitism with invading hyphae [32]. Similarly, pre-inoculation with autochthonous consortia of mycorrhiza has been shown to confer resistance to *Verticillium dahliae* infection in tomato [33] and olive plants [25].

Diagnostic tools for fungal communities

In order to understand the diversity of soil and plant-associated fungal communities and their impact on plant health, it is crucial to have effective diagnostics tools. This is particularly important to keep track of newly emerging pathogens. In the past decade, an array of technologies has become available to identify and analyse fungal communities. These tools have much-improved precision and sensitivity of detection and can provide near real-time information about the composition of fungal disease complexes [34,35].

Fungal species have traditionally been morphologically identified by culturing on selective media followed by microscopic examination and reinoculation. However, these methods are time-consuming and require expertise in fungal pathology. Moreover, many species are not amenable to culturing and isolation [36,37]. Other methods include immunology-based (antigen–antibody binding) diagnostics such as enzyme-linked immunosorbent assays, immunofluorescent staining and immunoblotting. Unfortunately, the detection of fungi with these assays has not been very effective due to high

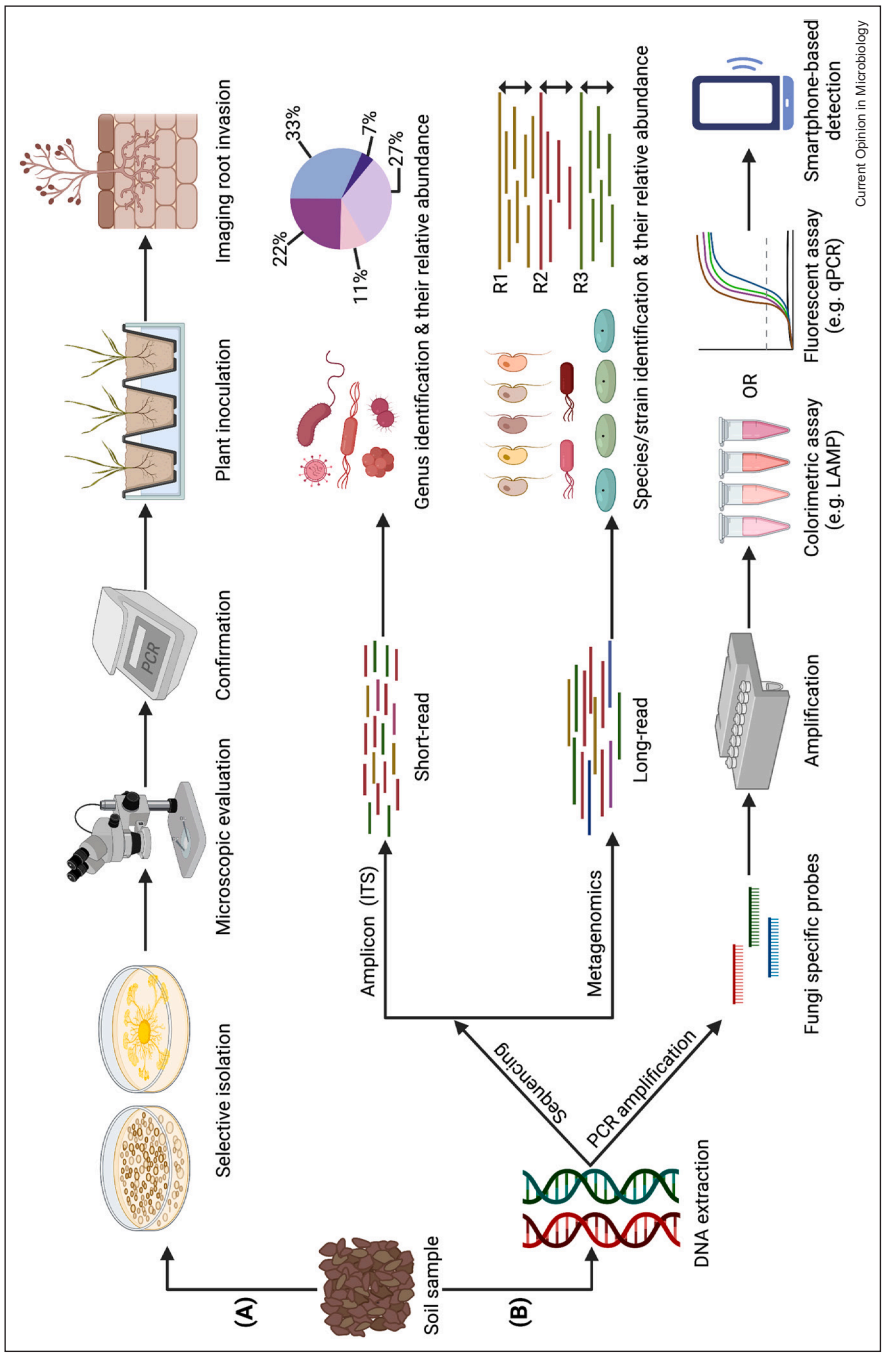


Figure 2

An overview of diagnostic tools for detecting fungal species from soil samples. **(a)** Soil culturing on selective media to isolate specific fungi species from which spores/mycelia are morphologically analysed followed by PCR confirmation. The isolated fungi can be further inoculated onto seedlings to study their colonisation and disease impacts. **(b)** DNA or nucleic acid-based diagnostics can be conducted either by amplicon/metagenomic sequencing, or by using specific primers for targeted PCR amplification (e.g. qPCR or LAMP) to identify and quantify species and specific strains of fungi.

inconsistency and phenotypic serological plasticity of fungi [38].

Molecular diagnostics based on nucleic acids are more popular and are either based on polymerase chain reaction (PCR) or high-throughput sequencing (HTS). These can be implemented on unculturable taxa and are both fast and sensitive. There are several recent examples of the successful use of PCR variants for cost-effective and targeted detection of fungal pathogens [34,39]. Increased sensitivity and specificity are provided by real-time quantitative polymerase chain reaction (qPCR), which provides both sequence information and quantification of a particular phytopathogenic fungi. qPCR assays have been widely developed for fungal and oomycete pathogens [40,41], for example, detection of the fungal pathogen *Cryphonectria parasitica* that causes disease in chestnut trees with a sensitivity of 2 fg of genomic DNA, equivalent to one spore of the pathogen [42]. Weighed against these advantages, qPCR requires specialised and costly instrumentation, which limits its application in the lab.

LAMP, or loop-mediated isothermal amplification, is more promising when it comes to developing point-of-care diagnostics. LAMP permits amplification of targeted nucleotide sequences at a constant temperature in a single tube and does not require any sophisticated instruments. Furthermore, the thermocycler is portable and can be linked to custom-designed, smartphone-compatible software for quantitative assay measurement and delivery of results [43]. These features make LAMP an attractive option for infield diagnostics. Examples of fungal pathogens detected using LAMP assays include *Uromyces betae* (sugar beet rust), *Fusarium circinatum* (causes pitch canker in pine and other conifers) and *Magnaporthe oryzae* (rice blast fungus) [44–46].

The major limitation of both PCR-based methods is that they are unsuitable to identify and study unknown species. This shortfall may be resolved by HTS methods, which fall into two categories. The first is short-read sequencing (Illumina) of small hypervariable regions in one or several genes, for example, internal transcribed spacer (ITS)1 and ITS2 regions in fungi using universal primers. This provides robust genus-level identification, but can lack precision for species identification [47].

Identification to species level is important for distinguishing closely related pathogenic fungi, which may show completely different behaviours on their host plant. A potential solution to this is to either amplify the complete ITS gene [48] or design species-specific primers against other parts of the genome. Examples of this include elongation factor 1-alpha to assess the diversity of *Fusarium* spp. [49] and *TEF-1 alpha* gene for detection and quantification of *Didymella pinodella* from pea root rot complex [•50].

An alternative solution is to apply random, untargeted metagenomic sequencing of the DNA present in a sample either using short (Illumina) reads or long-read sequencing (PacBio/Oxford Nanopore) [51,•52]. The long-read sequencing can provide information at species and strain level. A nice example of using Oxford Nanopore technologies for infield diagnostics is provided by Radhakrishnan and co-workers [53], who developed a portable, genomics-based and point-of-care diagnostics approach called MARPLE (Mobile And Real-time PLant disEase) to identify individual strains of complex fungal-plant pathogens. An overview of these diagnostics is shown in Figure 2.

Concluding remarks

Recent advances in the invention and optimisation of microbial detection methods, coupled with the availability of fast bioinformatics tools and deep-learning algorithms to rapidly analyse sequence data, are revolutionising our understanding of rhizosphere fungal diversity and biocontrol.

Conflict of interest statement

None.

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