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Dark septate endophytes: mutualism from by-products?

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Glossary

Biotrophy: organism feeding on other organism.

By-product mutualism*: mutually beneficial interaction between individual organisms equipped with traits that primarily benefit the bearer and benefit the other individual only as a side effect.

Mutualistic association: mutually beneficial interactions between two individual organisms.

Mycorrhizal symbiosis: symbiosis between plant roots and fungi, in which the fungus facilitates nutrient uptake from soil into the plant and gets carbon in return.

Pseudoreciprocity*: organisms's investment on itself benefits another organism as a by-product. Insect mating gifts, where the male investment on their own reproduction benefits female mating behaviour is an example of pseudoreciprocity.

Reciprocal investments*: continuous reciprocal investments between individual organisms. Investment can come in many forms, for example (1) improving physical access to partners and their resources/services, (2) improving/manipulation of the partners ability to provide beneficial services/resources, and (3) improving ability to effectively utilize the received services/resources for own survival and reproduction.

Saprotrophic capacity: ability to feed dead organic material.

Symbiosis: living together. A close interaction between two organisms. The outcome of the interaction can be positive (mutualism), neutral (commensalism) or negative (parasitism).

*Modified after [35,41]

Abstract

Plant roots are abundantly colonized by dark septate endophytic (DSE) fungi in virtually all ecosystems. DSE fungi are functionally heterogeneous and their relationships with plants range from antagonistic to mutualistic. Here, we consider the role of by-product benefits in DSE and other root-fungal symbioses. We compared host investments against symbiont-derived benefits for the host plant and categorised these benefits as by-products or benefits requiring reciprocal investment from the host. By-product benefits may provide the variability required for the evolution of invested mutualisms between the host and symbiont. We suggest that DSE could be considered as "by-product mutualist transitional phase" in the evolution of cooperative mycorrhizal symbionts from saprotrophic fungi.

DSE - root fungal symbionts between mycorrhizal and saprotrophic habit

Dark septate endophytic fungi (DSE, [1] (Box 1) colonize plant roots in most taxonomic groups in all major biomes of the world [2]. Although DSE colonization has been shown to be able to improve growth and nutrition of the host plant [3,4] and thus resemble **mycorrhizal symbiosis** (Glossary), the nature of DSE symbiosis – whether beneficial or harmful for the host plant- has remained largely unknown. Here, we define DSE as fungi that colonize living plant roots by melanized septate (ascomycetous) hyphae and sometimes microsclerotia (Box 1, Figures 1 and 2). DSE is an unambiguous form group and may represent several orders within ascomycetous fungi [5]. Recent studies have also found DSE to be characterized with a marked proportion of saprotrophic genes in their genomes [6-8]. High-throughput sequencing of soil has revealed an abundance of DSE fungi in plant rhizospheres (e.g., [9-12]). Interest towards applications in plant production [13-15], phytoremediation [16-23] and in carbon sequestration into soil [24] (https://www.theland.com.au/story/5344438/soil-survival-benefits-from-a-fungi/) would benefit from improved understanding of the biology of DSE symbiosis.

DSE fungi thus colonize roots of healthy plants by forming both superficial and intraradical fungal hyphae (Figures 1 and 2) and by forming intraradical microsclerotia (Figure 2). Bidirectional translocation of carbon and nutrients between host plants and root-associated fungi is the core definition of mycorrhizal symbiosis [25] and therefore, is also of major interest when studying nutritional benefits of DSE for plants. The transfer of carbon from the host plant to DSE fungi has been detected to take place [10,26] although it is not clear whether all the transfer is due to intraradical fungal colonization [27]. Improved nitrogen acquisition of the host plant, which is often reported resulting from DSE colonization, is neither necessarily directly associated with DSE colonization in plant roots [27]. In addition, the ecological significance of carbon and nutrient translocation between the host plant and DSE fungi - if it takes place - is not well understood because plant responses to DSE fungal colonization are context dependent and vary from negative to positive (e. g, [3,4,28,29]). Lack of knowledge of taxonomy and function in relation to morphological definitions is typical in the research field of mycorrhizal and other root-associated fungi.

Here, we focus on eco-evolutionary evidence indicating that DSE fungi have properties from both mycorrhizal and saprotrophic fungi [7,8,10,30,31]. Similar to mycorrhizae, DSE fungi form close associations with plants but, similar to soil saprobes, they may also be independent of their host plant because of **saprotrophic capacity**. Furthermore, in DSE symbioses, benefits and costs may not be limited to nutrient and carbon trade to the same extent as in mycorrhizal symbioses (see e.g., [29]). Therefore, theories of mycorrhizal symbioses based on reciprocal investments [32] and on the theory of biological markets [33,34] may not be directly applicable to DSE. Instead, soil properties and resource pools related to the plant-soil interface may play a more significant role. In this opinion, we propose that DSE symbiosis could be better understood by considering **by-product benefits** [35,36] and briefly discuss DSE symbiosis in relation to the evolution of mycorrhizal symbioses.

Benefits of mutualism

Benefits of mutualism

Connor [35] classified benefits of **mutualistic associations** into three categories: (i) by-product benefits, (ii) invested benefits and (iii) purloined (i.e., stolen) benefits. By-product benefits are traits or other attributes of an organism that incidentally benefit the other organism. In by-product mutualism one organism receives benefits that another organism produces as a by-product of its self-serving traits [35,37], such as in the case of certain micro-organisms that utilize the metabolic waste products of their hosts [37,38]. By-product mutualism has been considered an important step and one explanation for the evolution of cooperation [35,36,39-41] (see also Harcombe et al. [38] for empirical results in an experimental bacterial system). In invested benefits one partner actively invests in the other. The benefit of the interaction is then considered to exceed the cost of the investment [35]. In reciprocal interactions both partners invest and benefit from the **symbiosis**. Mycorrhizal symbiosis is often considered a classic example of a mutualistic interaction based on **reciprocal investments** and benefits i.e., interspecific transfer of nutrients and carbon between the host and fungi [25,32]. Benefits and reciprocal investments are, however, only rarely symmetrical in nature [42]. For example, similarly to all other biological interactions, mycorrhizae-host interactions are based on reciprocal exploitation [43] and despite generalizable expressions (such as “exchange” and “trade”) mycorrhizal symbioses include diverse dynamics [44]. In addition, mycorrhizae have also been regarded as an example of **pseudoreciprocity** where the investment from a host plant into root growth increases the availability of new root tips for mycorrhizal colonization, which can be seen as a by-product benefit for the plant [41]. The extreme case are purloined benefits, where symbionts also exploit (i.e, steal) resources that were intended to increase the fitness of the plant partner in addition to by-products. Despite stealing of resources by the symbiont (parasite), the symbiosis may be mutualistic if the parasite produces other benefits - either by-products or invested - for the host plant that exceed the costs of purloined benefits [35].

Window for by-product benefits

As above stated, in plant-root fungal symbiosis by-products could include resources that the host plant has an excess of or are waste products of the plant. In particular, plants may have excess carbon products and these may enter the soil via many routes (respiration, plant litter, secretion of organic acids and dead root biomass) [45-48] (Figure 3).

Although the evolution of plant-microbial symbioses can be assumed to be driven by competition for resources between organisms, selection may favor properties that also benefit the other organism. If by-product benefits are present in plant-fungal symbiosis, the optimal investment of the plant to the symbiont may decrease even to the theoretical level where the optimal (best) investment is zero and the plant exploits by-product benefits without investing in the symbiont (Figure 4, highest curve). Contrary to that: when by-product benefits do not exist at all, it may be optimal for plants to either have no investment at all or, alternatively, a relatively high investment into the symbiont is required for symbiosis to be profitable for the host (Figure 4, lowest curve). In cases where the by-product benefits of symbionts is not in either of the previously mentioned extremes, we suggest that it is best for the plant to either not invest at all (i.e. to obtain lesser, but still positive, by-benefits from the symbiont) or to invest in the symbiont, but a lower level of investment is optimal than in the situation where no by-product benefits exist at all and all benefits are only gained via investment (Figure 4).

Based on this schematic presentation, the presence of by-product benefits may decrease the threshold of selection for costly traits in plants and increase their dependency on the microbial partner, such as DSE. It may be especially important if, in the absence of by-product benefits, investment costs are higher than the corresponding benefits for low investment levels (Figure 4, lowest curve). By-product benefits can thus help plants to overcome this initial bottleneck. Because our presentation only includes treatment from “plant’s view”, it is important to note that as far as the obtained benefits for the host plant require costly investments from the symbionts (for example, altered physiological functions or structural investments) the joint evolution of both of them will determine the outcome (i.e, whether the local plant optima in the can be reached or not).

DSE fungi and by-product benefits

DSE fungi have a wide variety of enzymes for organic matter degradation [30,49,50] and therefore they resemble free-living soil saprotrophs. DSE fungi have also been found to have a positive impact on plant growth in the presence of organic nutrients [27,51,52] and the colonization of plant roots by DSE fungi in the field correlates positively with the amount of organic matter in soil [53-57]. Intraradical colonization of DSE however suggests a special, differentiated relationship with host plants because root colonizing fungi have to cross physical and chemical barriers during entering the root and be able to tolerate conditions inside the roots. Root colonizing fungi thus have to have the ability for a complex cross-talk with the host [58,59]. Intraradical colonization also increases opportunities for close interactions between hosts and fungal symbionts, for example in carbon and nutrient translocation or other potentially beneficial impacts, such as hormonal signalling [60]. By having both the saprotrophic capacity for organic matter degradation in the soil and the capability of colonizing roots, DSE fungi could therefore fit well into the category of by-product mutualists which enhance the performance and fitness of their host plants by providing benefits, but not requiring major investments from the host. In addition, among Pleosporales, which is an order including many DSE fungi, a transfer from saprotrophic to hemibiotrophic and **biotrophic** states during evolution has been suggested [61].

Dark septate endophytes – mutualism from by-products?

Evolution in the fungal tree of life comprises a spectrum of symbiotic (mycorrhizal) and saprotrophic lifestyles largely arising from ancestral features of fungi, such as hyphal cell structure, hyphal growth embedded into substrate, extrahyphal enzymes and symbiosis with photosynthesizing organisms. Although evolution of lifestyles rather consists of continuums than “man-made” categories, we compared key characteristics of root-associated and rhizosphere fungal groups to contrast the general differences between mycorrhizal, DSE and saprotrophic fungi (Table 1). This simple comparison shows that DSE symbiosis resembles free-living saprotrophic fungi more than mycorrhizal fungi. When paying specific attention to symbiont-derived benefits for the host and specificity of the association between the host and the symbiont (Table 1), DSE symbiosis can be merely considered as an intermediate, transitional form. Thus, DSE fungi are more beneficial for their hosts and have higher host-specificity than free saprotrophs, but they are less beneficial and have lower host-specificity than mycorrhiza-forming fungi (especially Glomeromycota, the ancestral form of plant-fungal symbiosis, see Jumpponen et al. [2] for DSE and Smith and Read [25] for Glomeromycota). In line with this, different fungal phyla are involved in divergent functional roles (Table 1) [62].

By-product benefits may also play a role in evolution of mycorrhizal symbioses in general [41,63-66]. For example, Martino et al. [59], based on a genomic analysis, showed that development from an endophytic state has taken place during the evolution of ericoid mycorrhizal symbiosis. Ericoid mycorrhizal fungi resemble DSE fungi: they both have developed enzymatic capacity for organic matter degradation and they both occur as non-mycorrhizal endophytes in the roots of a wide variety of plant groups. However, in contrast to DSE, ericoid mycorrhizal fungi also form a highly specialized and functionally well-characterized mycorrhizal symbiosis with ericaceous plants [24]. Similarities in the life strategies between DSE fungi and ericoid mycorrhizal fungi give support for hypotheses about a relatively recent transition between symbiotic and saprotrophic growth habits among the fungal lineages. There are also other mycorrhizal groups which may be less known but have well-developed saprotrophic capacity, such as orchid [25] and sebacinoid mycorrhiza [65,66]. It is possible that our treatment/discussion is applicable to this kind of mycorrhizal fungi as well.

Similar to other biotic interactions, fungal symbioses are dynamic and context dependent continuums of interactions with host plants [43]. Consequently, mutual dependency between the fungus and the host plant may be less likely to evolve in heterogeneous environments where the benefits of the cooperation vary [64,67]. Chamberlain et al. compared interaction types (competition, commensalism, mutualism) and showed that mutualism was most likely to change to neutral or antagonistic according to the context [68]. More recent analyses based on phylogenetic data indicated that evolutionary history would better explain large-scale mutualism breakdown/speciation events than context, in particular when nutritional-type symbioses are considered [67, see also 69]. It could be that if the amount of by-products varies in space and time it could favor organisms that are flexible in investment strategies and are able to change the amount of investments according to the availability of by-products and the range of symbiotic partners available. This could well be the case in most terrestrial ecosystems. By-product benefits should therefore be seen as a potential step towards mutualistic relationships in the evolution of plant-fungal interactions.

Concluding remarks

We conclude that the presence of by-product benefits may increase options for mutualism to evolve. In ecological contexts, by-product benefits may lead positive association of species purely due to improved local population growth rates without particular adaptations to fortificate the positive reciprocal effects. The first evolutionary steps towards the increased dependence might involve costly adaptations (investments) to (i) improve physical access to partners and their resources/services or (ii) improve the ability to effectively utilize the received services/resources for own survival and reproduction. These adaptations may not require reciprocal investments but may eventually lead to the dependency on the presence of the partner and eventually to the coevolution of the interacting species. Secondly, the costly investments/adaptations may specifically involve improvement/manipulation of the partners ability to provide beneficial services/resources in quantity and/or quality above the level of by-product benefits. Our schematic model outlines some hypothetical possibilities for the shape of benefit curves for such costly investments in the presence and the absence of by-product benefits.

Biology of DSE fungi fits into the general definition of by-product mutualism and contrasts to key characteristics of mycorrhizal fungi (in particular Glomeromycota, arbuscular mycorrhiza-forming fungi) and, on the other hand, to free living saprotrophs in soil. The contrast to arbuscular mycorrhizal symbiosis is of interest because of well-known co-colonization of arbuscular mycorrhizal fungi and DSE fungi in herbaceous plants. DSE fungi possess intermediate characteristics which may be indicative of differentiation from free saprotrophy towards mutualism in this fungal group (Outstanding questions). Similar suggestions have been made also of other endophytic fungi, in particular Sebacinales [65,66]. Prescott et al. [48] recently hypothesised the role of plant excess carbon as a driver of plant- soil interactions, especially in nutrient-limited conditions. The question whether the amount of surplus carbon is enough to promote mycorrhizal fungi capable to degrade these compounds remains to be solved. Excess carbon in plants, carbon in the litter and in particular exchanged carbon via mycorrhizal route give support to a hypothesis that excess carbon and by-products drive the evolution of mycorrhizal symbioses (Outstanding questions).

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Figure legends

Figure. 1. Dark septate endophyte (DSE) hyphae in the root cortex of *Deschampsia flexuosa* Root preparation bleached and stained with trypan blue. 400x magnification in compound (light) microscope.

Figure. 2. Microsclerotium in the root cortex cell of *Deschampsia flexuosa* Root preparation bleached and stained with trypan blue. 400x magnification in compound (light) microscope.

Figure 3. By-product mutualism in DSE symbiosis Deposits from the host plant (C) are utilized by dark septate endophytic (DSE) fungi in soil. Activity of DSE fungi associated to host plant roots increases nutritional and potentially other benefits (N) for the plant. This figure was partly created using BioRender (https://biorender.com/).

Figure 4. Symbiont derived benefit for the plant in relation to the plant investment A schematic presentation on symbiont-derived benefit for the plant (solid curves) in relation to the plant investment for maintenance of the symbiosis (dashed line). Open dots show the worst and closed dots the best of the plant benefit-cost balance for the plant profits. Investment optima, "best solutions", for the plant: black dot = no investment, red dot = highest investment level, green dot = investment level with some by-product benefits on the symbionts (if a minimum occurs at lower investment level but above 0). Blue dots = by-product benefits received without any investments from the plant partner. This figure was partly created using BioRender (https://biorender.com/).

Table 1. Contrasting symbiosis-related characteristics in plant-fungal associations with mycorrhizal fungi, dark septate endophytes (DSE) and free saprotrophs

Symbiosis-related characteristics	Mycorrhiza[a]	DSE	Free saprotrophs
Potential role of by-products in association	Low	High	High
Structural investment cost	High	Low	No or low
Symbiont derived benefit	High[b]	Intermediate	No or low
Dependency on host	High-intermediate	Low	Low
Host-Specificity	High[b]	Intermediate	Low
Taxonomic groups involved	Ascomycota, Basidiomycota, Glomeromycota, Mucoromycotina[c]	Ascomycota	Diverse, with potentially all phyla represented

[a]Ericoid, orchid and sebacinoid mycorrhizal fungi also have advanced saprotrophic capacity.
[b]Benefit and specificity in mycorrhizal symbioses varies according to the mycorrhizal type.
[c]Hoystedt et al. [70]

Box 1. Dark septate endophytic (DSE) fungi

DSE fungi colonize living plant roots by melanized and hyaline septate hyphae (septa = cell wall, hyaline = colourless) (Figure 1). In addition, microsclerotia, intraradical resting structures of the fungus are characteristic to DSE (Figure 2). Based on root colonization morphology alone, DSE cannot be identified, therefore investigations by laboratory and molecular techniques are needed [5]. But distinctive hyphal morphology inside young, healthy roots without visible symptoms in host plants is considered indicative of DSE colonization.

Functioning of the DSE symbiosis in the roots has remained obscure, i.e. whether or not fungal-mediated exchange of carbon and nutrients takes place between the host and the fungi. Specialized structures for carbon and nutrient exchange between the plant and the fungus, which are typical to mycorrhizal symbiosis, do not exist in DSE.

DSE fungi have highly developed capacity to degrade organic matter (saprotrophy).

DSE fungal cultures can produce conidia (= asexual spores) and certain macrofungi, such as *Mollisia* and *Pyrenopeziza*, have an association to DSE. However, there is no comprehensive knowledge of life cycles of DSE fungi in the wild.

Figure 1.

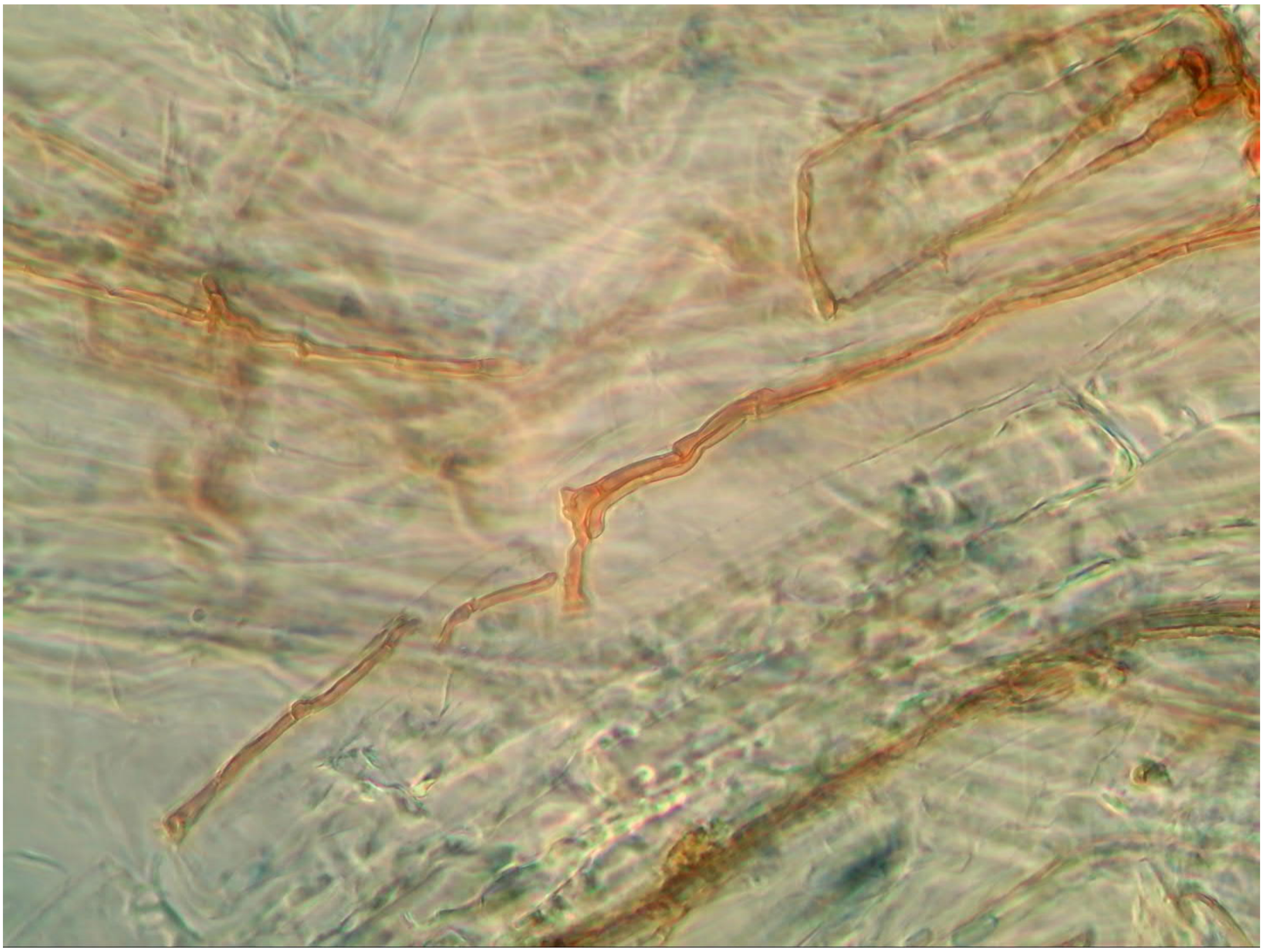


Figure 2.

Figure 3.

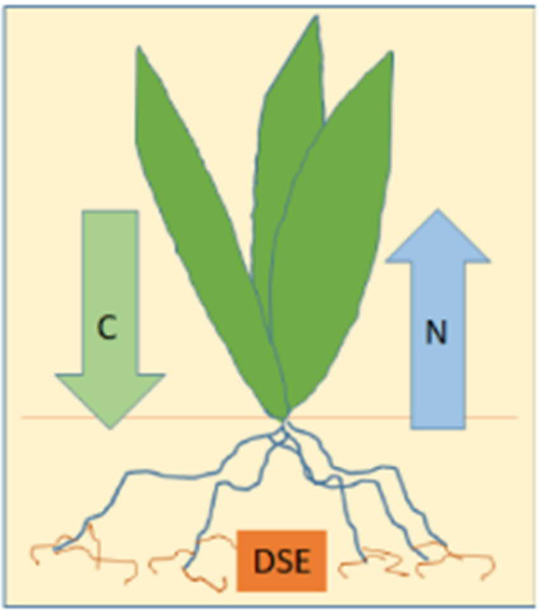
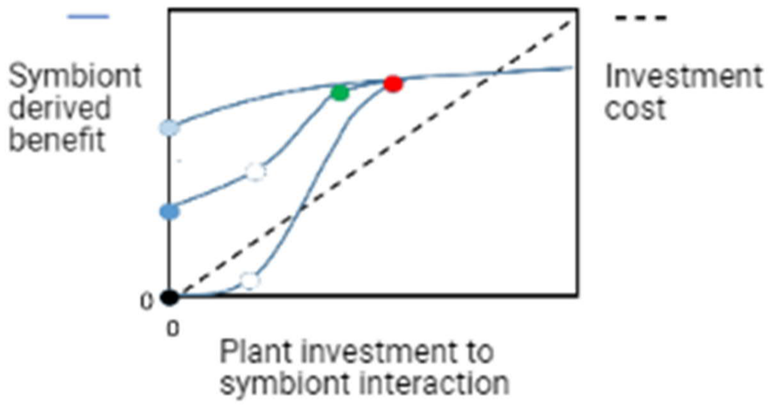


Figure 4.


Symbiont
derived
benefit
Investment
cost
0
0
Plant investment to
symbiont interaction