Fungal superhighways: do common mycorrhizal networks enhance below ground communication?

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In many natural communities communication between plants and other organisms below ground drives community dynamics. This communication is primarily through the release and detection of infochemicals, which must traverse the soil matrix to be effective. In this opinion article, we propose the Network Enhanced Bioactive Zone (NEBaZ) model, which posits that common mycorrhizal networks (CMNs) increase the bioactive zones of infochemicals by serving as superhighways directly connecting plants below ground. Here we argue that infochemical transport *via* CMNs allows for systemic defense signaling across plant populations and directed allelochemical delivery to target plants. Plant–animal interactions may also be facilitated by CMNs, suggesting that these fungal networks may be crucial components of many natural ecosystems.

Below ground communication

Communication between plants, microbes, and soil animals below ground is driven by the exchange of substances released by one organism and detected by another [1]. We use the term 'infochemical' to encompass all substances, generally secondary metabolites and plant hormones, used in communication. For below ground communication to be effective, enough of the infochemical must be produced by the 'sending' organism to get through the soil matrix and reach the 'receiving' organism in large enough concentrations to trigger a response. These infochemicals mediate many types of communication between plants and, for example, their plant neighbors [2,3], parasitic plants [4], symbiotic rhizobia and fungi [5,6], plant growth promoting bacteria [7], and other soil organisms [8]. Once infochemicals reach the soil, whether through root exudation or by leaching during decomposition, available concentrations quickly decline due to biotic and abiotic degradation [9], sorption to organic matter, and the formation of complexes with metals [10]. Such mechanisms create a substantial barrier to the reception of bioactive levels of infochemicals by receiving organisms. In this opinion article we present a new NEBaZ model, which proposes that rapid infochemical movement through the soil matrix via common mycorrhizal networks (Figure 1) may protect infochemicals from

degradation, sorption, and complex formation by limiting their exposure to the soil environment.

CMNs

Many filamentous microbes inhabit the soil, but for the purpose of this article we focus primarily on mycorrhizal fungi. Other filamentous soil organisms, such as bacteria and saprobic or parasitic fungi, can also form networks in soil [11–13], but these have not been reported to connect plants. Mycorrhizal fungi, biotrophic pathogens, and endophytic fungi (i.e., *Sebacinales*) are more likely to form connected hyphal networks linking plants, but to date the only evidence of infochemical transport along networks pertains to arbuscular mycorrhizal fungi (AMF) [14,15]. For this reason, we focus on AMF networks while also calling for more research involving other types of fungi.

Formation of CMNs

CMNs are formed by mycorrhizal fungi and exist as large, interconnected networks of fungal hyphae [16]. Most mycorrhizal fungi are not host specific and will associate with multiple plant species simultaneously [6], creating direct fungal links between plants. AMF are obligately symbiotic and take up carbon only from their host plants, so it is not surprising that they possess mechanisms to ensure that connectivity to host plants remains high, including risk spreading by simultaneously connecting with multiple hosts, and forming frequent anastomoses with related fungi to increase network connectivity [17,18].

Movement of substances via CMNs

Nutrients such as phosphorus (P) and nitrogen (N) are taken up by mycorrhizal hyphae and delivered to host plants [6]. Carbon (C) is also moved around ecto- and ericoid mycorrhizal networks [19], although direct evidence of C transfer by AMF is lacking. Similarly to nutrients, water is taken up by fungal hyphae and delivered to host plants. However, water transport via CMNs is bidirectional and cycles diurnally, moving toward transpiring plants during the day, but toward dry soil areas at night [20,21]. In addition to water transport within hyphae, a layer of water forms on hyphal exteriors; this water also flows along water potential gradients and a larger volume may be moved than that transported within hyphae [17].

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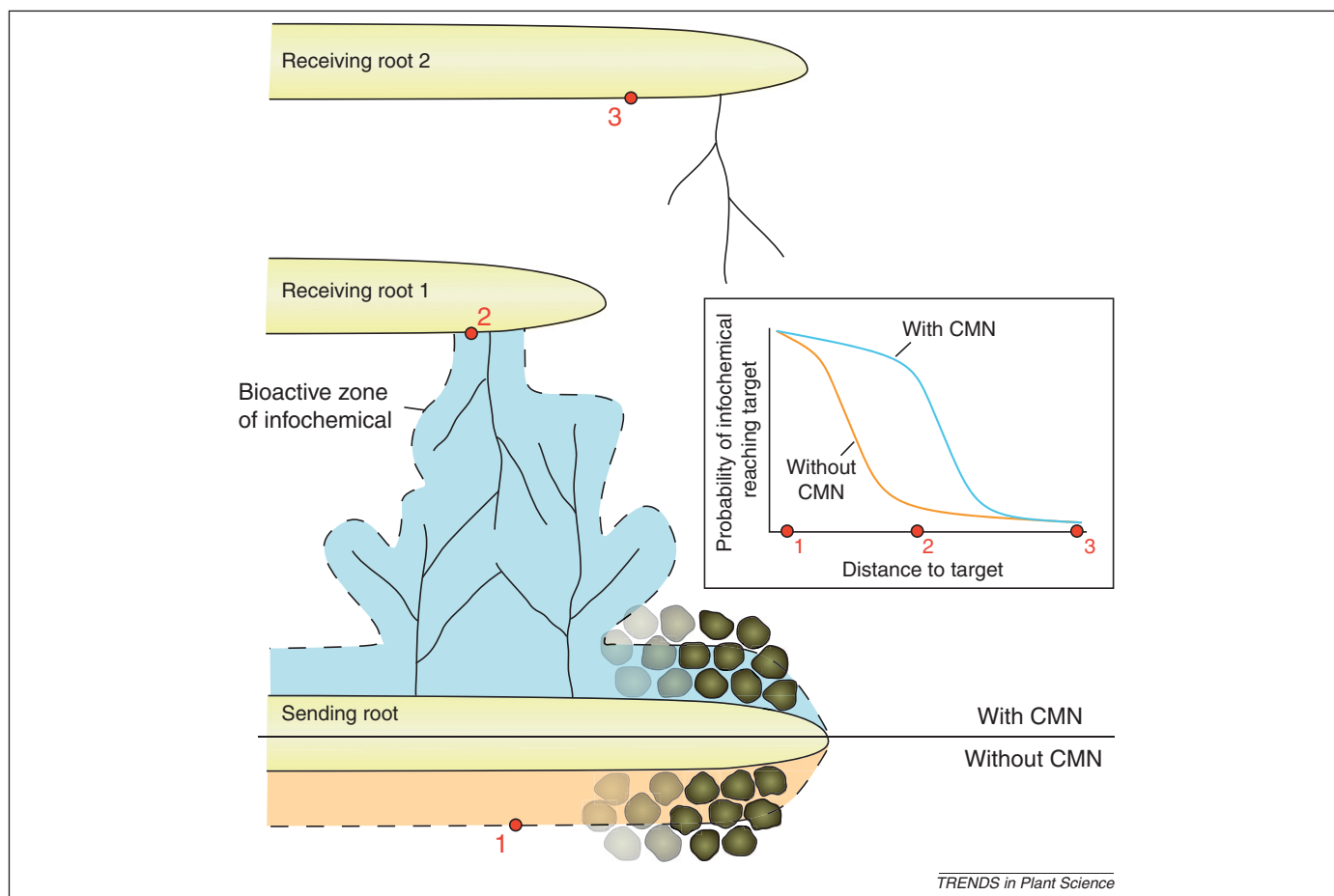http://dx.doi.org/10.1016/j.tplants.2012.06.007 Trends in Plant Science, November 2012, Vol. 17, No. 11   633

Figure 1. The Network Enhanced Bioactive Zone model showing a larger bioactive zone for infochemicals when a common mycorrhizal network (CMN) is present. We propose that this is due to the greater probability of infochemicals reaching the receiver at greater distances from the sender when a CMN is present than when it is absent.

An appreciation that infochemicals may also be transported via CMNs has emerged in the literature, with transport occurring along one of five possible pathways (Box 1). Three recent studies enable us to further speculate on the role of these infochemical exchanges. An organic fluorescent dye has been used to demonstrate water flow along hyphae, thereby also showing that movement of organic substances (e.g. infochemicals) is possible via CMNs [21]. Subsequently, it has been demonstrated that induction of defenses in one plant can induce defenses in a second plant sharing a CMN with the first [15]. Finally, it was demonstrated that the bioactive zones of allelochemicals were extended if CMNs were present [14]. We propose that these experiments, which we discuss in greater detail below, raise the possibility that transfer of infochemicals via CMNs occurs at levels sufficient to affect plant interactions.

Importance of infochemical transport via CMNs for plant science

Plant defense

CMNs provide a pathway for systemic defense signaling within plant populations, analogous to systemic signaling among clonal plants connected by runners or rhizomes [22]. A study of potted plants demonstrated that uninfected neighbors of tomato (*Solanum lycopersicum*) plants inoculated with *Alternaria solani* showed increases in the activity of several defense-related proteins and enhanced disease resistance [15]. This effect required intact CMNs and persisted even when volatile signaling above ground was restricted. Although undocumented, the authors speculated that the effect was due to transport of signals associated with systemic induced resistance through CMNs.

Benefits of plant defense signaling through CMNs may counterbalance the competitive costs of plants growing in close proximity, because induction of defenses in surrounding plants could insulate the inducer from further attack by creating a shield of healthy plants around it. As suggested in [22] regarding clonal plants, large patches of interconnected plants exhibiting induced resistance may better ward off attack. For example, if production of green leafy volatiles associated with parasitoid attraction is part of the induced response [23], a patch of interconnected plants may better attract parasitoids through the production of a larger volatile plume than single individuals could produce. This intriguing possibility awaits experimental testing, and at present the 'plume-enhancing' effects of CMNs are strictly hypothetical.

Allelopathy

The soil environment is an important barrier in allelopathic interactions, because allelochemicals must survive transit through the soil in sufficient concentrations to affect target plants. Transport via CMNs would greatly reduce transit times, simultaneously protecting allelochemicals

Box 1. Routes of infochemical transport via CMNs

There are five possible routes of infochemical transport via CMNs (Figure I). (**a**) Infochemicals could be transported cytoplasmically, following active uptake by hyphae or passive movement across fungal cell membranes. Hyphal cell walls are hydrophobic and well sealed so it is unlikely that infochemicals traveling cytoplasmically will leave hyphae before reaching roots or hyphal tips. This route is perhaps the least likely because membrane transporters capable of handling organic infochemicals have not yet been identified in AMF. All other routes of transport occur outside the cytoplasm. (**b**) Infochemicals could diffuse through the fungal cell wall (apoplastic). Given the hydrophobic nature of the cell wall [17] this pathway is likely to be available only for nonpolar infochemicals. (**c**) Infochemicals could move by dissolving in the layer of water on the surface of the hyphae (surficial). By definition, water-soluble infochemicals will be more common along this pathway than hydrophobic infochemicals. (**d**) Mycorrhizal hyphae can twine together to form cords when exiting plant roots [33], creating channels at the interior of the cord where water or air can collect. Cord interiors filled with water are likely to carry hydrophilic infochemicals. Alternatively, a cord filled with air would be expected to carry volatile hydrophobic compounds. Hyphal cords are likely to be less abundant in soil than individual hyphae, so this route of transport may be less important overall than the others. (**e**) Hyphal modification of the soil environment through increasing soil aggregation and conductivity and through modification of the microbial community may alter hyphosphere conditions in ways that speed infochemical movement. Transport via routes (a)–(d) is expected to be orders of magnitude faster than bulk diffusion through the soil simply because the length of the flow path will be shorter due to its decreased tortuosity (Figure 1) [34]. Flow rates would be orders of magnitude higher in water flowing on hyphal surfaces [35], or inside hyphae due to cytoplasmic streaming [36]. In addition to providing different rates of transport, the flow paths described above also provide differing degrees of protection from the soil environment. Movement via CMNs along routes (a)–(d) is likely to provide some protection from most soil 'hazards', but infochemicals moving along the surface may still be exposed to metals and organic matter in the soil. Due to the bacterial biofilms common on hyphae [37], surficial and hyphal cord flow paths may provide little protection from biodegradation.
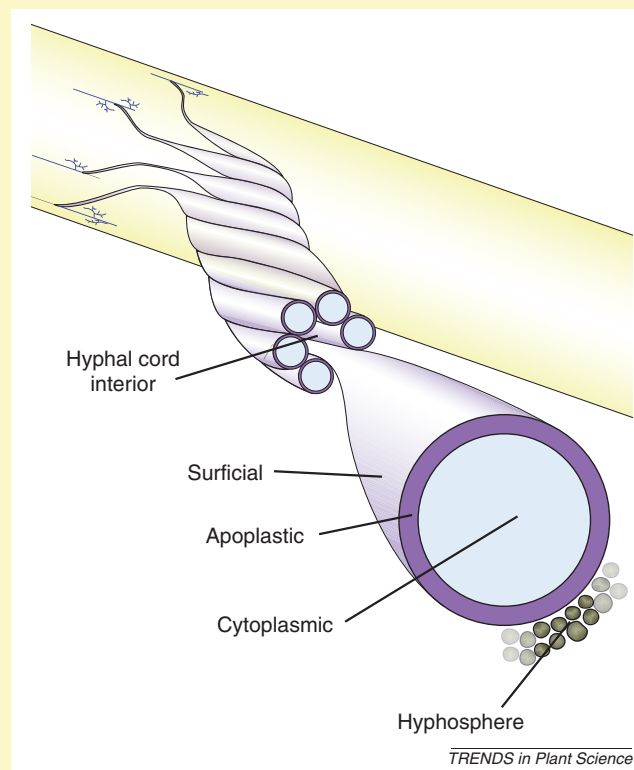

Figure I. Routes of infochemical transport via common mycorrhizal networks.

from many soil hazards, and thereby offering a plausible mechanism for the observed effectiveness of allelopathic interactions in some natural environments [24].

The soil volumes over which CMNs act to increase the bioactive zone of allelochemicals may be small in relation to the plant, but the importance of CMNs for infochemical transport is emphasized by the fact that infochemicals seem unable to traverse these small distances without a CMN. In a study manipulating the presence of a CMN in root exclusion compartments (RECs) marigold (*Tagetes tenuifolia*) plants were grown in pots containing RECs that were rotated or left undisturbed [14]. The RECs had a radius of 1.5 cm, and even over this small distance allelochemical concentrations were two to four times higher if a CMN was present. The allelochemicals produced by marigolds are hydrophobic and therefore not expected to diffuse quickly through soil. However, even the hydrophilic herbicide imazamox was found in higher concentrations in target plants connected to dosing sites by a CMN. CMNs existed in both pot-halves and were separated only by a moveable 2-mm soil zone between them. It is remarkable that requiring diffusion across 2 mm of soil, especially for a highly water-soluble compound, was enough to reduce delivery of the infochemical by one-half.

Allelochemical delivery via CMNs may be further enhanced when the allelopath modifies the AMF communities of surrounding plants so that target plants share a CMN with the allelopath. This CMN modification has been documented for two invasive grasses [25] and the invasive spotted knapweed (*Centaurea stoebe*) [26]. Although the NEBaZ model has not been explicitly tested using knapweed, this mechanism could help explain results reported in another experiment. The biomass of Idaho fescue (*Festuca idahoensis*) was lower when grown in competition with knapweed only when AMF were present, in a pattern consistent with hyphal transport of allelochemicals facilitating allelopathic effects [27]. However, a study using a mesh barrier to separate plants could not replicate the allelopathic effect [28], and the results were explained as greater utilization of AMF by *C. stoebe* and greater uptake of P through those AMF. This greater utilization of AMF by *C. stoebe* could also lead to efficient dispersal of allelochemicals towards neighboring plants. Experiments similar to those just described, but in which allelochemical concentrations are also measured, would help to resolve this issue.

Concluding remarks

Three main areas require focused research in the future: the development of new analytical tools to enable detection and quantification of low, dynamic concentrations of infochemicals (Box 2); experimental designs to elucidate the underlying mechanisms of the NEBaZ model; and its ecological relevance.

As in much of plant science research, multiple approaches ranging from high experimental control to high ecological realism will be necessary to test the functional

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Box 2. New analytical tools

Traditional soil analysis uses solvent extraction to measure static allelochemical concentrations. Sampling is destructive, making repeated analysis over time problematic. Furthermore, this provides no information on allelochemical dynamics, which are likely to be crucial to their signaling and defensive functions. New techniques show promise for measuring allelochemical fluxes in soil (and through CMNs).

Materials based on polydimethylsiloxane (PDMS) are widely used to monitor hydrophobic pollutants [38]. PDMS in several forms, including PDMS tubing, PDMS-coated optical fiber (matrix solid phase microextraction), and glass stir bars coated with PDMS (stir bar sorptive extraction [SBSE]), detected the root-exuded phytotoxin sorgoleone in soil beneath sorghum–sudangrass hybrids [39]. Materials were buried in soil at planting, and removed at harvest 1–3 months later. In a subsequent study, 10-cm lengths of PDMS tubing buried in garlic mustard (*Alliaria petiolata*) plots for 3-month periods detected a flavonoid glycoside from the plant [40].

PDMS materials have been successfully used to measure lipophilic thiophenes produced by the roots of the cultivated marigolds *T. patula* and *T. erecta* [41,42] and the related *T. tenuifolia* [14]. Novel soil probes made by inserting stainless steel wire into PDMS tubing recovered microgram quantities of the two major thiophenes produced by marigold when inserted into soil for 24 h beneath *T. patula* plants growing in garden beds [41]. Silicone tubing microextraction (STME) was developed by placing one-meter coils of PDMS microtubing beneath *T. erecta* plants [42]. The tubing ends were left out of the soil so that methanol could be washed through the tubing to collect thiophene samples for HPLC analysis. STME was used to demonstrate thiophene transport in soil through CMNs [14]. This technique provides a relative measure of thiophene concentrations in soil, but not a direct measure due to the fact that the soil volume extracted by the tubing is unknown. A key advantage of STME is the ability to sample soil non-destructively and repeatedly.

PDMS is not suitable for the many polar compounds implicated in plant communication and defense. Microdialysis techniques show potential here, having recently been used to measure organic acids in soil. Flow-through microdialysis samplers with a spatial resolution of 200 μm were constructed [43] and used to monitor concentrations of 12 amino acids as well as nitrate and ammonium in agricultural and forest soils [44].

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significance of the NEBaZ model in additional plant/infochemical systems. It is especially important not to rely on only one experimental design, because many of those already used have unavoidable drawbacks. Using waterproof membranes to separate pot compartments ensures that there is no CMN [15], but results are confounded by the fact that movement of water and microbes is also limited, so degradation of infochemicals is likely to vary with treatment. Rotated RECs create systems in which the microbial community and soil moisture are constant across treatments [14], but rotation itself may create air gaps and cause soil compression that could impede infochemical flow. Inoculating pasteurized soil with AMF to create CMNs has the benefit of needing no manipulations during the experiment (i.e., rotation) to maintain the treatment [15], but results are confounded by the fact that AMF have many effects on plant growth and behavior (i.e., infochemical production). Using combinations of designs will help ensure that future conclusions are not based on artifacts of the experimental design. Because CMNs affect nutrient transport, care should be taken to ensure that the putative effects of infochemical transport are not due to differential nutrient transfer instead. This can be accomplished by ensuring that nutrients are not limiting, by monitoring nutrient levels in soils and plant tissues, and by including treatments using activated C to remove infochemicals and identify any nutrient effect.

Questions that need to be addressed with highly controlled set-ups include determining which transport routes are used by different infochemicals, and determining any polarity or size restrictions on the types of compounds that can travel via each transport path. Given the importance of microbial degradation in regulating infochemical concentrations, it is important to determine how the bacterial communities growing in biofilms on AMF hyphae contribute to this degradation. Some compounds (e.g., phenolic acids) appear to be broken down by many bacterial species, but degradation of other compounds (e.g., juglone) is performed by specialists [29], making it imperative to characterize hyphal biofilm bacterial communities.

Many outstanding questions should also be addressed using more realistic pot and field approaches. We need to determine how robust infochemical transport via CMNs is to network disturbance by soil biota such as hyphal grazers (e.g., collembola, mites) and soil architects (e.g., earthworms). It is also unclear what the functional role of AMF diversity is, and how increased fungal diversity will affect transport. The length of AMF hyphae in soil increases with increasing fungal diversity [30], suggesting that transport will increase with increasing diversity. However, increasing diversity may also limit overall connectivity by increasing the number of distinct but overlapping networks in a community. Our model also suggests new predictions for how climate change may impact plant community dynamics. Increased CO_2 often increases AMF hyphal length in soil [31,32], possibly increasing connectivity and therefore delivery of infochemicals.

We have presented a NEBaZ model of below ground communication whereby infochemicals move through the soil via CMNs. We propose that this new framework can be applied to plant–plant interactions, both beneficial (defense) and antagonistic (allelopathy). This model may also apply to plant–animal interactions involving root pests that find their plant hosts by honing in on infochemicals released by plants, but we are awaiting the first test of this. At present, only three publications have clearly tested the model we propose, so more work is needed to support or refute the model and determine whether it is a general property of fungal networks. We have only begun to scratch the surface of our understanding of the mechanisms behind infochemical transfer via CMNs and to appreciate its importance in natural systems. Research on interactions in plant science would better emulate natural systems by explicitly including mycorrhizal fungi in experimental designs (as treatments) and allowing sufficient time for CMNs to develop, because evidence is increasing that they are important facilitators of many kinds of interactions.

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