Cover sheet

Arbuscular mycorrhizal fungi, Collembola and plant growth

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Arbuscular mycorrhizal fungi are ubiquitous in field soils, as are mycophagous animals such as Collembola. It has been suggested that these animals reduce the functioning of the mycorrhiza and are thereby detrimental to plant growth. However, recent choice experiments suggest that Collembola preferentially feed on non-mycorrhizal fungi in the rhizosphere. If these preferences also occur in field soils, then Collembola may indirectly benefit plants through an enhancement of mycorrhizal functioning and indirect multitrophic links to foliar-feeding insect herbivores.

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There is an intriguing paradox in arbuscular mycorrhizal (AM) fungal ecology. Although there are numerous laboratory studies that have shown a variety of benefits to plants in forming a mycorrhizal association, there have been far fewer occasions when these benefits have also been demonstrated in natural situations[1]. Positive effects of AM fungi on plants include enhanced nutrient uptake, protection against pests and pathogens, and relief of drought stress[2]. Recently, it has been suggested that the response of any plant to AM colonization lies along a continuum, from positive to negative[3]. Therefore, the question naturally arises as to whether laboratory studies, at the positive end of this continuum, are realistic mimics of field situations, which appear to lie in the null (no response) area. There is an urgent need to understand this problem, so that the ecology of mycorrhizas might be better described and the future management of the symbiosis improved. In laboratory studies, many factors may be controlled, and some or all of these may be responsible for reducing the efficacy of AM fungi in field conditions. These include soil nutrient levels, plant stress factors (e.g. drought), plant diseases, herbivorous and mycophagous animals[4]. An important group in the latter category is the Collembola (springtails).

Collembola are abundant microarthropods in virtually all soils, feeding on a range of materials, including fungi, bacteria, lichens, decomposing vegetation and detritus. The feeding ecology of most species is poorly known[5], but there appears to be a preference for fungal hyphae over other food types. By consuming dead vegetation and hyphae, these animals can play an important role in decomposition processes[6]. In many cases, Collembola may enhance the decomposition process, as hyphal grazing stimulates growth and respiration of the fungi[7]. The fact that most of the subterranean species feed (at least in part) on fungi has led to their being regarded as important regulators of the mycorrhizal symbiosis. Reviews of AM-soil fauna interactions suggest that Collembola have the potential to restrict mycorrhizal functioning in the field[8,9], but null and stimulative effects of their feeding have also been recorded. Other authors question their importance[4], so it is timely to ask whether Collembola are responsible for the disruption of AM associations, and whether they are one reason for the failure of field experiments to match results obtained under controlled conditions.

Laboratory studies

It is apparent that one species, *Folsomia candida,* has been used in almost 50% of laboratory pot trials (Fig 1a). The reason is simple: *F. candida* is exceptionally easy to culture, unlike many other species. However, to quote from a recent review[10], using this species as representative of all Collembola "is about as ecologically sound as choosing a mole as a 'typical' mammal". It is interesting that the frequency distribution of AM fungal species used shows a less skewed distribution (Fig. 1b), but this also represents species that are generally amenable to pot culture. There have been remarkably few attempts to recreate a field situation in the laboratory, by using co-occurring species of Collembola and AM fungi from one field site (but see Ref. 11).

Collembola densities in pot trials are usually given in numbers of individuals per dm^3, but these have been converted to numbers per m^2 for ease of comparison with known field densities (Table 1). With few exceptions[12], the density of animals used has been at, or below, that normally encountered in comparable field situations[21]. A feature of this summary is that only the two early studies recorded a negative effect on plant growth resulting from collembolan grazing on the mycorrhiza[18,19]. In these laboratory trials there are three instances of positive and two of negative effects. Six experiments produced no effect on plant growth, even though Collembola apparently reduced AM colonization in five of these[13,14,16,17]. Therefore, the notion that mycorrhizal grazing by Collembola disrupts the functioning of the symbiosis is not entirely supported by the literature.

Perhaps the greatest problem with most pot trials is that they are set up with a single plant-fungus-Collembolan combination. Such a situation is most unrealistic of field conditions. Of crucial importance is the fact that the soil used would not have contained as diverse an array of non-mycorrhizal fungi as would be found in the field. While none of the trials in Table 1 were performed in sterile conditions, it is likely that the most abundant fungal hyphae would have been mycorrhizal, as these were inoculated in each case. When fungal preference trials have been performed[22,23], it has been shown that Collembola consistently graze on other soil fungi, in preference to AM species. Furthermore, if different Collembola species are offered a selection of AM fungal species, distinct preferences are seen by each Collembolan species, but these are not consistent between Collembola[24,25]. Therefore, Collembola will graze on AM fungi, but not through choice. The conclusion is that future pot trials involving Collembola, AM fungi and plants should also include a known non-mycorrhizal fungal complement. If a situation is set up in which the animals have little choice but to feed on the mycorrhizal species offered, then at reasonably high density, we would expect to see a detrimental effect on the functioning of the symbiosis. However, if other fungal species are preferentially grazed, then the outcome of the experiment could be positive for the plant (see below).

Field experiments

To understand the role of Collembola in AM associations, we need to perform studies in field situations. However, manipulation of soil communities is extremely difficult because there is no biocide specific to either Collembola or AM fungi. In the few situations where insecticide application has been used to reduce numbers of Collembola[19,20] (Table 1), there was some evidence that reduction in grazing resulted in increased phosphorus (P) uptake and plant growth. This may be circumstantial evidence that the Collembola were grazing on the AM fungi, thereby reducing plant P uptake. It is also possible that the death of many soil animals resulted in a flush of nutrients for plants, resulting in higher P content in the biocide treatment. Furthermore, the insecticide used in both studies was broad-spectrum and the observed effects could have resulted from the removal of other larger rhizophagous insects, which feed on roots and also disrupt the mycorrhizal mycelium.

Instead of attempting to reduce numbers of a target group, one can take the alternative approach and augment a community with a particular species. Technically, this is much easier than reduction, but is open to criticism in that the resulting densities might be unrealistically high. One experiment has taken this approach, with the addition of Collembola to microcosms surrounding soybean plants[12]. In this case, the augmented densities were not excessive (a 26% increase), possibly owing to predation of the Collembola. However, what is interesting is that mycorrhizal colonization of the plants was enhanced in Collembola addition treatments, although no effect on plant growth was recorded. It is plausible that this study provides the first field evidence to prove that, at moderate densities, Collembola are actually beneficial to mycorrhizas rather than detrimental. These effects could have been caused by enhanced grazing on fungi, which compete with, or are antagonistic to, AM species - similar to the laboratory experiments described in the next section.

Positive effects on plants

Several controlled studies have shown a positive effect on plant growth of collembolan feeding on the mycorrhiza[12,17,19]. In each case, the response of the plant at increasing collembolan densities has been bell-shaped, remarkably similar to that seen in situations where Collembola graze on non-AM fungi[10]. For the plant-AM fungal association, stimulation of plant growth at intermediate densities is thought to result from either an increase in hyphal growth or mineralization of nitrogen (N) and P. At low collembolan densities, the hyphae are not stimulated to grow, but at high densities the grazing is detrimental. At intermediate densities, the preferential removal of small diameter hyphae towards the exterior of the mycelium[22] may result in proliferation and a consequent increase in benefit to the plant through mineral uptake. Alternatively, the release of N and P from Collembola faeces[26], and subsequent plant uptake, might be sufficient to more than compensate for hyphal loss at intermediate animal densities.

These studies[12,17,19] also suffer from oversimplification, because Collembola were presented with only one mycorrhizal species as a food source. A much more realistic experiment[11] examined the effects of feeding by a variety of microarthropods on AM fungi and growth of sugar maple (*Acer saccharum*). Here, the simultaneous addition of three mite and three Collembolan species, at densities similar to those found in the field, had no effect on maple growth. However, addition of the microarthropods and some decaying maple leaf litter resulted in a 59% increase in arbuscular colonization and a 32% increase in shoot biomass. Because the microarthropods preferred to feed on the non-AM fungi in the experiment, it appears that providing them with an alternative food source allowed mycorrhizal growth. It was suggested that by feeding on fungi that might compete with the mycorrhiza for root space and by the release of minerals from the ingested hyphae, greater AM colonization of roots and nutrient uptake occurred, with a benefit to plant growth. Therefore, this study[11] (like the previously mentioned field experiment[12]) raises the intriguing possibility that Collembola might be beneficial rather than detrimental, to mycorrhizal functioning.

Mechanisms

There is no doubt that Collembola are capable of grazing on mycorrhizal hyphae, but these are probably not their preferred food[23]. Choice experiments in which Collembola are fed AM and non-AM fungi are extremely limited[22,27]. As a result, it is unknown as to why AM fungi appear to be relatively unpalatable to these animals, compared with saprophytic fungi. One reason, based on the optimal foraging model[28] has been put forward[27]. According to this model, one would expect Collembola to preferentially feed on the food source which is most energetically rewarding and which maximises reproductive success. It is interesting that the one study to address this question has found that reproductive success was indeed greater on non AM fungi[27], when consumed in preference to AM fungi. However, the actual mechanism determining palatability remains unknown. It may be due to hyphal thickness and architecture, since most of the intricate AM mycelium is composed of hyphae > 10mm[29] and Collembola preferentially attack thin hyphae, < 0.5mm in diameter[22]. Alternatively, AM hyphae may be low in nutrients or high in antifeedants, compared with saprophytic fungal hyphae, but these possibilities have yet to be examined.

At some of the extremely high field densities that have been reported for these animals[21], it is possible that preferred fungal food resources could be depleted to the extent that AM fungi become heavily grazed. However, we have yet to see a field experiment with Collembola and AM fungi in which the animal densities reach their recorded upper range[21] of 1×10^5 m^{-2}. If, at these extraordinary densities, AM fungi are eaten in the field, then the nature of the grazing interaction becomes important. If the ends of hyphal elements are severed, then regeneration may occur and the chance for proliferation of the hyphae exists. However, a mycorrhiza has an internal hyphal element in a root and an external one in the soil. If the hyphae are severed at the root surface, then this could have serious consequences for the plant. The internal mycelium would still receive carbon compounds from the host but the reciprocal transaction, namely the provision of mineral nutrients, is lost. Because the internal mycelium might represent as much as 20% of the biomass of a root[8], it would become parasitic rather than mutualistic. The very limited evidence we have is that even at high densities, Collembola do not sever the larger hyphae at the root surface, but instead attack thin walled hyphae away from the root[22,23]. Such a pattern of attack might limit the ability of the fungus to forage for nutrients, but the effects on the plant will be far less severe than if the hyphae were severed at the root surface. Therefore, it is possible that in most field situations, Collembola do not have a sufficiently negative effect on mycorrhizal functioning to be manifest in directly reduced plant growth.

Instead, they are more likely to have positive effects on plant growth and there are several mechanisms by which this might occur. Grazing on non-AM fungi might result in increased N mineralization, however this might not always result in an increased uptake of nitrate (NO_3^-) by the mycorrhiza[26]. This is because other microbes in the rhizosphere might take up the N and immobilize it. Grazing on non-AM fungi, which compete with the mycorrhizal fungus for root space, might allow greater colonization of the root by AM fungi. There is little evidence for this (Table 1), but if it did occur, it may be of indirect benefit to plants, through enhanced nutrient uptake or protection against root pathogenic fungi[30].

A fascinating possibility is that Collembola might have indirect effects on plant growth by causing changes in the performance of foliar-feeding insects on the same plant. Although not a mycorrhizal experiment, it has recently been shown that the presence of Collembola in soil can lead to a decrease in reproduction of the aphid *Myzus persicae* when feeding on *Trifolium repens*[31]. The causal mechanism was unclear. If, at moderate densities, Collembola can enhance mycorrhizal colonization, this might lead to improved plant growth because AM fungi increase the resistance of foliar tissues to chewing insects[32]. The mechanism is thought to be one in which the C:N ratio of the plant is increased by the mycorrhiza, leading to an increase in carbon-based defence compounds that are active against generalist chewing insects[33]. Meanwhile, if high densities of Collembola do reduce AM colonization, then the performance of foliar-sucking insects might also be reduced because AM fungi have recently been shown to increase aphid performance[34].

A final mechanism by which Collembola might positively affect AM fungi is through their dispersal. Several studies have shown that AM fungal spores can be present in the guts of Collembola[8] and the first demonstration of AM dispersal in soil by Collembola has recently been presented[35]. The effect again depended on the species of mycorrhiza involved.

Prospects

Evidence for the disruption of the AM mutualism by Collembola is equivocal. Indeed, the opposite may be true; Collembola might allow enhanced mycorrhizal growth and thereby be of indirect benefit to plants. However, there is an urgent need for the design of microcosm experiments which are ecologically realistic so as to understand better this fascinating interaction. In particular, densities of Collembola need to mimic those found in the field, with ecologically realistic combinations of AM and non-AM fungi. The mechanism determining fungal palatability needs to be established and experiments conducted to determine whether preferential feeding is caused by chemical or morphological differences in AM and non-AM fungi. Finally, in order to determine if Collembola do reduce mycorrhizal functioning in the field, technologically difficult experiments need to be done, in which populations of animals and fungi are manipulated, while other soil organisms are unaffected. Given that AM fungi can affect the structure of plant communities, enhancing diversity and productivity[36], an understanding of the interactions between Collembola, AM fungi and herbivorous insects will be an important step forward in our knowledge of community structuring forces.

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Table 1. Investigation of grazing by Collembola on AM fungi and the consequences for plant growth[a,b]

Type of experiment	Collembolan density, x 10^3 m^{-2}	Effect on AM fungus	Effect on plant growth	Refs
Laboratory	17-150	Not recorded	Positive	12
	24	HD: negative RC: --	--	13
	21-42	HD: negative RC: --	--	14
	2.8-11	Not recorded	--	15
	9.5	RC: negative	--	16
	6	RC: negative	Positive	17
	1.9	RC: negative	--	
	3.2	RC: negative	--	
	3-22	--	Negative	18
	< 5	--	Positive	19
	7-25	--	Negative	
Field	0.99	RC: positive	Positive	12
	5	Not recorded	Negative	19
	5	--	Negative	20

[a] Key: HD, hyphal density; RC, density of root colonization; --, no effect.

[b] References obtained from the ISI database. To put the Collembolan densities used into perspective, an approximate average value for the temperate ecosystems simulated here is 48 x 10^3 individuals m-2 (Ref. 21). Most studies have used densities towards the lower end of the abundance scale and only one study[12] has used the very high numbers which can occur in nature[21].

Fig. 1. Frequency that Collembola and arbuscular mycorrhizal (AM) species are used in laboratory experiments reported in the ISI database. (a) The frequency histogram of Collembola is heavily skewed, being dominated by the easily cultured *Folsomia candida*. Key: F.C., *Folsomia candida*; O.a., *Onychiurus ambulans*; F.p., *Folsomia penicula*; P.m., *Proisotoma minuta*; T.c., *Tullbergia clavata*; O.e., *Onychiurus encarpatus*; O.fi., *Onychiurus fimatus*; O.fo., *Onychiurus folsomi*; S.c., *Sinella coeca*; T.g., *Tullbergia clavata*; X.g., *Xenylla grisea.*

(b) The frequency histogram of AM fungi is also heavily skewed and dominated by species widely available in culture. Key: Gl.f., *Glomus fasciculatum*; Gl.int., *Glomus intraradices*; Gl.ca., *Glomus caledonium*; Gl.d., *Glomus deserticolum*; Gl.e., *Glomus etunicatum*; Gl.cl., *Glomus clarum*; Gl.inv., *Glomus invermaium*; Gl.mac., *Glomus macrocarpum*; Gl.man., *Glomus manihotis*; Gl.mon., *Glomus monosporum*; Gl.mos., *Glomus mosseae*; Gl.o., *Glomus occultum*; Gl.t., *Glomus tenue*; E.s., *Entrophosphora schenkii*; Gi.m., *Gigaspora margarita*; Gi.r., *Gigaspora rosea*; A.d., *Acaulospora denticulata*; S.c., *Scutellospora calospora.*