The impact of dispersal, plant genotype and nematodes on arbuscular mycorrhizal fungal colonization

Pil U. Rasmussen a,*, Anupol Chareesri b, Roy Neilson c, Alison E. Bennett d, Ayco J.M. Tack e

a Department of Ecology, Environment and Plant Sciences, Stockholm University, SE-106 91, Stockholm, Sweden
b Department of Soil Quality, Wageningen University and Research, PO Box 47, 6700AA, Wageningen, the Netherlands
c Ecological Sciences, The James Hutton Institute, Dundee, DD2 5DA, UK
d Department of Ecology, Evolution and Organismal Biology, The Ohio State University, 318 W. 12th Ave., 300 Arenoff Laboratory, Columbus, OH, 43210, USA

ARTICLE INFO

Keywords:
Arbuscular mycorrhizal fungi
Colonization ability
Dispersal
Genotype
Nematodes
Plantago lanceolata

ABSTRACT

While the majority of parasitic and mutualistic microbes have the potential for long-range dispersal, the high turnover in community composition among nearby hosts has often been interpreted to reflect dispersal constraints. To resolve this apparent contradiction, we need further insights into the relative importance of dispersal limitation, host genotype and the biotic environment on the colonization process. We focused on the important root symbionts, the arbuscular mycorrhizal (AM) fungi. We studied AM fungal colonization ability in a controlled mesocosm setting, where we placed Plantago lanceolata plants belonging to four different genotypes in sterile soil at 10, 30 and 70 cm from a central AM fungal inoculated P. lanceolata plant. In part of the mesocosms, we also inoculated the source plants with nematodes. AM fungi colonized receiver plants < 1 m away over the course of ten weeks, with a strong effect of distance from source plant on AM fungal colonization. Plant genotype influenced AM fungal colonization during the early stages of colonization, while nematode inoculation had no effect on AM fungal colonization. Overall, the effect of both dispersal limitation and plant genetic variation may underlie the small-scale heterogeneity found in natural AM fungal communities.

1. Introduction

Within the last decade it has become clear that many microbes have limited biogeographical distributions, and that microbial communities can have a high turnover even at small spatial scales (Choudoir et al., 2018; Ettema and Wardle, 2002; Evans et al., 2017; Hanson et al., 2012). The distribution of microbes at the meso- and microscale may be limited by several factors, such as dispersal, the environment and species interactions (Chaudhary et al., 2008; Ettema and Wardle, 2002). Even though microbes make up a large portion of the global biodiversity (Torsvik et al., 2002) and play an important role in ecosystem functioning (Clemmensen et al., 2013; van der Heijden et al., 2008), the factors that shape the spatial distribution of microbes is still poorly understood. Understanding the factors that shape the spatial distribution of microbes allows us to make predictions about how fragmentation and environmental change may affect future distributions of microbes.

AM fungi are an important group of symbiotic root fungi colonizing the majority of terrestrial plants (Brundrett and Tedersoo, 2018). These symbionts are well-known for sharing essential nutrients such as phosphorus and nitrogen with plants in exchange for carbon derived from photosynthesis (Smith and Smith, 2011). While it has been argued that many AM fungi exhibit a cosmopolitan distribution (Davison et al., 2015; but see Bruns and Taylor, 2016), studies have also reported high turnover at small spatial scales (e.g. Rasmussen et al., 2018; Sosa-Hernández et al., 2018). Previously, it has been suggested that this high turnover in AM fungal communities at small spatial scales is an indicator of dispersal limitation (Vályi et al., 2016). However, spatial heterogeneity in the abiotic and biotic environment may be an equally, or more important, driver of the high turnover of AM fungi at small spatial scales (e.g. Bainard et al., 2014; Rasmussen et al., 2018).

AM fungal colonization of new plants occurs by three different propagule types: spores, mycelium and colonized root fragments (Smith and Read, 2008). These propagules may be dispersed by multiple processes, with the importance of each mechanism dependent on the spatial scale of the dispersal process. AM fungi can spread through mycelial growth, which may be an important dispersal mechanism at the sub-metre scale (Jumpponen and Egerton-Warburton, 2005).
Furthermore spores and root fragments can be dispersed by invertebrates and small mammals at distances ranging from cm to km, or potentially even further (Gange, 1993; Klironomos and Moutoglis, 1999; Mangan and Adler, 2002). Dispersal of mycelium, spores and root fragments at larger scales may be mediated by water movement (Harner et al., 2011), large mammals (Allen, 1987), wind (Egan et al., 2014; Kivlin et al., 2014; Warner et al., 1987) or by human-mediated soil movement. Several studies have targeted the dispersal of AM fungi within an empirical setting (Table S1), and these indicate a strong effect of distance, especially in studies targeting mycelial growth as the dispersal mechanism.

Alongside dispersal, the abiotic and biotic environment can have a major impact on the colonization ability and community composition of AM fungi (Chaudhary et al., 2008). Indeed, the abiotic and biotic soil environment are highly variable at scales ranging from less than a m to several hundreds or thousands of km (Ettema and Wardle, 2002; Rasmussen et al., 2018). In particular, the abiotic environment (Treseder, 2004), plant species (Dickson, 2004; Helgason et al., 2002) and plant genotype (An et al., 2010; Bennett et al., 2015; Kaeppler et al., 2000) have been shown to influence AM fungal colonization levels. Likewise, other soil organisms, like nematodes, may play a role in the colonization ability of AM fungi. Nematodes are one of the most abundant groups of soil organisms, they display a large range of feeding styles (Yeates et al., 1993) and play an important role in soil ecosystem processes and plant community structure, thereby making them interesting for ecological studies (Bardgett and van der Putten, 2014; Nielsen et al., 2014). Studies have shown variable interactions between nematodes and AM fungi: plant-parasitic nematodes can reduce AM fungal colonization, while AM fungi have been shown to both positively and negatively impact nematodes, though the latter effect was dependent upon the mode of parasitism (Borowicz, 2001; Frew et al., 2018; Hol and Cook, 2005). While there are no studies on how and if nematodes influence dispersal of AM fungi, they could potentially affect the successful establishment and colonization of AM fungi through e.g. mycophagy or their direct effects on plant cells and nutrient transfer within plants (Hol and Cook, 2005). Other soil organisms such as root feeding insects and collembolans can influence AM fungal distributions through impacts on dispersal, mycophagy, competition and facilitation (Chaudhary et al., 2008; Klironomos and Moutoglis, 1999), and similar results for nematodes may therefore be observed. As plant genotypes differ in their response to AM fungal inoculation (An et al., 2010; Bennett et al., 2015; Kaeppler et al., 2000), plant genetic variation could underlie the small-scale variation found in AM fungal colonization in natural settings. Furthermore, plant genetic variation may interact with dispersal and mediate interspecific interactions between root-associated organisms. While these factors have mostly been studied in isolation, the joint impact of dispersal limitation, plant genotype and interspecific interactions may have important implications for the small-scale distribution of AM fungi.

To identify the impact of dispersal limitation, plant genotype and nematodes on AM fungal colonization, we set up a mesocosm experiment. More specifically, we planted Plantago lanceolata individuals belonging to four different genotypes in sterile soil at multiple distances (10, 30 and 70 cm) from a central AM fungal inoculated P. lanceolata plant. To study interspecific interactions, part of the mesocosms were inoculated with nematodes. We asked two key questions:

1) What is the impact of distance, plant genotype and their interaction on AM fungal and nematode colonization at the sub-metre scale?
2) Do nematodes influence AM fungal colonization?

Our a priori expectations were: 1a) AM fungi (Table S1) and nematodes (de la Peña et al., 2011; Gorman et al., 2018; Patschek et al., 2018; Taylor et al., 1994) are dispersal limited at the sub-metre scale, resulting in reduced colonization by AM fungi and nematodes the further away from the source plant. 1b) Considering that AM fungal colonization and nematode community composition can differ between plant genotypes (An et al., 2010; Palomares-Rius et al., 2012), we expect that some plant genotypes will be colonized earlier or more heavily than others. 1c) Given the lack of studies that have assessed the joint impact of distance and plant genotype on AM fungal and nematode colonization, we have no a priori expectation of the role of the interaction between, or relative importance of, distance and plant genotype in shaping root colonization by AM fungi and nematodes. At a broader taxonomic scale, studies on plant-pathogenic microbes have shown that distance and host plant genotype may interact in shaping the colonization process (e.g. Tack et al., 2014). 2) While we know of no studies linking nematodes to AM fungal dispersal, previous studies have reported a negative effect of nematodes on AM fungal colonization levels (Borowicz, 2001; Hol and Cook, 2005). Hence, we expect that plants in nematode-free mesocosms will be colonized earlier or more heavily than plants in mesocosms with nematodes.

2. Materials and methods

2.1. Study system

Plantago lanceolata is a wind dispersed perennial herb found in all continents except Antarctica. It is an obligatory outcrossing species (Cavers et al., 1980; Krohne et al., 1980). It is most commonly found in dry grasslands, road sides and disturbed habitats (Cavers et al., 1980), and is known to associate with a large number of mutualistic and antagonistic microbes and invertebrates, including AM fungi and nematodes (e.g. Cavers et al., 1980; De Deyn et al., 2004; Rasmussen et al., 2018).

2.2. Collection and pre-experimental treatment of plants, AM fungi and nematodes

In order to investigate the influence of plant genotype on AM fungal dispersal, we used P. lanceolata seeds originating from four mother plants, each originating from a different population in the Åland Islands, Finland (labelled genotype A – D). Seeds were pre-germinated in sterilized coir and after four weeks individual plants were transferred to 250 ml pots with a mixture of double autoclaved (121 °C; 2 h) potting soil and vermiculite (70:30) and fertilized with 10 ml Hoagland’s solution (2.5 ml 2M KNO3 and 1.5 ml 1M NH4NO3 per litre water). When plants were transferred to individual pots, sixteen individuals of plant genotype A were inoculated with AM fungi to function as ‘source plants’. A commercial AM fungal inoculum was used (RootGrow Professional, Kent, UK) which contained Claroideoglomus etunicatum, Funneliformis mosseae, and Rhizophagus irregularis. The inoculum was added as a granulate powder around the roots of each source plant. Eight weeks after sowing, the plants were placed in the mesocosms (see section below). To validate whether autoclaving the soil had resulted in AM fungal free plants, we harvested excess plants before transplantation of plants into the mesocosms. Root staining and scoring of these plants (see below for the methods) confirmed that double-autoclaving the soil excluded AM fungi.

Nematodes were extracted from a P. lanceolata population from the grounds at the James Hutton Institute, UK. The inoculum contained a typical mixed community of nematodes found in grasslands, mainly consisting of plant, bacterial and fungal feeding and omnivorous nematodes (Neilson et al., 1997). We inoculated the source plants with nematodes in twelve out of the sixteen mesocosms three weeks after the plants had been transplanted into the mesocosms (mean ± sd density of nematodes per source plant: 1186 ± 59; n = 4 mesocosms were not inoculated to serve as a control). Inoculations were conducted by pouring the nematode inoculum into a small hole next to the source plant. As a caveat, we note that while autoclaving reduces nematode density, even double-autoclaving will not effectively remove all remaining individuals; hence, in contrast to AM fungi, there will be a
2.3. Study design

To investigate the impact of dispersal, plant genotype and nematode treatment on AM fungal colonization, we set up sixteen mesocosms each with a diameter of 180 cm. Mesocosms were placed in two polytunnels and watered regularly with mist spray. Each mesocosm was each with a diameter of 180 cm. Mesocosms were placed in two poly
treatment on AM fungal colonization, we set up sixteen mesocosms

2.3. Study design

To investigate the impact of dispersal, plant genotype and nematode treatment on AM fungal colonization, we set up sixteen mesocosms each with a diameter of 180 cm. Mesocosms were placed in two polytunnels and watered regularly with mist spray. Each mesocosm was each with a diameter of 180 cm. Mesocosms were placed in two poly
treatment on AM fungal colonization, we set up sixteen mesocosms

2.3. Study design

To investigate the impact of dispersal, plant genotype and nematode treatment on AM fungal colonization, we set up sixteen mesocosms each with a diameter of 180 cm. Mesocosms were placed in two polytunnels and watered regularly with mist spray. Each mesocosm was each with a diameter of 180 cm. Mesocosms were placed in two poly
3. Results

3.1. AM fungal dispersal

AM fungal colonization increased over time, with low colonization in the first six weeks and much higher levels in weeks 8 and 10 (Figs. 1 and 2). AM fungal presence and root colonization decreased with distance, but the pattern changed through time (Table 1 and S2; Figs. 2 and 3). In week 2, root colonization sharply decreased with distance: while 47% of the plants were colonized by AM fungi at 10 cm distance, only a single plant at 30 cm was colonized, and none of the plants at 70 cm were colonized (Fig. 3a). During weeks 4 and 6, the pattern of decreasing root colonization with distance remained, with a larger number of colonized plants at the distances 10 and 30 cm and higher average colonization levels (Fig. 3b and c). While the relationship between AM fungal root colonization with distance was non-significant at week 8, the pattern of decreasing colonization levels with distance was more pronounced at week 10, when all plants were colonized (Fig. 3d and e).

AM fungal colonization differed among plant genotypes during weeks 2 and 4 (Table 1, Fig. 3a and b) but not at later times. We detected no interaction between plant genotype and distance at any time point (Table 1, Fig. 3). The inoculation of mesocosms with nematodes did not significantly influence AM fungal colonization (Tables 1 and S2; Fig S2).

AM fungal spore densities were low (less than one spore per gram). Spore production was not influenced by distance, plant genotype or nematode treatment (Table S3). Spore densities differed among species, with higher densities for Rhiisopus irregularis than for Claroideoglomus etunicatum and Funneliformis mosseae (Table S3 and Fig. S3).

3.2. Nematode dispersal

Similar to the pattern of AM fungal colonization in the end of the experiment (at week 10), nematode density decreased with increasing distance from the source plant, irrespective of plant genotype (Table 1; Fig. 4). Interestingly, the pattern also suggested a larger variance in nematode density with increasing distance from the source plant (Fig. 4), a pattern that was not apparent for AM fungi (Fig. 3e).

3.3. Correlation between AM fungi and nematodes

We found no correlation between AM fungal root colonization and nematode density ($r = -0.17$, $t = -1.51$, df = 77, $P = 0.135$).

4. Discussion

As expected, we found that both AM fungi and nematodes were dispersal limited at the sub-metre scale, with reduced levels of root colonization with increasing distance from the source plant. While we predicted that AM fungal and nematode colonization levels would differ between plant genotypes, this pattern was only apparent during the initial phase of the colonization process for AM fungi, and we detected no effect of plant genotype on nematode colonization. We found no effect of distance on the colonization of the distinct plant genotypes (i.e. an interactive effect), indicating that the effect of plant genotype on colonization was not affected by the decreasing colonization pressure at increasing distances from the source. Lastly, and contrary to our expectation, we found no effect of the presence of nematodes on AM fungal colonization. Overall, distance was the main driver of AM fungal colonization over time, with plant genotypic variation contributing to the variation in colonization. Distance and plant genetic variation may thereby explain part of the large variation in the AM fungal communities associated with neighbouring plants in natural systems.

Our findings support that AM fungi are dispersal limited at the sub-metre scale: after two weeks, the AM fungi had colonized half of the plants at 10 cm distance, and only one plant at distances beyond that; at week 10, when all plants were colonized, there was still a clear pattern of decreasing levels of colonization with increasing distance. Dispersal limitation has been proposed to be one of the main drivers of large small-scale variation in AM fungal community composition in descriptive field studies (Mummey and Rillig, 2008; Rasmussen et al., 2018; Wolfe et al., 2007), and our findings confirm that dispersal limitation may indeed play a role in shaping these patterns. In an experimental study using P. lanceolata, Klironomos and Moutoglis (1999) investigated the dispersal of AM fungi through hyphal growth from a source plant to receiver plants. They similarly found a clear effect of distance on AM fungal colonization at small scales, with colonization of plants up to 40 cm from the source plant within 5–10 weeks depending on the AM fungal species. This matches results of other experimental studies focusing on dispersal by hyphal growth (Table S1). In contrast, studies that focused on wind and animal dispersal showed less consistent evidence for dispersal limitation (Table S1). For example, Garcia de Léon (2016) found that AM fungal richness in trap pots located at a range of distances (5–36 m) from a quarry edge was not related to distance.

Nematodes showed higher densities at plants close to the source, a pattern similar to that found for AM fungi. At short distances (at the
sub-metre scale like our mesocosm experiment), nematodes possess limited mobility, which is strongly dependent on the porosity, habitable pore space and moisture content of the soil (Robinson, 2002; Taylor et al., 1994; Yeates et al., 2002). Given the uniform soil conditions within the experiment, the pattern of decreasing densities with increasing distance from the source plant then matches our expectation of nematode dispersal limitation. Patterns may differ at larger spatial scales (hundreds of m up to tens of km), where nematodes are dependent upon wind (Nkem et al., 2006), machinery (Boag, 1985) and overland water flow (Baxter et al., 2013) for dispersal.

Previous studies have shown that plant genotype can influence AM fungal colonization and this in turn may have large impacts on plant performance, plant-associated organisms and ecosystem functioning (Rasmussen et al., 2017; Smith et al., 2010; van der Heijden et al., 1998). In line with this, we found that colonization differed among plant genotypes. While several studies have focused on the impact of

### Table 1
The impact of distance, plant genotype, the interaction between distance and genotype, and nematode treatment on arbuscular mycorrhizal (AM) fungal colonization and nematode density in *Plantago lanceolata* plants.

<table>
<thead>
<tr>
<th>Distance</th>
<th>Genotype</th>
<th>AM fungi colonization week 2</th>
<th>AM fungi colonization week 4</th>
<th>AM fungi colonization week 6</th>
<th>AM fungi colonization week 8</th>
<th>AM fungi colonization week 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Df</td>
<td>F/χ²</td>
<td>P</td>
<td>Df</td>
<td>F/χ²</td>
<td>Df</td>
<td>F/χ²</td>
</tr>
<tr>
<td>Distance</td>
<td>Genotype</td>
<td>Distance × Genotype</td>
<td>Nematode treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------</td>
<td>----------</td>
<td>--------------------------</td>
<td>-------------------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nematodes</td>
<td>Density 1 1</td>
<td>14.47</td>
<td>&lt; 0.001</td>
<td>3</td>
<td>1.93</td>
<td>0.587</td>
</tr>
</tbody>
</table>
| Degrees of freedom: Numerator/denominator. Significant P-values (p < 0.05) in bold.

Fig. 3. Arbuscular mycorrhizal (AM) fungal colonization of *Plantago lanceolata* plants as a function of distance (10, 30 or 70 cm) from an AM fungal inoculated source plant at (a) week 2, (b) week 4, (c) week 6, (d) week 8, and (e) week 10. Data points represent the AM fungal colonization percentage for individual plants and are jittered horizontally to avoid overlap between data points; the colour of the data points represents the four plant genotypes. The significant relationship between AM fungal colonization and distance is shown with a trend line, with significant and non-significant relationships shown with a solid and dashed trend line, respectively. Trend lines were plotted using the function *geom_smooth* (method = lm) in the R-package *ggplot2*. Note that the range of the y-axis differs between the figures. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
plant genotype on AM fungal colonization in both agricultural (Bennett et al., 2015; e.g. Hetrick et al., 1993; Kaeptler et al., 2000) and natural systems (e.g. Johnson et al., 2010), we lack studies on how the relative importance of plant genotype changes over the course of the colonization process. We found that plant genotype mattered most during the early phase of AM fungal colonization (i.e. the lag phase when colonization is low; Smith and Read, 2008), but played an insignificant role during and after the rapid increase in AM fungal root colonization. We hope that our findings will stimulate studies that will explore this temporal pattern, and investigate how the relative influence of plant genotype and distance may change during the progression of the AM fungal colonization process.

An interesting question is whether distance from inoculum source and genotype interact to influence AM fungal colonization. For example, plant genotypes may differ in the relationship between root colonization levels and colonization pressure in the form of hyphae or spores; as colonization pressure decreases with distance from the source, the relative levels of colonization may differ among plant genotypes at different distances from the source plant. Such interactions may leave a strong imprint on the small-scale distribution of AM fungi. Our findings suggest the absence of such an interaction. However, we need comparable studies in this and other study systems to generalize the conclusion that distance and genotype do not interact during the colonization process.

Despite studies showing that nematodes and AM fungi may interact (Bakhtiar et al., 2001; Borowicz, 2001; Hol and Cook, 2005), we found no direct effect of nematode treatment or nematode density on AM fungal colonization. This discrepancy may be due to the fact that previous studies on the interaction between AM fungi and nematodes focused predominantly on the biocontrol of plant parasitic nematodes (e.g. Schouteden et al., 2015). In contrast, we focused on a diverse natural nematode community, which may have diluted the more negative interaction between AM fungi and plant parasitic nematodes.

5. Conclusion

Our findings demonstrated that dispersal limitation and plant genotype jointly shape plant colonization at the sub-metre scale, with decreasing levels of colonization with increasing distance and differences among plant genotypes at the early stages of colonization. Given dispersal limitation, not all AM fungal species may reach all plant individuals at the same point in time, creating small-scale variation in the AM fungal community colonizing neighbouring plants, a pattern that may persist due to e.g. priority effects. Likewise, the effect of plant genotype during the early stage of colonization may leave an imprint on subsequent community dynamics. As such, both dispersal limitation and plant genetic diversity may play a role in the large variation found in AM fungal distributional patterns at small spatial scales in natural systems. While the main dispersal mechanism studied here was hyphal spread, other dispersal mechanisms such as animal and wind dispersal may contribute to the spatial patterns of AM fungi over larger scales. Moreover, small-scale spatial heterogeneity in the abiotic environment – like pH and N (e.g. Dumbrell et al., 2010; Rasmussen et al., 2018) – may play a major role. While in this study we used a uniform abiotic soil environment to isolate the effect of distance, plant genotype and nematodes, future studies may assess the relative importance of spatial abiotic heterogeneity and dispersal limitation on the temporal dynamics of AM fungal colonization. To allow for generalizations, such studies would be replicated across plant species and across multiple spatial and temporal scales.

Declarations of interest

None.

Acknowledgements

The authors are grateful for all the assistants at the James Hutton Institute, who helped out with sampling, and Agata Kaczmarek and Linnea Ström for help in the lab. The authors acknowledge funding from the Maj and Tor Nessling foundation (2014211 to AJMT) and the Swedish Research Council (2015-03993 to AJMT). The James Hutton Institute receives financial support from the Scottish Government, Rural and Environment Science and Analytical Services Division.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.soilbio.2019.01.018.

References


De la Peña, E., Vandegehuchte, M.L., Bonte, D., Moens, M., 2011. Nematodes sur


Brown, D.J.F., Boag, B., 1988. An examination of methods used to extract virus-vector


Brown, D.J.F., Boag, B., 1988. An examination of methods used to extract virus-vector


Brown, D.J.F., Boag, B., 1988. An examination of methods used to extract virus-vector


De la Peña, E., Vandegehuchte, M.L., Bonte, D., Moens, M., 2011. Nematodes sur


Brown, D.J.F., Boag, B., 1988. An examination of methods used to extract virus-vector


Brown, D.J.F., Boag, B., 1988. An examination of methods used to extract virus-vector


De la Peña, E., Vandegehuchte, M.L., Bonte, D., Moens, M., 2011. Nematodes sur


