Soil Biology & Biochemistry 99 (2016) 85–93

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Arbuscular and ectomycorrhizal root colonisation and plant nutrition in soils exposed to freezing temperatures

Jouni Kilpeläinen [a, *], Mauritz Vestberg [b], Tapani Repo [c], Tarja Lehto [a]

[a] University of Eastern Finland, School of Forest Sciences, P.O. Box 111, FI-80101 Joensuu, Finland
[b] Natural Resources Institute Finland (Luke), Green Technology, Antinniementie 1, FI-41330 Vihtavuori, Finland
[c] Natural Resources Institute Finland (Luke), Natural Resources and Bioproduction, P.O. Box 68, FI-80101 Joensuu, Finland

ARTICLE INFO

Article history:
Received 3 December 2015
Received in revised form
4 April 2016
Accepted 30 April 2016
Available online 9 May 2016

Keywords:
Arbuscular mycorrhiza
Cold climate
Dual mycorrhiza
Ectomycorrhiza
Frost
Nitrogen
Phosphorus

ABSTRACT

Arbuscular mycorrhizas (AM) are adapted to soils with higher pH, less organic matter and more available nitrogen than ectomycorrhizas (EM). We hypothesise that also climatic factors have a direct role in the relative success of the two types. We tested their colonisation rates after soil freezing. Soil was taken from forest and meadow sites to comprise propagules of both types of mycorrhizal fungi. The soil was sieved (6 mm) and mixed. Soil batches were exposed to +5 °C (control), −12, −25, −48 or −130 °C, time at target temperature was 6 h. Silver birch (*Betula pendula*), grey alder (*Alnus incana*) and white clover (*Trifolium repens*) were sown to these soils and grown in favourable conditions for 11 weeks. EM colonisation in birch and alder was similar in all treatments. Arbuscule formation in alder and clover was not affected, but vesicles, AM fungal hyphae and spores in roots were reduced with decreasing temperature. Soil soluble phosphorus (P) was increased by freezing as were also the foliar concentrations in birch. By contrast, clover had less foliar P in the lowest temperature treatments which may be due to less efficient functioning of AM. To conclude, the development and function of AM were impaired to some extent by very low temperatures, while EM were not affected. The low-temperature tolerance of EM may be another explanation to their predominance in cold climates.

1. Introduction

Plants with the most common mycorrhiza types, arbuscular mycorrhizas (AM), ectomycorrhizas (EM) and ericoid mycorrhizas, predominate in distinctive global vegetation zones and soil types. This is thought to result from differences in the availability of different nutrients, which is controlled indirectly by climate through soil formation (Read, 1991; Smith and Read, 2008). AM are very efficient in taking up inorganic phosphorus (P), and therefore dominant in warm, dry climates where P is often a limiting factor. EM are more efficient in taking up N than AM are, and they are the most common in the boreal zone and the humid parts of the temperate zone, where the low temperatures and high humidity promote the accumulation of organic matter, decreasing pH and low N availability. However, it is difficult to separate the importance of N and P availability from direct climatic effects. It is also possible that the major climatic factors, humidity and temperature, are directly responsible for some of the differences in the occurrence of different mycorrhiza types.

Most plant species form only one type of mycorrhizas, and therefore the occurrence of the mycorrhiza types is coupled with their host plant species. However, some woody plants can form both AM and EM, such as several *Alnus*, *Salix*, *Populus* and *Eucalyptus* species. Dual mycorrhizal plants provide an experimental model system for direct comparisons of AM and EM, although they often have a predominance of either type. AM are more common in young plants (Arveby and Granhall, 1998), and their formation is favoured by high pH and low P/N ratio (Van der Heijden and Kuyper, 2001). Water availability can have an effect on the mycorrhiza type (Allen et al., 1995), but the effect of temperature has not been explored.

Climatic temperature and humidity regimes are usually closely correlated and high-altitude and -latitude climates combine both high humidity and low temperatures. Decreasing AM fungal (AMF) colonisation has been found with increasing altitude while EM fungal (EMF) colonisation does not decrease either in dual mycorrhizal plants (Gehring et al., 2006) or in vegetation comprising

* Corresponding author.
E-mail addresses: jouni.kilpelainen@uef.fi (J. Kilpeläinen), mauritz.vestberg@luke.fi (M. Vestberg), tapani.repo@luke.fi (T. Repo), tarja.lehto@uef.fi (T. Lehto).

http://dx.doi.org/10.1016/j.soilbio.2016.04.025
0038-0717/

grasses, herbs and *Salix* shrubs (Read and Haselwandter, 1981). Similarly, in a recently deglaciated chronosequence only traces of AMF colonisation were found in grasses, and none in usually AM trees or herbaceous plants. This was attributed to low temperatures in addition to scarcity of inoculum (Helm et al., 1996, 1999).

The threshold temperatures for EMF or AMF or their mycorrhizas have not yet been tested. Typically, organisms adapted to adverse conditions tend to have a much higher tolerance to the critical environmental factors than they actually encounter in their habitats. For instance, the needles of Scots pine (*Pinus sylvestris*) may tolerate even −90 °C in their most frost-hardy stage even though such temperatures do not occur in the distribution range of this species (Zhang et al., 2003). Therefore, in order to assess the threshold of tolerance to a specific factor, more extreme conditions are needed than those occurring in the field. The freezing tolerance of organisms is usually tested by exposing them to a series of low temperatures for 4–6 h, the lowest temperatures being lethal (Burr et al., 2001).

Previous studies on the freezing tolerance of EMF or AMF have mostly not reached lethal temperatures. Some specimens of all four tested EMF species survived −30 °C after growing in pure culture without acclimation, and two out of the four survived −48 °C, which was the lowest test temperature (Lehto et al., 2008). AMF mycelium survived freezing temperatures of −12 °C in the field (Addy et al., 1994) and −5 °C (Klironomos et al., 2001) and −12 °C (Addy et al., 1998) in controlled environments, but lower temperatures have not been tested. The infectivity of hyphae that were severed from roots was not reduced at soil temperature −2 °C (Addy et al., 1997); however, the amount of viable AMF hyphae was halved during a winter with the lowest soil temperature reported as high as −3.7 °C (McGonigle and Miller, 1999).

In addition to mycorrhizal fungi, the communities of other soil microbes and fauna are affected by soil temperature and moisture which leads to fluctuations in nutrient conditions. Frost can affect the soil nutrient availability by physical breakage of organisms and litter plus disruption of linkages between particles; freezing-thawing processes are also major weathering agents in cold climates (Briggs and Smithson, 1986).

Here, we tested the hypothesis that EMF are more tolerant to freezing temperatures than AMF. Batches of a homogenous soil mix containing propagules of both types of mycorrhizal fungi were exposed to a range of freezing temperatures. Subsequently, grey alder (*Alnus incana* (L.) Moench), silver birch (*Betula pendula* (L.) Roth) and white clover (*Trifolium repens* L.) seedlings were grown from seeds in these soils in favourable conditions, and the extent of EM and AM formation was recorded, plus the N-fixing nodule formation. The soil and foliar nutrient concentrations were determined to assess direct effects of frost on soil nutrients and thereby plant nutrition versus nutrient effects caused by changes in the root symbioses.

2. Materials and methods

2.1. Soil, plants and conditions

Soil at 0–8 cm depth was collected in a *B. pendula* stand and under *Sorbus aucuparia* and *A. incana* trees in a mixed deciduous stand in Kukkola, Joensuu, Finland (62°33.7′N, 29°49.3′E) in October 2013. Meadow soil was collected in Ikolanaho (63°4.5′N, 29°49.7′E) and Havukanaho (63°3.75′N, 29°52.3′E) in Koli National Park at 0–15 cm depth in November 2013. The meadows date back to former slash and burn agriculture but are nowadays managed regularly by cutting with a scythe or a clearing saw. These meadows are not flooded and their vegetation included different grasses such as *Deschampsia cespitosa*, *Poa pratensis*, *Festuca rubra* and *Nardus stricta*, and herbaceous plants, e.g. *Campanula* spp., *Trifolium* spp., *Galium* spp., *Centaurea* spp., *Alchemilla* spp., *Leucanthemum vulgare*, *Geranium sylvaticum*, *Convallaria majalis* and *Fragaria vesca*. The drier parts of the meadows grow for instance *Antennaria dioica*, *Dianthus deltoides* and *Hieracium pilosella*. The collected soils were sieved (6 mm) and homogenised, and mixed in the volume proportion of 1:2:2:2 (meadow soil and soil near *Betula*, *Sorbus* and *Alnus* trees) to get a soil mix containing both EMF and AMF inocula.

The soil mix was placed in aluminium trays as a 4 cm thick layer. Each tray was allotted to one of five temperature treatments: + 5 °C (control), −12 °C, −25 °C, −48 °C and −130 °C. The temperature was first lowered to +5 °C within 1 h, and then the rate of decrease was 5 °C h^{-1} except that the temperature was kept for 8 h at −3 °C before further lowering it to the target temperature to ensure that the soil was frozen. The target temperature was maintained for 6 h, and subsequently, the temperature was raised again at the rate of 5 °C h^{-1}. At the end, the temperature was kept at +5 °C for 1 h before opening the chamber. In the −130 °C treatment the samples were first cooled to −50 °C with the above described procedure in the same freezing chambers as the other treatments (ARC 300/−55 + 20, Arctest, Finland) and after that the rate of temperature decrease to −130 °C and increase to −50 °C was faster: 10 °C h^{-1} in a liquid-nitrogen cooled chamber (GCC30, Carbolite, Chelmsford, UK). The soil was then warmed from −50 to +5 °C in the Arctest chamber at the rate of 5 °C h^{-1}. The treated soils were blended with perlite in the volume proportion of 30:70 in each treatment. Plastic pots with 84.2 g soil (water content 31% of dry matter) in the filling volume of 185 ml were used; pot height was 80 mm, top diameter 61 mm and base diameter 48 mm.

There were three bait plant species: grey alder (*A. incana* (L.) Moench), silver birch (*B. pendula* (L.) Roth) and white clover (*T. repens* L.). Ten plants of each species per treatment were grown in individual pots. Additionally, fifteen pots per treatment were left without seeds to test whether the treatments affected the soluble-nutrient concentrations in soil. Other than test species were removed from all pots. Thus, there were 5 (treatments) × 3 (plant species) × 10 (plant individuals) + 5 × 15 = 225 pots in total. All pots were placed in a randomised arrangement in the growth room.

Plant seeds were surface sterilised before sowing. The seeds were left in tap water overnight. Next day, one drop of Tween 80 was added and the seeds were shaken for 5 min. Next, the seeds were kept in 30% H_2O_2 for 20 min and were rinsed 5 times in sterilised distilled water. Five seeds were placed in each pot, covered with a thin soil layer and Clingfilm and germinated in a walk-in growth chamber (Conviron GR77, Controlled Environments, Winnipeg, MB, Canada) under fluorescent tubes (VHO 215 W, Sylvania Cool White, Sylvania, USA) in 90% relative humidity, 22 °C, 20-h day, 4-h night. After germination the growing conditions were 20-h day at 22 °C in 60% relative humidity and 4-h night at 17 °C in 80% relative humidity. Cooling/warming rate was 5 °C h^{-1}. Day/night irradiance was ca. 350/0 μmol m^{-2} s^{-1} PAR from incandescent lamps (60 W, Oy Airam, Finland) and fluorescent tubes. At the beginning and end of a day, light intensity was changed stepwise during 2 h.

The seedlings were thinned to one per pot 4.5 weeks after the sowing. Starting at 6.5 weeks after sowing, the seedlings were fertilised with Kekkilä irrigation fertiliser N-P-K 17-4-25 and all other nutrients so that N dose was 1 mg in 10 ml water/week. The pots were watered after they reached weight corresponding to 60% water holding capacity (see Section Soil properties and plant nutrients). Plants were harvested 10 weeks and 4 days (for clarity, denoted 11 weeks) after sowing. One alder died before harvest in soil treated with −12 °C and another with −130 °C. The shoots were cut at soil surface.