ELSEVIER

Soil Biology & Biochemistry 39 (2007) 400–408

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

Root colonisation by arbuscular mycorrhizal, fine endophytic and dark septate fungi across a pH gradient in acid beech forests

Jacqueline W.M. Postma*, Pål Axel Olsson, Ursula Falkengren-Grerup

Plant Ecology and Systematics, Ecology Building, Lund University, S-223 62 Lund, Sweden

Received 5 June 2006; received in revised form 4 August 2006; accepted 14 August 2006
Available online 10 October 2006

Abstract

Colonisation by root endophytes can be beneficial to plants growing on acid, nutrient-poor soils. Arbuscular mycorrhizal (AM) fungi can supply herbs with nutrients and may give protection against aluminium toxicity. Two other root colonising fungi, fine endophytes (FE) and dark septate fungi (DSE), are less well known but are potentially of benefit to their host plant. AM fungi are the most prevalent symbionts in herbs at neutral to acidic soil pH. At extremely low pH, fungal growth can be limited and AM colonisation is usually rare. Fine and dark septate endophytes, on the other hand, have been observed more often under these conditions. In order to relate endophyte colonisation to a gradient in soil pH, we investigated root colonisation by AM, FE and DSE in *Maianthemum bifolium*, *Galium odoratum*, *Mercurialis perennis* and *Stellaria nemorum*, from a range of acidic beech forests. With decreasing pH, colonisation by AM decreased, whereas the other two endophytes increased. AM and FE colonisation were inversely correlated in *Maianthemum bifolium*. We compared changes in root colonisation with those in chemical composition of soil and leaf samples and found a positive correlation between leaf magnesium concentrations and the presence of DSE in *Galium odoratum*. Aluminium concentration in *Maianthemum bifolium* tended to be lower when FE colonisation was high, suggesting a possible role for the fungi in plant protection against Al. We suggest that FE and DSE may replace AM fungi in herbaceous vegetation at extremely low pH, counteracting some of the negative effects of high soil acidity on plants.

Keywords: Acid soil; Arbuscular mycorrhiza; Dark septate endophytes; Fine endophytes; Forest ground vegetation; Linear mixed-effects model; pH gradient; Root colonisation

1. Introduction

Acid soils are harsh environments for plants; high concentrations of Al, H and Mn, and low availability of Ca, Mg, K, P and NO_3 decrease the chances for many species to establish and survive. Aluminium and H can replace Ca in the cell wall of plant roots, thereby inhibiting root elongation and subsequently the uptake of nutrients (Koyama et al., 2001; Barceló and Poschenrieder, 2002; Postma et al., 2005). High concentrations of Al, Mn and H can also inhibit uptake of other nutrient cations, such as Ca, K and Mg, by competing for uptake sites or by changing membrane surface potentials (Kinraide et al., 1992; Horst, 1995). Manganese phytotoxicity on acid soils may occur at extreme Mn concentrations and causes wrinkled leaves with necrotic spots (Marschner, 1995; Nogueira et al., 2002).

Still, many plants thrive at a soil solution pH below 4, on soils with high metal concentrations and low in nutrients (Falkengren-Grerup, 1994). Plants may have special mechanisms to counter-act the acid environment, such as exudation of organic anions that chelate toxic metals or release soil P (Ström et al., 1994; Schöttelndreier et al., 2001; Kochian et al., 2004 and references therein). Another way for plants to overcome adverse soil conditions is the symbiotic association with fungi. Ectomycorrhizal fungi, for example, have the potential to decrease Al toxicity in trees by retaining Al in the fungal hyphae and mantle around the root tip and by improving nutrient uptake (Ahonen-Jonnarth et al., 2003; Moyer-Henry et al., 2005). Arbuscular mycorrhizal (AM) fungi do not form a

*Corresponding author. Tel.: +46 46 2228968; fax: +46 46 2224716.
E-mail address: jacqueline.postma@ekol.lu.se (J.W.M. Postma).

0038-0717/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2006.08.007

protective mantle and their effect on Al and Mn concentrations in colonised plants varies; concentrations of Al in shoots and roots may even increase compared to in non-mycorrhizal plants (Clark, 1997; Lux and Cumming, 2001). Yet, AM fungi may alleviate acid soil stress in plants, either by improved nutrient uptake or possibly by sequestering Al (Clark and Zeto, 1996; Lux and Cumming, 2001; Yano and Takaki, 2005). Many mycorrhizal fungi are, however, sensitive to low pH and high Al concentrations and the pH range in which plants can benefit from the mycorrhizal symbiosis may be rather narrow (Bartolome-Esteban and Schenck, 1994; Clark, 1997; van Aarle et al., 2002; L.O. Nilsson, doctoral thesis, Lund University, 2004).

Besides AM fungi, there are often two other types of root colonising fungi in forbs that may influence plant performance. Fine endophytes (FE) and dark septate endophytes (DSE) can co-occur with mycorrhizal fungi, but they occurred more frequently than AM under harsh climatic conditions, such as high altitude and semi-arid conditions (Barrow, 2003; Olsson et al., 2004; Addy et al., 2005). FE dominated at pH 4.5 but grew poorly at pH 6.5–7 in a soil liming experiment (Wang et al., 1985). DSE were able to hydrolyse P sources and make them available to the plant. They also improved a plant's ability to withstand drought and reduced infection by pathogenic fungi (Jumpponen and Trappe, 1998; Addy et al., 2005; Mandyam and Jumpponen, 2005). DSE may be beneficial to their host plant but it is not clear to what extent the colonisation is similar to that of mycorrhizal fungi or if the symbiosis is mutualistic (Addy et al., 2005).

Acidification of forest soils, as seen for example in Sweden, may restrict the distribution of plant species that are sensitive to acid environments (Falkengren-Grerup et al., 1995; Brunet et al., 1996; Olsson and Kellner, 2002). It is important to understand how such acidifuge plants interact with the soil, including possible mediation by root fungi, to be able to understand the impact of a changing environment. Read (1991) hypothesised that the importance of AM colonisation decreases with decreasing pH, when limitation of N becomes more important than that of P. The aim of our study was to relate the fungal colonisation of AM, FE and DSE in four forbs to a pH gradient in beech forest soils in southern Sweden. The plant species, *Galium odoratum*, *Maianthemum bifolium*, *Mercurialis perennis* and *Stellaria nemorum*, are typical for the ground vegetation in acid beech forests in the studied area (Falkengren-Grerup, 1990). The ground vegetation is believed to shift with decreasing pH from AM in deciduous forests to ericoid mycorrhizal in boreal forests (Read and Perez-Moreno, 2003) and our investigation focused on a pH gradient over two pH units in the former. We hypothesised that the abundance of AM is lower at low pH, because of the sensitivity of the fungi to high H concentrations, and that FE and DSE colonisation are more abundant at low pH, based on the ability of these fungi to withstand extreme environmental conditions.

2. Material and methods

2.1. Plant and soil material

In an earlier study specimens of *Maianthemum bifolium* L., *Galium odoratum* L., *Mercurialis perennis* L. and *Stellaria nemorum* L. were collected in late June–early July 1987 in beech forest sites in Skåne (southernmost Sweden), with a soil pH (0.2 M KCl) between 2.98 and 5.35 (Falkengren-Grerup, 1990). Ten sites, with two 0.5×0.5 m plots per site, were used for each species. Several sites had more than one species, resulting in 80 plots from 28 sites. Soils at these sites were mull soils, with the exception of the most acidic *Maianthemum bifolium* sites, which had mor soils. The soil at 0–5 cm below the litter layer was sampled and all individual plants collected for each plot. The plants were pressed between papers sheets, dried at 40 °C and stored at room temperature.

Fresh soil samples were extracted with 0.2 M KCl for pH measurement, and with ammonium acetate (pH 7.0) for analysis of exchangeable cations (Ca, Mg, K, Mn, Zn). Soil organic matter (SOM) was measured as loss-on-ignition. Clay content was determined by a combined hydrometrical and gravimetrical technique. Leaf material was digested in concentrated HNO_3 and analysed for Al, B, Ca, Fe, K, Mg, Mn, P and Zn, using inductively coupled plasma—atomic emission spectroscopy (ICP–AES), with the exception of leaf N, which was analysed with the Kjeldahl method. The chemical composition of leaf samples was determined for 17–19 out of 20 plots for each plant species. See Falkengren-Grerup (1990) for further details. Data for soil and plant analyses are given in Table 1. The soil pH correlated with the soil concentrations of exchangeable Ca ($r = 0.77$, $P < 0.001$), Mg ($r = 0.49$, $P < 0.001$) and Mn ($r = -0.58$, $P < 0.001$). Soil K and Zn did not show a consistent change with pH.

2.2. Fungal root colonisation

In 2005, fine roots along the rhizomes of two to four plants per species were pooled for each plot, for determination of root colonisation. *Stellaria nemorum*, as a member of the *Caryophyllaceae*, was not expected to be colonised with AM fungi (Harley and Harley, 1987) and represented in this investigation the non-mycorrhizal plant species. Roots were cut in 1–2 cm pieces and stained, using a modification of the method described by Phillips and Hayman (1970). Roots were cleared for 30 min in 10% KOH at 80 °C and subsequently rinsed with demineralised water and then soaked in 1% HCl. Samples were stained overnight in 0.1% Trypan blue in 1:2:2 lactic acid:glycerol: water. The roots were again rinsed with demineralised water, left for 3–4 days in 1:1 glycerol:water for destaining and mounted on microscope slides.

The magnified intersections method (McGonigle et al., 1990) was used for counting the proportion of root length colonised with AM, FE and DSE. For each sample,