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Root colonization of bait plants by indigenous arbuscular mycorrhizal fungal communities is not a suitable indicator of agricultural land-use legacy

Jan Jansa[a,*], Veronika Řezáčová[a], Petr Šmilauer[b], Hans-Rudolf Oberholzer[c], Simon Egli[d]

[a] Laboratory of Fungal Biology, Institute of Microbiology, Academy of Sciences of the Czech Republic, 142 20 Prague, Czech Republic
[b] Faculty of Science, University of South Bohemia, Branišovská 31, 37005 České Budějovice, Czech Republic
[c] Agroscope Reckenholz-Tänikon ART, Reckenholzerstrasse 191, 8046 Zürich, Switzerland
[d] Swiss Federal Research Institute for Forest, Snow & Landscape WSL, Zürcherstrasse 111, 8903 Birmensdorf, Switzerland

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ABSTRACT

Arbuscular mycorrhizal (AM) fungi are present in all soils and comprise an important component of soil biota with respect to plant nutrition and growth as well as soil quality. Previous research in a number of agroecosystems had documented large impacts from various management practices (e.g., tillage, fertilization, crop rotation) on the levels of host root colonization and/or composition of the AM fungal communities in the roots and soil. Here, we tested whether a standardized mycorrhizal bioassay could contribute to deciphering soil-use legacy through detection of consistent changes in the colonization pattern of a mycorrhizal bait plant (leek). To this end, we grew the leek in a large number (154) of soils under uniform environmental conditions. Most of the variation in the mycorrhizal colonization of bait plants grown in the different soils could be attributed to soil properties and sampling site altitude, whereas the occurrence of specific structures (vesicles) was particularly correlated with abundance of certain AM fungal taxa such as *Rhizophagus* sp. Contributions of the root colonization of bait plants to explaining agricultural management practices and soil heavy metal concentrations (frequently used as indicators of soil quality) were comparatively small and partly counterintuitive. For example, higher incidence of arbuscules was detected in soils to which mineral fertilizers were applied as compared to organically farmed soils. Moreover, the explanatory power of the bioassay was much less than was that for soil respiration, a well established bioindicator of soil quality. Therefore, the mycorrhizal bioassay tested here appears not to be suitable as an interpreter of soil-use legacy, even though it does uncover very clear trends in colonization patterns of the bait plants by indigenous AM communities across large environmental gradients.

1. Introduction

Arbuscular mycorrhizal (AM) symbiosis is an evolutionarily ancient type of plant–fungal coexistence. Globally widespread and present in virtually all soils, it is established by a majority of extant plants species, including many crop plants (Smith and Read, 2008; and references therein). The fungi involved in AM symbiosis form a taxonomically and functionally well delimited group (Schüßler et al., 2001; Tisserant et al., 2013), with a multitude of important and well-documented roles in ecosystem nutrient and carbon fluxes, as well as maintenance of ecosystem diversity and resilience (van der Heijden et al., 1998; Helgason et al., 2007; Johnson et al., 2010; Lendenmann et al., 2011).

In most soils, the AM fungal communities are composed of dozens of species or molecular phylotypes (Jansa et al., 2002; Dumbrell et al., 2011; Verbruggen et al., 2012; Moora et al., 2014; Kohout et al., 2015), and it has been demonstrated that AM fungal community composition and/or activity (expressed for example as the intensity of host root colonization or development of AM hyphal networks in soil) may depend on such various factors as soil properties, climate, geography, and such land management practices as mineral fertilization, crop rotation, and tillage (Hendrix et al., 1995; McGonigle et al., 1999; Jansa et al., 2003, 2014; Moora et al., 2014; Säle et al., 2015; Soudzilovskaia et al., 2015). The AM fungi would thus be particularly well suited as universal bioindicators of soil quality, environmental

* Corresponding author.
E-mail address: jansa@biomed.cas.cz (J. Jansa).

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deterioration, and/or ecosystem resilience if the contribution of the soil and climate could be unequivocally separated from the land-use impact. Although some previous studies had identified potential (qualitative) bioindicators of land-use intensity among the taxa within indigenous AM fungal community profiles (Oehl et al., 2003; Tchabi et al., 2008; Verbruggen et al., 2010; Moora et al., 2014), others had indicated that the effect of land management was comparatively small relative to such other contributing factors as soil type, soil physico-chemical properties, and geography (Oehl et al., 2010; Jansa et al., 2014) and that the richness rather than the exact composition of AM fungal communities would correlate with land-use intensity (Verbruggen et al., 2012; Xiang et al., 2014; Valyi et al., 2015). The latter means that analyses of entire AM fungal community profiles would be required to decipher land-use legacy. That may be impractical due to high costs and time requirements as well as prone to a number of biases associated with current molecular approaches (Cotton et al., 2014; Kohout et al., 2014; Řezáčová et al., 2016). Moreover, to uncover the true potential of AM fungal communities as bioindicators of soil quality, validation studies on large spatial scales covering long soil and climate gradients are necessary.

The extent of root colonization by mycorrhizal hyphae, arbuscules and vesicles, or other structures such as coils assessed by various microscopic approaches on stained or unstained roots (Trouvelot et al., 1986; McGonigle et al., 1990; Vierheilig et al., 2005) is still the most commonly recorded parameter of AM fungal abundance *in planta* (Treseder, 2013). Nevertheless, this approach has been recognized to have certain limitations. These limitations are mostly due to the differential staining properties of the various AM fungal taxa (Vierheilig et al., 2005) and, at least in case of the so-called magnified intersection method (McGonigle et al., 1990), flattening differences in colonization intensity per root fragment (Gamper et al., 2008; Thonar et al., 2012). The abundance of AM fungi in roots and soil has been proposed to correlate positively with soil quality and sustainable farming practices and negatively with intensive land use, particularly with frequent and deep tillage or other types of soil disturbance and with mineral fertilization (Alguacil et al., 2010; Bedini et al., 2013; Spurgeon et al., 2013). The levels of root colonization also depend, however, on the identity of the host plants (Wilson and Hartnett, 1998), and it has recently been postulated that the patterns of root colonization by mycorrhizal fungi (in fields) are strongly governed by climate (Soudzilovskaia et al., 2015).

Thus, we address here whether a carefully conducted mycorrhizal bioassay under controlled conditions could indicate soil quality, land-use intensity, and/or environmental pollution across large environmental gradients, and whether this information is sufficiently robust as to be implemented in soil conservation monitoring. To this end, we grew the same bioassay bait plant (leek) in pots filled with the different field soils under strictly controlled environmental conditions, thereby avoiding potential climate- and plant identity-driven biases associated with measurement of mycorrhizal root colonization in fields. We asked the following questions:

1. Is there significant variation in mycorrhizal activity (here defined as the capacity of the indigenous AM fungal communities to colonize the roots of a bait plant in a mycorrhizal bioassay) among the different soils covering large spatial, soil, and land-use gradients?
2. If yes, what explains the variation recorded among the sites? Could the variation be specifically attributed to spatial dispersion of the sites, soil properties, land-use features and/or local AM fungal community composition?

2. Material and methods

2.1. Mycorrhizal bioassay

Soils samples were collected from 154 individual sites (described previously by Jansa et al., 2014; see Appendix A for details) in spring and summer 2010 within two weeks after regional snowmelt and then processed as described earlier (Jansa et al., 2009, 2014). Selection of sampling sites covered large spatial and environmental gradients. Distances between sampling sites were as much as 273 km, and altitudes of the sites ranged between 270 and 2240 m a.s.l. Land-use variables for the sampling sites and soil physico-chemical properties are reported in Tables 1 and 2, respectively. Soil was collected from each sampling site using a soil auger (3 cm in diameter) from a depth of 0–20 cm. Specifically, multiple (30–100) soil cores were collected from a 10 × 10 m plot at each sampling site, totaling approximately 5 kg of fresh soil per site. Within four days of soil collection, unsterile field soil (passed through a 5-mm sieve) from each of the sites was filled in six replicate pots, 250 ml each, and the remainder was stored either fresh at 4 °C for soil respiration and microbial biomass assessments, as described by Fließbach et al. (2007), or dried at room temperature, sieved down to 2 mm, and used for other soil analyses as described previously (Jansa et al., 2014). The pots were sown with 15–20 leek (*Allium porrum* L. cv. Dubouchet Selma) seeds and incubated for a total of 60 days in a growth chamber. Seedlings were thinned to four individuals per pot at three weeks after sowing. The growth conditions were as follows: temperature 24/20 °C day/night, 16 h photoperiod, 100 μmol m^{-2} s^{-1} photosynthetically active radiation, and 60% aerial humidity. The pots were watered daily with deionized water to maintain the soil water content between 60% and 80% of the water holding capacity. Following the 60-day growth period, the shoots were harvested from each individual pot, dried at 70 °C for 48 h, and the dry weights recorded. Thereafter, the shoot samples from the replicate pots established with the same soil were pooled, milled and the carbon and nitrogen (N) concentrations measured in these pooled samples using a FlashEA 1112 NCS Analyzer (ThermoElectron, Ecublens, Switzerland). The roots from each individual pot were washed free of soil, cut into 1 cm fragments and mixed. The roots from each pot were split into two subsamples and frozen at −80 °C. One root subsample was used for molecular profiling of AM fungal communities as described earlier (Jansa et al., 2014) and the other for microscopic estimation of the root colonization by AM fungi. This microscopic estimation was carried out following root staining according to Hartwig et al. (2002), employing the magnified intersection method (McGonigle et al., 1990), scoring 50 intersections per sample and recording the proportion of root intersections (equaling the proportion of root length) colonized by hyphae, arbuscules, and vesicles (see Appendix B for the data).

2.2. Mycorrhizal fungal community profiling

The AM fungal communities in the leek roots (three randomly chosen pots analyzed separately for each soil) were profiled by quantitative real-time polymerase chain reaction (qPCR) with hydrolysis probes as described previously (see Table C1 in Appendix C for details). To this end, we analyzed abundances of six AM fungal taxa, including both generalists and specialists (*Funneliformis mosseae* Walker & Schüβler, *Claroideoglomus claroideum* Walker & Schüßler, *Rhizophagus irregularis* Walker & Schüßler, *Gigaspora margarita* Becker & Hall, *Cetraspora pellucida* Oehl, Souza & Sieverding, and *Diversispora celata* Gamper, Walker and Schüβler), for which we had the qPCR markers ready and validated. The qPCR results were corrected for recovery of internal