Determining the existence, direction and intensity of a common mycelial network (CMN) in a desert truffle symbiosis

Introduction

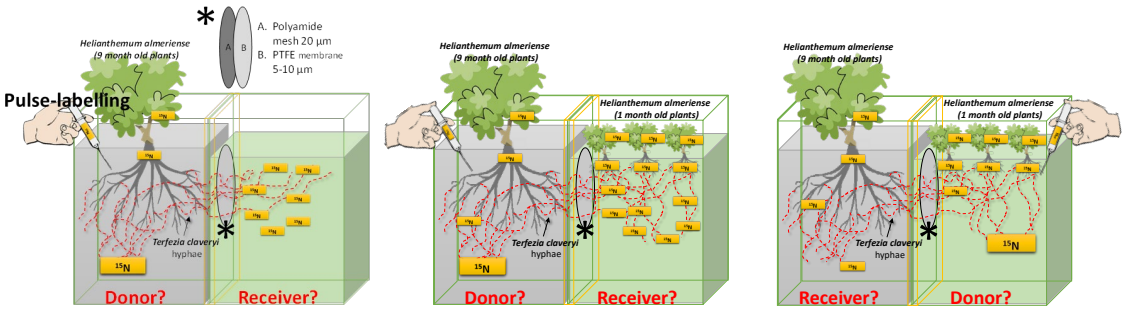
Plant productivity and community succession may be facilitated by an extensive CMN, favouring the performance among plants, where a larger nurse/donor plant may provide resources, such as water, nitrogen (N), phosphorus (P) and C, to new seedlings that become more rapidly colonized, thus improving their establishment. The facilitation process may have a special importance in Mediterranean ecosystems, where early-successional shrubs (*e.g.* Cistaceae) undergo summer drought that drastically limits plant establishment (Richard *et al.* 2009). It is therefore essential to develop an understanding of the basic functioning of these CMNs in such climate-sensitive environments, as they could play a key role both in slowing down desertification processes and in the ecological succession of plant communities under a climate change scenario.

The main objective of Prof. Asunción Morte's visit is to become familiar with and learn the nitrogen stable isotope labelling technique, which is routinely conducted at the Soil Science Institute in Hanover. Prof. Morte's visit was framed within the experiment on the existence of a common mycelial network (CMN) between *Helianthemum almeriense* plants forming mycorrhizal symbiosis with the desert truffle *Terfezia claveryi* and its role in nitrogen exchange between the symbionts. The experiment is part of the work developed by PhD student Aline Fernandes Figueiredo, in the framework of the DFG project 1798 (Signalling at the Plant-Soil Interface).

Material and methods

To elucidate the existence of a CMN between *H. almeriense* plants mediated by *T. claveryi*, three complementary tests were performed, as described below in Figure 1. Forty-two seedlings of *Helianthemum almeriense* were inoculated with *Terfezia claveryi*. Mycorrhizal colonization was checked before transplanting into mesocosms. A two-chamber mesocosm system allows the fungal mycelium to develop in a separate compartment (Fig. 2a, b). Both compartments are separated by a polyamide (PA) mesh (20 µm) (Franz Eckert GmbH) and a polytetrafluoroethylene (PTFE) membrane (5-10 µm) (Pieper Filter GmbH). The PA mesh stops root growth, while the hydrophobic PTFE membrane allows the hyphae to cross but prevents mass flow and diffusion of ions into the other compartment, being the fungus the only responsible for transporting the stable isotopes from one compartment to the other.

Mycorrhizal plants were cultivated in these mesocosms during four months in growth chamber at 24ºC and 16 h photoperiod and watered when needed (Fig. 2c). After his period, the presence of *T. claveryi* mycelium, in the adjacent compartment of the mesocosm, was verified by DNA extraction from soil, using DNeasy PowerSoil Kit, and quantified by real-time PCR using specific primers (Arenas 2021). After that, surface sterilized *H. almeriense* seeds were placed in the adjacent chambers of the mesocosms. These seedlings were grown in this compartment for two months (Fig. 2b), after which they were found to be mycorrhizal with the mycelium having penetrated the mesocosm membranes. To determine the existence of a CMN, a ^{15}N pulse-chase labelling, using tracer ammonium nitrate-$^{15}N_2$ (Sigma 366528) in a solution of 6.2 mM, was performed in a two-chamber mesocosm system.



Test 1: Is the CMN homogeneously spreading the nutrients (N) or only where it is really required?

Test 2: Does the mycorrhiza in the compartment of the larger plant behave as an N donor with the seedlings, with which it shares a CMN, when it detects an N-rich patch?

Test 3: Does the mycorrhiza in the compartment of the seedlings behave as an N donor with the larger plant, with which it shares a CMN, when they detect an N-rich patch?

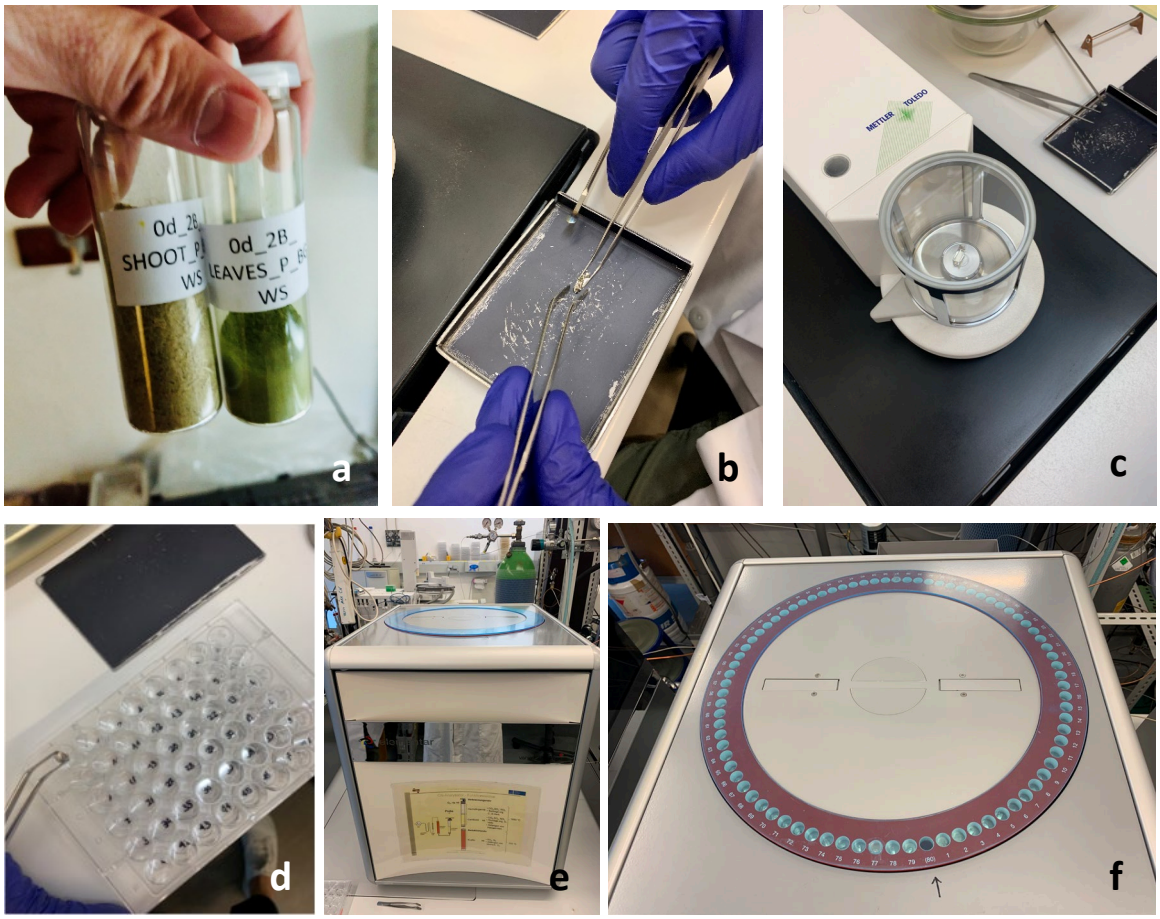
Figure 1. Set of different ^{15}N labelling tests to determine the existence and directionality of a common mycelial network (CMN) between *Helianthemum almeriense* plants and the fungus *Terfezia claveryi.*

The aim is determining the direction and intensity of N exchange through the mycelial network. In order to establish the temporal dynamics of ^{15}N, four biological replicates were sampled of each mesocosm per test, at time intervals covering up to 0 (on the day labelling to detect the ^{15}N background values with which to calculate the acquisition of ^{15}N in the different tissues), 7 and 14 days.

Figure 2. Mesocosm with adult mycorrhizal plant in one compartiment and young seedlings in the other compartiment separated by PA and PTFE membranes (a, b). Growth chamber with the mesocosms at

24ºC and 16 h photoperiod (c). Mycorrhizal colonization of H. alemriense roots with T. claveryi (d). ^{15}N labelling in the soil of the different compartiments (d).

Shoots with leaves, roots, and substrates from both compartments were sampled after each harvesting time point. The samples were freeze-dried and ball-milled, and aliquots were weighed into tin capsules for isotope ratio mass spectrometry analysis. Total N contents plus the $\delta^{15}N$ ratio were determined with the elemental analyzer Isotope cube (Elementar GmbH, Hanau, Germany) connected to an Isoprime 100 isotope ratio mass spectrometer (Elementar GmbH, Hanau, Germany) (Figs. 3 d,e,f).



Figue 3. Freeze-dried and ball-milled samples (a). Aliquots weighed into tin capsules for isotope ratio mass spectrometry analysis (b, c). Total N contents plus the $\delta^{15}N$ ratio were determined with the elemental analyzer Isotope cube connected to an Isoprime 100 isotope ratio mass spectrometer Elementar GmbH (d, e, f).

Results and discussion

T. claveryi mycelium, in the adjacent compartments of the mesocosm, was quantified by real-time PCR with values ranged between 5.48 – 29.12 mg mycelium/ g soil and was only not detected in two samples (Table 1). These values are similar to other found in soils from *T. claveryi* plantations (Arenas 2021). Therefore, we can state that the mesocosm conditions did not affect the mycelial development of *T. claveryi*.

Table 1. Total mycelial biomass of *T. claveryi* (mg mycelium/g soil) detected by real-time PCR in 22 mesocosm compartiments without mycorrhizal plants, before planting the seeds.

sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
mg myc/ g soil	12.04	17.73	ND	9.64	12.90	15.23	5.78	ND	17.54	13.60	22.97
sample	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**
mg myc/ g soil	17.33	11.05	7.21	13.30	12.31	9.76	29.12	5.48	9.84	18.25	6.26

In addition, this mycelium was able to mycorrhizal colonize the seedlings germinating in the adjacent compartments, mycorrhizal colonization of the young seedling, after two months and before the ^{15}N labelling, varied from 30% until 35% (Fig. 2d).

Results of the total ^{15}N content of shoots, leaves, roots and soils from the three tests are being analyzed.

References

Arenas F (2021) Analysis of mycelial growth and development of the desert truffle *Terfezia claveryi* Chatin and microorganisms associated to desert truffle mycorrhizal plants. Doctoral Thesis, Universoty of Murcia.

Richard F, Selosse MA, Gardes M (2009) Facilitated establishment of *Quercus ilex* in shrub-dominated communities within a Mediterranean ecosystem: Do mycorrhizal partners matter? FEMS Microbiol Ecol 68:14–24

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