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Ecology of vertical life in harsh environments: The case of mycorrhizal symbiosis with secular cliff climbing trees (*Juniperus phoenicea* L.)

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ABSTRACT

The Phoenician juniper tree (*Juniperus phoenicea* L.) is emblematic of Mediterranean ecosystems. Secular trees from a relict population are present in the cliffs of the Ardèche gorges (protected natural reserve). This population shows a remarkable adaptability to major physical, nutrient and water availability constraints. The basis of its adaptation to hostile environmental conditions is poorly understood. The aim of the study is to decipher the diversity and structure of the Phoenician juniper mycorrhizal community in order to highlight mycorrhizal characteristics related to the particular ecology of this relict population. We hypothesized that a long-term co-evolution between arbuscular mycorrhizal (AM) fungi and the plant partner, emphasized by a geographic isolation, may have selected a specific mycorrhizal community playing a key role in *J. phoenicea* adaptation. 454-sequencing of AM fungal community from Phoenician juniper secular trees revealed a complex community, notably composed of *Rhizophagus* and poorly-affiliated *Glomeraceae* clades. The AM fungal community characterized was mainly related to those found in arid and semi-arid habitats, strengthening the ecological specificity of the Phoenician juniper AM fungal communities to harsh environmental conditions.

1. Short note

Juniperus phoenicea L. (family *Cupressaceae*) is a small (8–12 m tall) gynodioic tree native to the Mediterranean basin, mainly distributed in its western part. It is a light-demanding tree resisting to dry climates and characterized by high pioneer properties (Quézel and Médail, 2003). Populations of *J. phoenicea* subsp. *turbinata* are characteristic of the coastal areas while *J. phoenicea* subsp. *phoenicea* are growing up to 2000 m (*e.g.* on the Atlas mountain slopes). The subsp. *phoenicea* shows a low level of geographic differentiation and a conservation of local characters by isolation from other subspecies (Boratynski et al., 2009).

In France, populations of *J. phoenicea* subsp. *phoenicea* stretch to the nature reserve of the Ardèche gorges (Mathaux et al., 2016), in the south of Rhône-Alpes. This reserve is characterized by a 200-m deep canyon composed of urgonian limestones and an extensive plateau mainly covered by typical Mediterranean forest. Old Phoenician juniper trees found on the large walls of the limestone cliffs have adapted their growth to the topological and physico-chemical constraints (Mathaux et al., 2016). This ecosystem, functioning for millennium – trees have been dated to be thousand-year-old (Mathaux et al., 2016) – is probably among the last native ones in France, representing a possible relict population (geographic and physical isolation, confined distribution). Its natural regeneration is reaching a critical threshold due to erosion and to the attested warming in the Rhône-Alpes region (Météo-France Centre-Est, 2011). Phoenician juniper trees establish a symbiosis with arbuscular mycorrhizal (AM) fungi (Boullard, 1986), a key parameter in ecosystem functioning (Smith and Read, 2008). Characterizing Phoenician juniper mycorrhizal diversity may represent a major insight to better understand the biological functioning of this particular ecosystem.

Three secular Phoenician trees (J1, J2, and J3; Fig. 1) located on the reserve canyon cliffs (Gaud, Saint-Remèze, France; Fig. A1) were

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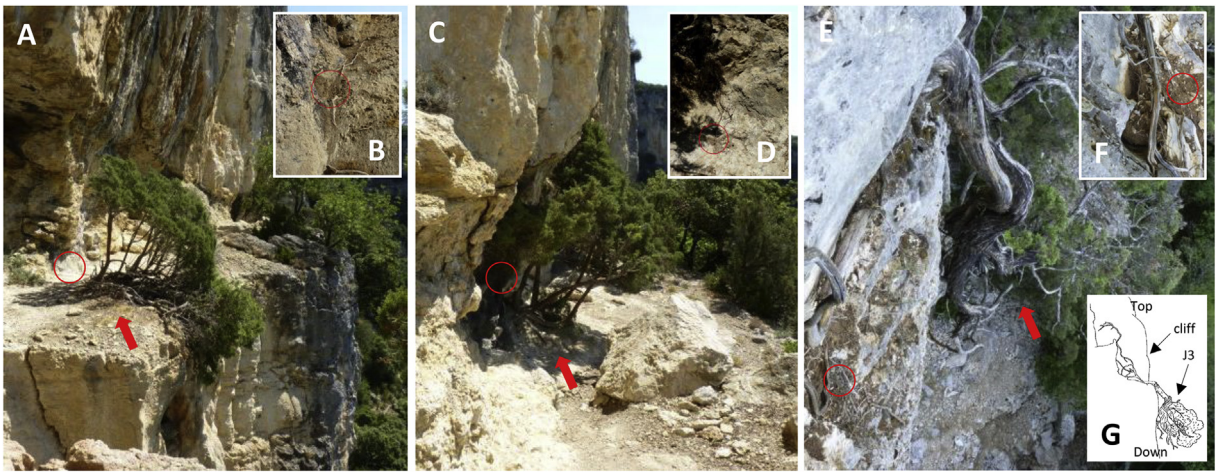

Fig. 1. Photos of selected *J. phoenicea* trees in the protected nature reserve of the Ardèche gorges (Gaud, Saint-Remèze, France). Phoenician juniper trees (A) J1, (C) J2 and (E) J3 are indicated with red arrows. Red circles indicate root sampling area for each tree, visualized more precisely in (B), (D), (F) for J1, J2 and J3, respectively. (G) Schema of the general morphology of J3 growing downwards on the cliff. The trees J1 (44°21′57″N – 4°27′06″E, 268 m altitude) and J2 (J2, 44°21′56″N 4°27′08″E, 268 m altitude) are distant from 25 m and are both located on a 2-m wide rocky shelf (A and C) used as pathway by feral goats. The tree J3 (44°21′44″N, 4°26′58″E, 190 m altitude) presents an original morphology since it grows down directly along the cliff (G). The juniper roots were shown to penetrate in rock crevices partially filled by soil deposits (B, D and E). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

reached using rock-climbing equipment. The selected trees are hundred-years-old (141, 128 and 216 years old for tree J1, J2, and J3, respectively), and are characterized by their extremely slow radial growth rates (0.37, 0.21 and 0.25 mm per year, respectively) (Appendix). Only three trees could be sampled because of the very low accessibility of root systems (rock crevices) and tree instability.

A 18S-based 454-pyrosequencing approach was applied to characterize the Phoenician juniper AM fungal community. The 18S rRNA gene as well as the primer set were selected because of their reliability to assess AM fungal diversity in environmental samples (Öpik et al., 2013; Van Geel et al., 2014). 454 data-processing (for more details, see Appendix file) lead to 4647 high quality sequences from approximately 10,000 raw sequences. All the analyses were performed both with and without singletons because it has been shown that half of the singletons may represent true target taxa (Brown et al., 2015). Finally, the sequence number between environmental samples was rarefied to improve statistical robustness.

The AM fungal community was composed of 77 molecular operational taxonomic units (MOTUs) (42 without singletons), based on a 97% sequence similarity. A high diversity coverage (>97%) was reached, with less than 10 MOTUs not retrieved (Boneh estimates) (Table 1), demonstrating a suitable sequencing effort for comparison of community richness, diversity and composition between environmental samples (Lemos et al., 2011). The AM fungal diversity and evenness among trees tended to be different, contrary to community richness (Table 1).

Approximately 80% of sequences were shared between at least two trees. Differences in the AM fungal community were only due to low abundant MOTUs membership (Fig. A2). On the other hand, the genetic structure of the AM fungal community was significantly different among trees ($P < 0.001$; Unifrac tests on genetic distances; Table A1). Phylogeny-based approaches are demonstrated as powerful approaches compared to similarity-based approaches to reliably analyse microbial community in environment (Senés-Guerrero and Schüßler, 2016). The differences observed in the AM fungal community structures among the juniper trees might be related to abiotic factors. The soil deposits surrounding the juniper roots showed variations for several chemical parameters ($CaCO_3$ content, SOC and SOM; Table A2) between J3 and the two other trees. Heterogeneity of physical and nutritive constraints and water availability are known as potential drivers of mycorrhizal communities (Tedersoo et al., 2003; Johnson et al., 2010; Jumpponen et al., 2010). The variation in abiotic factors were postulated as the main factor driving both plant and AM fungal communities in

Table 1
Richness and diversity indexes.

Juniper tree[a]	Number of MOTUs	Chao (lci; hci)[b]	Shannon (lci; hci)[b]	Inverse simpson (lci; hci)[b]	Simpson evenness	Shannon evenness	Coverage (%)[c]	Boneh estimation
J1	42	70 (52; 121)	1.55 (1.46; 1.64)	3.10 (2.93; 3.28)	0.07	0.41	97.69	7.58
J1(- s)	21	21 (21; 24)	1.41 (1.34; 1.49)	2.96 (2.82; 3.13)	0.14	0.46	99.79	3.29
J2	26	61 (36; 152)	0.96 (0.87; 1.06)	1.59 (1.50; 1.69)	0.06	0.30	98.49	4.37
J2(- s)	21	21 (21; 21)	0.90 (0.85; 0.96)	1.56 (1.50; 1.63)	0.07	0.29	99.95	0
J3	20	48 (27; 133)	0.51 (0.43; 0.60)	1.22 (1.18; 1.27)	0.06	0.17	98.89	3.26
J3(- s)	13	14 (13; 24)	0.45 (0.39; 0.52)	1.20 (1.16; 1.24)	0.09	0.18	99.75	1.34

[a] J1, J2, J3 indicate the juniper trees 1, 2 and 3, respectively. (- s) indicates the data without the singletons. All data correspond to normalized data based on random subsamplings.
[b] lci and hci indicate the lower and higher 95% confidence intervals, respectively.
[c] Good's coverage: sum of probabilities of observed classes calculated as $(1 - (n/N))$, where n is the number of singleton sequences and N is the total number of sequences.