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Effect of temperature on ectomycorrhizal fungi associated with *Pinus sylvestris* L. in organic *vs* mineral soils.

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ABSTRACT
Ectomycorrhizal fungi of *Pinus sylvestris* L. were characterized in two contrasted soils. The effect of temperature on mycorrhization and fungal diversity was also tested. Eight fungi were identified by morphotyping and a total of thirteen by sequencing. Both soils had a similar number of fungi, although completely differed in the identity of the dominant ones. The number of fungi and ectomycorrhizas per seedling, together with fungal diversity significantly decreased at high temperature. Nearly half of fungi were not found forming mycorrhizas at the highest temperature and contrarily, only one fungus significantly increased in frequency with this factor. Our results emphasize the strength of environmental conditions affecting host mycorrhizal rates and structuring EM communities, which might have profound implications in forest dynamics.

INTRODUCTION
Pinus sylvestris is a representative ectomycorrhizal (EM) tree species highly spread in Europe. Ectomycorrhizas are the symbiotic association between roots and fungi in which basically, the fungus improves plant water and nutrient uptake in exchange of carbohydrates (Smith and Read, 1997). The EM symbiosis plays an essential role in plant nutrition and forest dynamics, which can be crucial especially under unfavourable environmental conditions such as those related to global change (i.e. drought, increased temperature) (Smith and Read, 1997). The objectives of this work were to characterize the EM fungi associated with *P. sylvestris* growing in two contrasted soils, and to check how different temperatures could affect the mycorrhizal status of seedlings and the diversity of their fungal associates.

MATERIAL AND METHODS
Soils were collected in a *P. sylvestris* forest (Valsaín, Segovia), in an altitudinal gradient: a) mineral soil at 1.250 m, and b) organic soil at 1.850 m. Seedlings were grown in containers filled with each soil, at three different temperatures (day/night): a) low 10/15 °C, b) medium 15/20 °C and c) high 20/25 °C. A total of six treatments were established with four replicates in each. Four months after, EM percentages were assessed, and ectomycorrhizas classified by morphotypes (Agerer, 1995) and collected for DNA extraction (Rincón *et al.* 2007). The internal transcribed spacer region (ITS) of the rDNA was PCR-amplified with the primers ITS1F/ITS4 (Gardes and Bruns, 1993), the PCR-products digested by endonucleases (*Hinf I*, *Msp I*), and restriction patterns (RFLPs) analysed. Samples sharing identical RFLP profile were classed as unique Operational Taxonomic Units (OTU). Each OTU was sequenced and tentatively identified by comparison with sequences in the GenBank database. The relative frequencies of each fungal OTU were calculated and analysed by t-Student test ($P \leq 0.05$). The EM percentages, the number of OTUs per seedling and the fungal diversity (Shannon index) were analyzed by one-way analysis of variance (ANOVA) and differences among treatments separated by the Tukey test ($P \leq 0.05$). All analyses were done with the SPSS 19.0 software.

RESULTS AND DISCUSSION

Characterization of ectomycorrhizal fungi of organic and mineral soils

Eight EM morphotypes were classified attending to morphological features, whereas sequencing allowed identifying a total of 13 different fungal OTUs. This highlighted the convenience of using a combined morphological and molecular approach for identifying EM fungi, which might have highly similar and/or cryptic characteristics leading to confusing identification (i.e. *Pezizales*) (Tedersoo *et al.*, 2006).

A total of 12 and 10 OTUs were respectively identified in the organic and mineral soil, with 69 % of OTUs found in both soils. *Thelephora terrestris* Ehrh., *S. luteus* and Unkown-5 were only found in the organic soil, and Unkown-9 only in the mineral one. In both soils, the fungal community structure was similar with few dominant fungi and many less frequent ones, although the specific composition clearly differed between soils. In the organic one, the most frequent fungi were: Unkown-3 (Archaeorhizomycetes), *Suillus bovinus* (Pers.) Roussel and *T. terrestris*, whereas in the mineral soil were Unkown-2 (*Atheliaceae*), Unknown-4, *Cenococcum geophilum* Fr. and *Wilcoxinia* sp. Fungi Unk-2 and Unk-4 significantly decreased in frequency in the organic soil compared with the mineral one ($P = 0,00$; $P = 0,01$), whereas the opposite happened to *T. terrestris* and *S. bovinus* ($P = 0,04$; $P = 0,04$). The results showed that both soils had a high potential of active EM fungal inoculum, but a totally different cohort of fungi distinctively adapted to mineral or organic conditions.

Effect of temperature on seedling mycorrhization and fungal diversity

The EM percentages ($P=0,00$), the number of fungi per sample ($P=0,03$), and fungal diversity ($P=0,04$) significantly decreased at high temperature. Reduced mycorrhization rates could indicate a lower symbiotic-dependence for nutrient uptake by the host, probably due to a enhanced photosynthetic activity at high temperature (Van derHeijden and Sanders, 2002). Nearly half of the fungi (46%) were not found forming mycorrhizas at the highest temperature, and three among them (Unk-5, *Tomentella* sp. and Unk-9) were only found at low temperature. The Unk-3 (Archaeorhizomycetes) was the only fungus that significantly increased in frequency in response to high temperature ($P =0,04$), even disappearing at the lowest one.

Replacements of species and changes in the relative dominance of particular fungi denoted a variable inter-specific response pointing out to temperature as an important driver structuring EM fungal communities, which might have important consequences for the functioning of forest ecosystems (Van derHeijden and Sanders, 2002).

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REFERENCES

Agerer, R. (1995). In: Varma A, Hock B (eds). Springer-Verlag. Berlin, Heidelberg. pp: 687-734.
Gardes, M., and Bruns, T. (1993). Molecular Ecology 2: 113-118.
Rincón, A., *et al.* (2007). Mycorrhiza 18: 23-32.
Smith, S., and Read, D. (1997). Mycorrhizal Simbiosis. Academic Press. New York. pp: 605.
Tedersoo, L., *et al.* (2006). New Phytologist 170:581-596.
Van der Heijden, M., and Sanders, I., eds. (2002). Mycorrhizal Ecology. Springer-Verlag. Berlin, pp: 469.