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Response of ectomycorrhizal communities to past Roman occupation in an oak forest

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ABSTRACT

The impact of past Roman occupation on the composition of ectomycorrhizal (ECM) communities was analysed in 12 Roman settlements in an oak forest in Central France. At each Roman settlement, soils and ECM roots were sampled from two plots (600 m^2 each), one plot close to the remains of the buildings (<100 m), supposed to be impacted by ancient Roman agriculture, and the second plot 250–500 m away from the remains of the buildings, supposed to be less intensively influenced by previous cultivation. Soils were analysed and ECM fungal taxa were identified by morphotyping and sequencing the rDNA ITS region. The soil properties were significantly affected by the past Roman occupation, in terms of nutrient availability, especially for P, N and Mg. The enhancement of soil nutrient levels by past Roman land-use had significantly modified alpha diversity and species composition of ECM communities. Among the 67 determined ECM morphotypes, 40 were shared by the occupied and non-occupied plots, 17 were found only in the occupied plots and 10 only in the non-occupied plots. Six morphotypes were significantly more frequent near the antique remnants. Our study showed, for the first time, that ectomycorrhizal communities are impacted by previous Roman land-use, even after nearly two thousand years of forest state.

1. Introduction

The strong impact of ancient agricultural land-use, dating from the last few centuries, on the plant species composition, ecological cycles and productivity of present day forests has been repeatedly evidenced in Europe and North America (Peterken, 1996; Hermy et al., 1999; Foster, 2002). Indeed, the complex modifications of structural and chemical properties of soils induced by past farming and manure inputs have impacted the forests which have replaced croplands (Koerner et al., 1997, 1999; Compton et al., 1998; Jussy et al., 2002). More recently, the impact of much older occupations, dating from the Roman period, on the present soil properties and biodiversity of forests has been revealed (Dupouey et al., 2002; Dambrine et al., 2007). Traces of Roman occupations have been discovered in exceptionally large areas of present forests in France (Peltre and Bruant, 1991; Dambrine et al., 2007). In the Tronçais forest of Central France, more than 100 Roman settlements have been found (Bertrand, 1996; Laüt, 2001). Dupouey et al. (2002) have shown that 200 years of farming during Roman times had induced gradients in soil nutrient availability and biodiversity that are still measurable in present forests almost 2000 years later. Up to now, phanerogamic communities only have been investigated, although other biota could also have been impacted.

In forest ecosystems, fungi are associated with the roots of trees, forming mixed symbiotic organs called ectomycorrhizas (ECMs), which perform the uptake of water and nutrients for the trees. The genetic and functional diversities of ECMs are considered key to forest soil ecosystem functioning through decomposition and mineralization of organic matter (Smith and Read, 1997). Numerous reports indicate that fertilization influences the composition and function of ECM communities. For instance, increased N deposition reduces the production and diversity of ECM sporocarps, and modifies the species composition of ECM fungi colonizing roots belowground (Taylor et al., 2000; Peter et al., 2001; Lilleskov et al., 2001, 2002; Avis et al., 2003). Moreover, Taylor and Read (1996) noted that, in the areas with high N deposition, the ECM fungi which can readily use organic N were replaced in areas with high N deposition by those which rely largely or solely upon inorganic N sources. Despite the increasingly evidences of shifts in the species composition of ECM communities following increasing N

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deposition during the last 20th century (Wallenda and Kottke, 1998; Cairney and Meharg, 1999; Lilleskov and Bruns, 2001), nothing is known about the influence of past agricultural use of soils on the ECM community composition when the formerly farmed sites have been re-colonized by forest during later historical periods. Yet, past changes in land-use are a major factor currently affecting the availability of nutrients in forest ecosystems all over the world.

Mycorrhizae play a central role in phosphorous supply and demand of plants (Koide, 1991). In a meta-analysis of mycorrhizal responses to nitrogen and phosphorus manipulation in field studies, Treseder (2004) showed that the decrease of mycorrizal abundance following phosphorus addition was stronger and more consistent across studies than the decrease following nitrogen addition. Moreover, phosphorus is the soil element whose concentration shows the most increase in the vicinity of ancient human settlements (Craddock et al., 1985). Traces of human occupation, even dating back as far as the Neolithic, are routinely mapped by soil phosphorus analysis. Thus, the hypothesis that mycorrhizae communities could be, in the long run, affected by ancient land use seems natural.

The aim of this study was to determine if the past Roman occupation of presently forested areas has durably modified the ECM community composition. For this purpose, we set up and sampled a network of paired sites, previously disturbed or undisturbed during the Roman period.

2. Materials and methods

2.1. Studied area and plot selection

The state forest of Tronçais is located in Central France (46°38'28.60″N, 2°42'58.91″E). The history of the Tronçais forest has been marked by a diversity of successive types of human occupation and management, as described in many historical documents. Recently (Bertrand, 1996; Laüt, 2001), 108 Roman settlements have been found by surface surveys of stones, tiles and ceramics, and have been dated from the 1st to the 4th century AD according to standard archaeological references. Each settlement is defined by the ruins of one or a few Roman buildings. Historical documents point to a very ancient forest recolonisation. From the 17th to the 19th century, the forest of Tronçais has been intensively exploited for wood and grazed, before being used for making charcoal. It is nowadays managed as a pure oak high forest and is the source of high-quality oak wood used for making wine casks. It covers 10,600 ha of a homogenous plateau with a mean elevation of 250 m. The canopy is dominated by sessile oak (*Quercus petraea* > 80%) mixed with beech (*Fagus sylvatica*). Hornbeam (*Carpinus betulus*) is frequent in the understory. The soils are mostly sandy acidic inceptisols developed from standstone or alluvial sand deposits with various degrees of hydromorphy. The humus form is mull to moder (see Dambrine et al., 2007).

Twelve Roman settlements (P1 to P12) were selected, evenly distributed across the forest. The main criteria of selection were the homogeneity of topography and stand characteristics, both in terms of species composition (mature sessile oak dominant) and structure (regular high forest) within the site. At each site, we made our observations in two plots (600 m^2 each), one plot close to the remains of the central building (<100 m), supposed to be impacted by ancient Roman agriculture, and the second plot at 250–500 m away from the central building, supposed to be out of the influence or at least less influenced by previous cultivation. The age of each plot was obtained from ancient forest management records, which provided the date of stand regeneration. Stands were between 60 and 160 year-old. The two plots at a given site always were the same age.

2.2. Soil and root sampling

For each of the 12 sites, at both the centre and the outer plots, five cylindrical soil cores (4 cm diameter, 20 cm deep) were collected within an area of less than 50 m^2 and more than 1 m from tree trunks, during the year 2006. The soil of each plot was air-dried and sieved (<2 mm) to discard roots and stones, and analysed for pH (H_2O), total C, N and P contents, available P according to the methods of Olsen (Olsen et al., 1954) and Duchaufour (Duchaufour and Bonneau, 1959), and exchangeable cations (Ca, Mg, K, Na) at pH 7 (ammonium acetate method) at the INRA Central Soil Analysis Laboratory in Arras, France.

For ECM analysis, sampling was performed twice in the year in each of the 24 selected plots (12 from centres and 12 from outer zones). Soil cores were extracted following the same protocol as for soil analyses. The soil cores were separately wrapped with polythene film and kept into isotherm boxes until arrival at the laboratory within 24 h. The soil cores were kept at 4 °C from 1 to 2 days after sampling. For each plot, the 5 cores were pooled in order to perform soil and ECM analysis. The roots were gently separated from the surrounding soil, washed and observed in water using a stereomicroscope. ECM tips were classified into morphotypes (MTs) based on distinctive macroscopic and microscopic features: branching, colour and texture of the mantle, presence or absence of external hyphae, mycelial strands or rhizomorphs, and sclerotia linked to ectomycorrhizae. In all samples, three ECM tips of each encountered MT were frozen in liquid nitrogen and stored at – 20 °C for later molecular identification. The relative abundance (in percent) of dominant MTs in each plot was determined from a subsample of 150 ECM tips randomly taken from the root sample. The frequency of each MT was calculated as the number of times it was encountered among the 24 samples (12 sites × 2 dates) from the centre and outer plots.

2.3. Molecular analyses

Total DNA was isolated from the three frozen ECM tips of each morphotype in each sample using the DNeasy Plant mini-kit following the manufacturer's recommendations (Qiagen SA, Courtaboeuf, France). The internal transcribed spacer (ITS) region of the fungal nuclear ribosomal DNA was amplified with the fungus-specific primer pair ITS1f - ITS4 (White et al., 1990). All amplifications were performed as in Diedhiou et al. (2004). Before sequencing, the amplified DNA was purified on a 96-well MultiScreen-PCR plate system following the manufacturer's recommendations (Millipore SA, Molsheim, France).

The ITS region was directly sequenced using ITS1/ITS4 or ITS1/ITS2 primers with CEQ DTCS-Quick Start Kit on 8-capillar sequencer CEQ 2000XL (Beckman, Fullerton, CA, USA). Forward and reverse DNA sequences of each MT were aligned to produce a consensus DNA sequence. In order to determine the taxonomical belonging of each MT forming fungus, DNA sequences were compared to the GenBank (http://www.ncbi.nlm.nih.gov/) and UNITE (http://unite.ut.ee/index.php) databases using the BLASTn algorithm (Altschul et al., 1990).

2.4. Statistical analysis

Since the study plots were paired, one element of each pair being in the centre of the site, the other one away from the zone of heavy disturbance, we tested the differences in soil characteristics and MT richness between the two ancient land uses by paired *t*-tests. In addition to individual soil variables analysis, a principal component analysis of the ten measured (see above) and two calculated (C/N ratio and sum of exchangeable cations Ca, Mg, K, Na, noted SEC) soil characteristics was conduced and differences in position along the first axis were tested. In order to simultaneously