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Long-term irrigation effects on Spanish holm oak growth and its black truffle symbiont

Ulf Büntgen[a,b,c,*], Simon Egli[a], Loic Schneider[a], Georg von Arx[a], Andreas Rigling[a], J. Julio Camarero[d], Gabriel Sangüesa-Barreda[d], Christine R. Fischer[e], Daniel Oliach[e], José A. Bonet[e,f], Carlos Colinas[e,f], Willy Tegel[g], José I. Ruiz Barbarin[h], Fernando Martínez-Peña[i]

[a] *Swiss Federal Research Institute WSL, Birmensdorf, Switzerland*
[b] *Oeschger Centre for Climate Change Research, Bern, Switzerland*
[c] *Global Change Research Centre AS CR, Brno, Czech Republic*
[d] *Pyrenean Institute of Ecology IPE-CSIC, Zaragoza, Spain*
[e] *Forest Sciences Centre of Catalonia CTFC-CEMFOR, Solsona, Spain*
[f] *University of Lleida-Agrotecnio Center (UdL-Agrotecnio), Lleida, Spain*
[g] *Chair of Forest Growth, Albert-Ludwigs University, Freiburg, Germany*
[h] *Arotz-Foods, Soria, Spain*
[i] *Research Unit of Forestry Mycology and Trufficulture, Cesefor Foundation, Soria, Spain*

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A B S T R A C T

The Périgord black truffle is an exclusive culinary delicacy, but its Mediterranean harvests have declined, despite cultivation efforts since the 1970s. The role of long-term irrigation, symbiotic fungus-host interaction, and microbial belowground progression remain poorly understood, because generally too short experimental settings miss the necessary degree of real world complexity and reliable information from truffle orchards is limited. Here, we conduct the first dendrochronological and wood anatomical assessment of 295 holm oaks, which have been growing under different irrigation intensities in the world's largest truffle orchard in Spain. The relationships between different climatic variables (monthly temperature means and precipitation totals) and dendro-parameters (ring width, vessel count and vessel size) of the oak hosts are utilized to disentangle direct and indirect drivers of truffle fruit body production. Irrigation at medium – instead of high – intensity is most beneficial for oak growth. Non-irrigated trees reveal overall lower stem increments. Warmer temperatures from February to April and wetter conditions from May to July enhance host vitality and possibly also its interplay with fungi symbionts via increased fine root production and mycorrhizal colonization. Adequately irrigated Mediterranean orchards may counteract some of the drought-induced natural truffle decline, and help stabilizing rural tourism, regional agriculture and global markets.

1. Introduction

The Périgord black truffle is the fruit body of *Tuber melanosporum* (Vittad.), an ectomycorrhizal hypogeous fungus considered a unique delicacy by gourmets worldwide (Hall et al., 2003). This species is characterized by a black peridium and a dark spore-bearing gleba that matures under cold conditions between November and February–March within its native Mediterranean habitat (see references hereafter). Naturally occurring *T. melanosporum* fruit bodies are mainly harvested in Italy, France and Spain (Delmas, 1978; Ceruti et al., 2003), where their occurrence is confined to calcareous soils without excesses of nitrogen and phosphorus, mild summer temperatures and suitable rainfall regimes (Bonet et al., 2009). The overall scarce distribution of fruit bodies across the greater Mediterranean region, together with its harvest dependency on trained dogs (and historically also pigs), have weaved a component of mystery (Olivier et al., 1996), which in turn contributed to the organoleptic appeal of the Périgord black truffle to gastronomy aficionados all over the globe.

In contrast to the increasing demand for black truffles, the bounty of its harvest has decreased over the second half of the

* Corresponding author at: Swiss Federal Research Institute WSL, Birmensdorf, Switzerland. Tel.: +41 44 739 2679.
E-mail address: buentgen@wsl.ch (U. Büntgen).

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20th century (Callot, 1999). A continuous long-term decline not only resulted in global price inflation, but also triggered local cultivation attempts as early as 40 years ago (Chevalier and Grente, 1979). Host tree inoculation facilitated the expansion of primary orchards, in which oak seedlings were successfully colonized by *T. melanosporum* (Chevalier et al., 1973; Palenzona, 1969). Since the 1980s, widespread plantations have begun to compensate for some of the loss in wild truffle growth (Le Tacon et al., 2014), offering rural landowners an economically interesting alternative to traditional crops (Samils et al., 2003, 2008). The still emerging Périgord black truffle business currently describes a multi-million euro industry, not only in France, Italy and Spain, but also in Australia (Reyna and Garcia-Barreda, 2014), for instance. Within the past decade, the prices for *T. melanosporum* ranged between 100 and 900 euros kg^{-1} (Reyna and Garcia-Barreda, 2014). The average annual production of *T. melanosporum* in Europe was about 68 t for the last ten seasons (2004/05 to 2013/14). The production in 2013/14 was 125 t with 45 t in Spain according to the European Group for Truffles (GET), Federación Española de Asociaciones de Truficultores (FETT) and G. Gregori, Experimental Centre for Trufficulture ASSAM Regione Marche Sant'Angelo in Vado (PU) Italy (personal communication), compared to 8 t in 2013 in Australia (A. Mitchell, President, Australian Truffle Growers Association, 2013, personal communication). Today, more than 40,000 ha are used globally for truffle cultivation, with 14,000 ha planted in Spain of which only 10–20% have the appropriate "age" to be in production (FETT, GET).

Despite a better understanding of the fungus' belowground life cycle (Kues and Martin, 2011), as well as advances in plantation management principles (Olivera et al., 2011, 2014a,b,c), the production of truffle sporocarps is not yet guaranteed, even when using well-inoculated seedlings on theoretically suitable ground (Guerin-Laguette et al., 2013; Molinier et al., 2013). Despite an immense plantation effort in many regions, the total harvest of this ectomycorrhizal ascomycete has declined in Europe compared with the production 100 years ago (Le Tacon et al., 2014). A satisfying explanation for this long-term dwindling of both natural and planted truffle fruit bodies must consider desiccation constraints in a warmer and dryer climate (Hall et al., 2003; Büntgen et al., 2012). In fact, *T. melanosporum* yields increased after a two-year summer irrigation experiment in southeastern France (Le Tacon et al., 1982). However, our understanding of long-term irrigation effects, symbiotic fungi-host interactions, and microbial belowground processes is, still limited. This knowledge gap partly originates from erratic and proprietary insight on truffle orchards, as well as the short-term nature of experimental settings that are not suitable to capture the complexity of long-term ecosystem processes.

Disentangling biotic (host plants, fungal partners and rhizospheric bacteria), abiotic (climate, pollution, land cover), and combined edaphic (soil, microbes) aspects of the mutualistic relationship between the ectomycorrhizal black truffle and its tree partners remains a challenging task. Given that both the host plant and fungal symbiont co-evolved under the Mediterranean climate

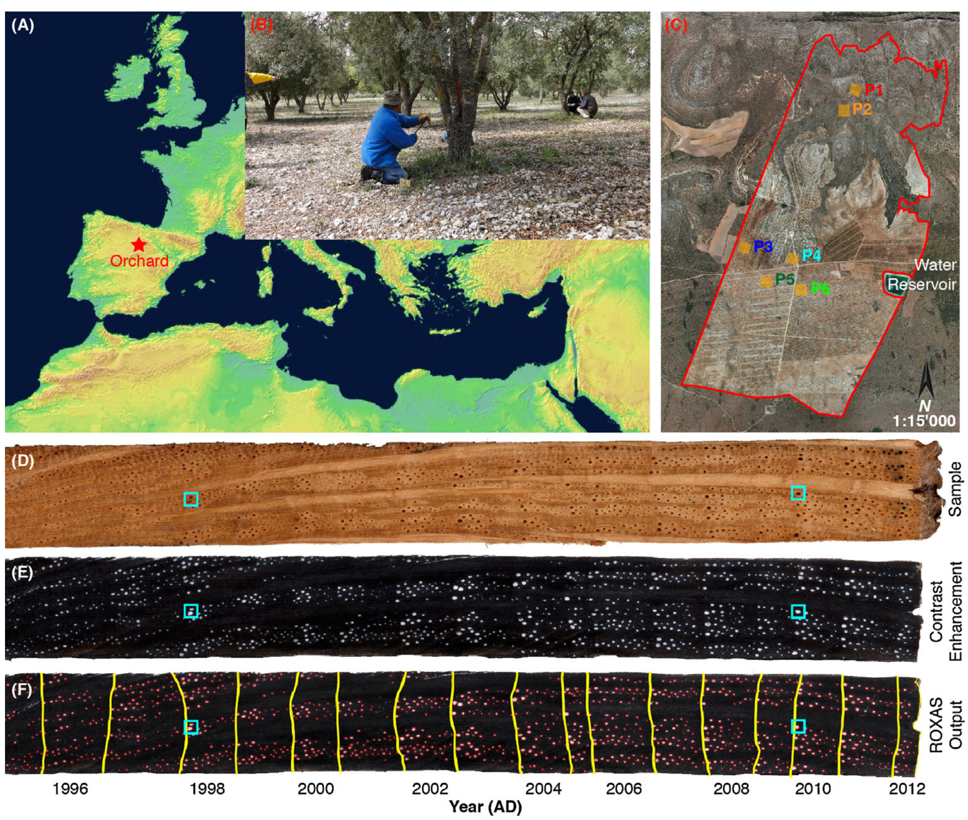

Fig. 1. (a) Location of the world's largest Périgord Black truffle (*Tuber melanosporum*) plantation "Los Quejigares" within the Spanish Province of Soria. (b) Sample collection in (c) the 600 ha large plantation situated between 1100 and 1400 m asl at the southern slope of the Sierra de Cabrejas, ~20 km west of the town of Soria, Central Spain (~41 °N and ~3 °W). Individual holm oak (*Quercus ilex*) trees were sampled in six sectors within the plantation (P1–P6), for which detailed information on irrigation intensity (none, medium, high) and truffle harvest (low, high) exists. (d) High-resolution (2400 dpi) scan of a holm oak wood sample (E57b) after surface preparation with a core microtome (Gärtner and Nievergelt, 2010). (e) The same wood sample after contrast enhancement using black staining and white chalk, and (f) application of the ROXAS software (von Arx and Dietz, 2005) on the wood sample to determine annual ring boundaries (yellow lines) and individual vessels (red polygons). The combination of surface preparation, contrast enhancement and image analysis yielded a wide range of different tree-ring parameters including ring width, as well as vessel number and size. Blue squares are simply to enhance visual orientation amongst the three images.