Lack of thinning effects over inter-annual changes in soil fungal community and diversity in a Mediterranean pine forest

Carles Castaño[a,b,*], Josu G. Alday[b], Björn D. Lindahl[c], Juan Martínez de Aragón[d], Sergio de-Miguel[b], Carlos Colinas[b,d], Javier Parladé[e], Joan Pera[e], José Antonio Bonet[b,d]

[a] Forest Bioengineering Solutions S.A., Ctra. de Sant Llorenç de Morunys, km. 2, E-25280 Solsona, Spain
[b] Departament de Producció Vegetal i Ciència Forestal, Universitat de Lleida-AGROTECNIO, Av. Rovira Roure, 191, E-25198 Lleida, Spain
[c] Swedish University of Agricultural Sciences, Department of Soil and Environment, SE-75007 Uppsala, Sweden
[d] Centre Tecnològic Forestal de Catalunya, CTFC-CEMFOR, Ctra. de St. Llorenç de Morunys km 2, E-25280 Solsona, Spain
[e] IRTA, Centre de Cabrils, Ctra. Cabrils km. 2, E-08348 Cabrils, Barcelona, Spain

ARTICLE INFO

ABSTRACT

Predicted changes in global climate might negatively affect the soil microbiome and associated ecosystem processes in Mediterranean forests. Forest treatments, such as forest thinning, have been suggested to mitigate climate change impacts on vegetation by reducing competition between trees, thus increasing water availability. Studies addressing the combined effects of climate and forest thinning on belowground fungal communities are still scarce, being fundamental to elaborate adaptive strategies to global warming.

The aim of this study was to evaluate the tree density reduction effects on soil fungal communities and their response to inter-annual changes in weather conditions. The temporal dynamics of soil fungal communities in relation to these two drivers (i.e., forest management and weather conditions) were studied from 2009 until 2014 in a set of 12 pairs of thinned and un-thinned plots dominated by *Pinus pinaster* Ait. Thinning (from 30% up to 70% reduction in stand basal area) was conducted in 2009 and soil fungal community composition was studied during 4 years. Here, we used autumn precipitation and temperature to describe the impact of inter-annual weather changes. We used Pacific Biosciences sequencing of fungal ITS2 amplicons to study fungal communities in soil samples. Forest thinning did not significantly affect fungal community composition nor fungal species richness and diversity, indicating that the soil fungal community is resistant to forest thinning regardless of its intensity. However, fungal species composition changed progressively across years, both at the species level and with regards to functional guilds. These changes in community composition were partly driven by inter-annual variation in precipitation and temperature, with free-living fungi increasing in abundance under wetter conditions, and symbiotic fungi being more prominent under drier and colder conditions. The results indicate that mycorrhizal communities in Mediterranean forest ecosystems can resist forest thinning, if enough trees and functional roots from thinned trees are retained.

1. Introduction

Soil fungi represent an important part of the soil microbial community, and are essential drivers of many ecosystem processes, such as soil organic matter (SOM) decomposition and nutrient release as well as plant nutrient uptake and production. Mycorrhizal fungi are one of the most important functional groups of the soil microbiome, playing an important role in tree nutrition and water acquisition (Smith and Read, 2008). The extramatrical mycelia (EMM) of these fungi explore the soil surrounding the host tree, foraging for nutrients and forming mycorrhizae with adjacent tree hosts (Cairney, 2012). In drier ecosystems, such as Mediterranean forests, mycorrhizal fungi contribute to plant water acquisition, by providing plant roots access to less accessible water and by improving soil structure, enhancing soil water retention (Allen, 2007; Querejeta, 2017). Besides the important role of mycorrhizal fungal species, other functional guilds, such as saprotrophs, also play a paramount role in litter degradation (Baldrian et al., 2011), which may be hampered during the dry and hot summer conditions of Mediterranean forest soils. Thus, fungal community changes in these ecosystems will have important consequences for nutrient cycling and water acquisition by plants and therefore impact plant communities (Sardans and Peñuelas, 2013).

* Corresponding author at: Departament de Producció Vegetal i Ciència Forestal, Universitat de Lleida-AGROTECNIO, Av. Rovira Roure, 191, E-25198 Lleida, Spain.
E-mail address: carles.castanyo@ctfc.cat (C. Castaño).

Global change is one of the most important threat for many Mediterranean ecosystems. In the Mediterranean basin, temperature has been forecasted to rise between 1.4 °C and 5.1 °C by 2055 (Nogués Bravo et al., 2008), and total annual precipitation projections show a tendency towards less precipitation, with more extreme rainfall events (García-Ruiz et al., 2011) and reduced soil moisture (Dai, 2013). Indeed, ecosystem alterations, local extinctions and phenological changes in these ecosystems have already been associated to current climate change (Peñuelas et al., 2002). Also, predicted drought increase in Mediterranean forests will likely reduce plant growth and aboveground biomass (Sardans and Peñuelas, 2013), probably with cascading effects belowground (Cairney, 2012; Alday et al., 2017a). Thus, changes in climate may alter the composition of soil fungal communities (Fernandez et al., 2016; Solly et al., 2017; Hartmann et al., 2017; Castaño et al., 2018) and cause alterations in ecosystem functioning with respect to plant nutrition, soil organic matter decomposition and carbon storage (Averill et al., 2014; Clemmensen et al., 2015).

Climate effects on fungi may be directly driven by changes in temperature and moisture (Voříšková et al., 2013; Santalahti et al., 2016) or indirectly by changes in host performance (Deslippe et al., 2011; Fernandez et al., 2016; Hartmann et al., 2017), host activity (Högberg et al., 2010), soil properties or litter input (Vašutová et al., 2016). In drier ecosystems, some mycorrhizal ascomycetes may be more abundant (Smith et al., 2007; Gordon and Gehring, 2011). Precipitation and temperature strongly influence positively fruiting body emergence and production (Hernández-Rodriguez et al., 2015; Alday et al., 2017b). Fire severity and thinning also affect fungal fruit body production (de-Miguel et al., 2014; Salo and Kouki, 2018) as well as community composition of fruiting bodies (Mediavilla et al., 2014). Recently, we observed that intra-annual changes in soil microclimate conditions strongly affected belowground fungal functional communities (Castaño et al., 2018). In addition, Léon Sánchez et al. (2017) studied the climate change effects on the belowground fungal community in a scrubland, and they observed negative effects of drier and warmer conditions on mycorrhizal species. However, studies focussed on belowground fungal responses to both thinning and climate changes in Mediterranean forests are still scarce. Forest thinning has been suggested as a forest management option to mitigate climate change impacts on Mediterranean forests, because its potential to increase water availability and water use efficiency of trees, thus changing soil microclimatic conditions. For example, Aldea et al. (2017) found that thinning increased radial growth of both conifer and oak species and led to increased resistance to drought and improved stand growth. Similarly, positive thinning effects have been observed on the fruiting body production of economically relevant fungal species (Shaw et al., 2003; Bonet et al., 2012), although the effects were species-dependent. Also, sustainable forest harvesting regimes have been predicted to positively influence mushroom production (de-Miguel et al., 2014). In contrast, clear-cutting and associated logging disturbances in clear-cut forests have been shown to have clear negative impact on soil mycorrhizal communities (Jones et al., 2003; Hartmann et al., 2012; Kyaschenko et al., 2017; Parladé et al., 2017). Forest management effects on mycorrhizal communities are likely to depend on whether these communities can survive in symbiosis with the remaining trees (Amaranthus and Perry, 1987; Rosenvald and Lõhmus, 2008). Tree removal may also affect belowground fungal communities via changes in environmental conditions, such as microclimate or soil biochemistry (Jones et al., 2003; Hartmann et al., 2012). Although forest thinning may have a less dramatic impact than clear-cutting, its impact on belowground fungal communities has yet not been assessed.

In this study we analysed the inter-annual dynamics of soil fungal communities during 4 years after forest thinning in 12 experimental plots dominated by *Pinus pinaster* Ait, with 12 paired non-thinned plots as a reference. The plots represented a gradient of retained stand basal area and number of trees (Bonet et al., 2012). In addition, we analysed potential correlations between autumn precipitation and temperature and the fungal community composition and structure. In recent studies, we analysed the soil microclimate effects on fungal communities from an intra-annual perspective, with significant effects found (Castaño et al., 2018). However, here we study both the climate and thinning effects from an inter-annual perspective. We specifically hypothesized that (i) light-medium thinning would not alter belowground fungal community composition or diversity. In contrast, (ii) changes in fungal species composition and the relative abundance of functional guilds would be expected after more intense thinning. We further hypothesized that (iii) fungal community composition would vary across years in relation to autumn precipitation and temperature, with mould species and yeasts being stimulated under wetter conditions.

2. Material and methods

2.1. Site selection

The study was carried out at a long-term experimental setup located in the natural area of PNIN-Poblet (Northeast Spain, 41°21′6.4728″ latitude and 1°2′25.7496″ longitude), where 12 pairs of thinned and non-thinned plots were established in 2009 to test the effect of forest thinning on mushroom production (Bonet et al., 2012). The plots consist of even-aged *Pinus pinaster* stands (60-years-old), with isolated *Quercus ilex* trees sometimes forming shrubs, while the understory is dominated by *Erica arborea*, *Arbutus unedo* and *Calluna vulgaris*. Mean annual temperature at the study site is 11.8 °C, and mean annual rainfall is 666.5 mm, with a pronounced summer drought that usually lasts for three months (June to August). Autumn precipitation (September to November) during the study years was similar across plots (136.8 ± 3 mm), but variable between years (136.8 ± 86.4 mm), whereas temperature variation was slightly higher across plots (16.06 ± 1.13 °C) than across years (16.06 ± 0.84 °C). Averaged autumn rainfall was: 2009 = 97.1 ± 3.4 mm, 2012 = 245.9 ± 22.7 mm, 2013 = 30.59 ± 0.3 mm, 2014 = 108.9 ± 18.6 mm. Yearly averaged autumn temperature was: 2009 = 17.1 ± 0.9 °C, 2012 = 16.4 ± 0.9 °C, 2013 = 15.3 ± 1.5 °C, 2014 = 15.4 ± 1.2 °C. Plots are similar in soil properties, but as a result of 2009 thinning, their characteristics differ considerably, with basal area ranging from 16.5 to 81.7 m^2 ha^{-1} and stand density from 350 to 2657 trees ha^{-1}. Soils are siliceous with sandy loam texture, average pH 6.7 ± 0.3, average total N 0.21 ± 0.06% and organic matter (OM) 5.5 ± 2%.

2.2. Thinning experiment

Initially, 12 mushroom inventory plots of 100 m^2 (10 m × 10 m) were established in 2008 (un-thinned plots) in an approximately 300 ha forest area (Fig. S1a). In 2009 12 additional inventory plots, scheduled for thinning (thinned plots), were established paired (with an average distance of 50 m. from controls) with the initial plots. Each thinned plot was 1600 m^2 in area (40 m × 40 m) with a central 100 m^2 sampling area, to reduce edge effects. In these thinned plots, three different thinning intensities were employed (light: 20–30% thinned, medium: 30%-50% thinned, and heavy: 50–70% thinned), resulting in basal area reductions of 30–70% (Fig. S1b). In un-thinned plots, stand structure was more or less homogeneous, with similar tree heights and diameters within plots. In thinned plots, trees were systematically removed without the use of heavy machinery to avoid confounding effects caused by soil disturbance, using a chainsaw and removing the cut trees from the plot. The most intense thinning resulted in a remaining stand basal area of 16.5 m^2 ha^{-1} and a stand density of 350 trees ha^{-1}, whereas the greatest standing basal area left was 81.7 m^2 (2552 trees ha^{-1}). Further information about the thinning treatments and the stand variables before and after the treatments is available in Bonet et al. (2012). A diagram of the experimental design is provided as Fig. S1.