Intercropping of aromatic plants in truffle orchards: short-term effect on extraradical truffle mycelium and aromatic plant growth

Sergi Garcia-Barreda [a,b*], Juliana Navarro-Rocha [a,b], Eva Gómez-Molina [c], Vasiliki Barou [d], María Ángeles Sanz [e,b], Sergio Sánchez [a,b], Javier Parladé [d]

[a] Departamento de Ciencia Vegetal, Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA). Avenida de Montañana 930, Zaragoza 50059, Spain.
[b] Instituto Agroalimentario de Aragón-IA2 (CITA-Universidad de Zaragoza), 50018 Zaragoza, Spain
[c] Centro de Investigación y Experimentación en Truficultura (CIET), Diputación Provincial de Huesca. Polígono Fabardo s/n, 22430, Graus, Spain.
[d] Protecció Vegetal Sostenible, Institut de Recerca i Tecnologia Agroalimentàries (IRTA). Ctra. Cabrils km 2, E-08348 Cabrils, Spain.
[e] Área de Laboratorios de Análisis y Asistencia Tecnológica, Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA). Avda. Montañana 930, 50059 Zaragoza, Spain.
* Corresponding author: sgarciaba@cita-aragon.es

ORCID
Sergi Garcia-Barreda: 0000-0002-7248-234X, Juliana Navarro-Rocha: 0000-0001-7975-9340, Eva Gómez-Molina: 0000-0002-2664-8484, Vasiliki Barou: 0000-0003-2939-2426, María Ángeles Sanz: 0000-0002-4513-3371, Sergio Sánchez: 0000-0003-4331-9794, Javier Parladé: 0000-0002-0867-3280

Abstract

Background and Aims

Intercropping of truffle-producing trees with aromatic plants is used to improve profitability of truffle orchards during the initial 4-7 years. However, after that period the viability of this system is challenged by the appearance of *brûlés*, an area around host tree characterised by scarce plant cover where the fungus exhibits allelopathic activity. We aimed to investigate the ecological interactions between these crops and between their associated mycorrhizal fungi in adult truffle plantations.

Methods

We simulated two intercropping systems, truffle oak – lavender and truffle oak – rosemary in their adult stage. We analysed and compared aromatic plants and soil samples inside and outside the *brûlés* during the first year of the aromatic plants in the field.

Results

We found a strong negative relation of *brûlés* with the growth of the aromatic plants, although not a decrease in the arbuscular mycorrhizal colonization of their roots. The essential oil yield and composition of aromatic plants was affected by *brûlés*. The extraradical truffle mycelium was not significantly affected by the presence of aromatic plants.

Conclusions

The growth and yield of aromatic plants was impaired during their first year growing in *brûlé*s, whereas no negative effect of aromatic plants on truffle fruiting potential was found. The study improves our understanding of the mechanisms influencing the viability of the truffle tree – aromatic plant intercropping and the possible technical challenges.

Keywords: *Tuber melanosporum*, *Lavandula × intermedia*, *Salvia rosmarinus*, intercropping, extraradical mycelium, medicinal and aromatic plants

Introduction

Agricultural lands in the Mediterranean basin face important challenges due to climate and societal changes, such as soil erosion, reduced water availability, and degradation of soil organic matter and the associated microbial communities (De Franchis 2003; Lagacherie et al. 2018). In this context, arboriculture has a high potential to offer ecosystem services such as enhancement of farmers' income and carbon sequestration, although in many cases there are conflicts between provisioning and regulating services, frequently linked to overfertilisation, rainwater runoff due to reduced groundcover or cultivation practices disrupting interactions responsible for pest control and pollination (Brunori et al. 2020; Demestihas et al. 2017, 2019). Intercropping of orchards with medicinal and aromatic plants (MAPs) could mitigate these conflicts through increasing agricultural production, carbon sequestration, soil water retention, soil biodiversity, populations of pollinators and control of pests and diseases (Chen et al. 2014; Durán-Zuazo et al. 2008; Morugán-Coronado et al. 2020; Song et al. 2010; Zhang et al. 2021).

The potential benefits of intercropping with MAPs could be particularly interesting for truffle cultivation. The prized black truffle (*Tuber melanosporum* Vittad.) grows and fruits below ground in ectomycorrhizal (ECM) association with tree roots. The productivity and sustainability of truffle orchards depends on the ecological relationships of truffle with soil components, host trees and soil microbial communities (Benucci and Bonito 2016; Le Tacon et al. 2013; Mello and Balestrini 2018; Splivallo et al. 2011). These relationships indicate the potential for agroecological approaches that integrate the diverse organisms interacting in the oak – truffle agroecosystem (Aumeeruddy-Thomas et al. 2016). Intercropping with MAPs could be an environmentally beneficial alternative (Schneider-Maunoury et al. 2020; Taschen et al. 2020), but the first step would be to develop farming practices harmonizing the association between these crops. A few experiences of intercropping of lavender (*Lavandula*

× *intermedia* Emeric ex Loisel.) and truffles exist in southern Europe (Martin-Chave 2019), although the scientific information on them is still very scarce (Geoffroy et al. 2018).

In truffle – lavender intercropping plantations, lavender is commonly planted in rows between truffle tree rows (Martin-Chave 2019). During the pre-productive stage of the truffle plantation –the first 4–7 years from tree plantation– the root systems of the crops do not overlap and intercropping seems easily feasible. However, from year 4–7 an area characterised by scarce plant cover (the so-called *brûlé*) is developed around the truffle trees (González-Armada et al. 2010; Streiblová et al. 2012). The biological mechanism behind the *brûle* formation has not been completely determined, but it likely involves competition among roots, allelopathic activity and/or root parasitism by *T. melanosporum* mycelium (Pacioni 1991; Plattner and Hall 1995; Schneider-Maunoury et al. 2018). To the extent that *brûlés* may affect MAP growth, they could jeopardise the productivity of the MAP crop or its aromatic profile, which is sensitive to other stress factors such as drought (Chrysargyris et al. 2016; Kulak 2020). This could be particularly problematic when MAPs are recently planted.

Plantations of lavender and rosemary (*Salvia rosmarinus* (L.) Schleid.) have a lifespan of 8-10 years, after which MAP rows must be replanted if the intercropping is going to be maintained (Fanlo et al. 2009).

On the other hand, most truffle fruitbodies are harvested within *brûlés*, with the extraradical truffle mycelium being more abundant in productive than non-productive *brûlés* (Parladé et al. 2013; Queralt et al. 2017). As *brûlés* stretch and reach a MAP row, the spread of truffle mycelium could be hindered by the competition with MAP roots and their associated fungi, which are arbuscular mycorrhizal (AM) fungi in the case of lavender and rosemary. Previous studies have frequently found a negative relationship between ECM and AM fungi (Chen et al. 2000; Knoblochová et al. 2017).

In this study, we explore the effect of intercropping on the growth of MAPs and on the abundance of truffle mycelium in the soil. Since the main challenges for the compatibility of these crops are likely to happen once the *brûlés* appear, and especially when MAPs are replanted, we studied these effects during the first vegetative period after planting MAPs in a ten-year-old truffle orchard with formed *brûlés*. We evaluated the survival, growth and essential oil characteristics of two common MAP species (lavender and rosemary) within the *brûlé*, as well as their root colonization by AM fungi. We compared them to MAPs planted outside the *brûlé*, where contact between tree and MAP roots is much more limited or null, just like in pre-productive truffle plantations. We also evaluated the effect of MAP occurrence on the abundance of *T. melanosporum* extraradical mycelium. Regarding the effect of intercropping on MAPs, we hypothesised that in the *brûle* the higher abundance of *T. melanosporum* mycelium would be associated with reduced survival and growth of MAPs and lower colonization of MAP roots by AM fungi. We also hypothesised that plants growing within the *brûlé* would suffer higher stress levels that would be associated with higher yields of essential oils (the main product of the cultivation of these MAPs) and altered volatile compounds profiles, since this is frequently the case for other stress factors such as drought (Chrysargyris et al. 2016; Kulak 2020; Sarmoum et al. 2019). Regarding the effect of intercropping on truffle, we hypothesised that the abundance of *T. melanosporum* mycelium would be impaired by the presence of MAPs, thus limiting the long-term compatibility of truffle – MAPs intercropping. We assessed two MAP species (lavender and rosemary) to test whether the truffle – MAP compatibility is dependent on the MAP species used.

Materials and methods

Experimental site and design

The experiment was conducted in the truffle orchard of Centro de Investigación y Experimentación en Truficultura (CIET) in Graus (Huesca province, north-eastern Spain, 42º 12.1’ N, 0º 22.4’ E, 520 m. a.s.l.). The climate is Continental Mediterranean, with a mean annual temperature of 12.2 ºC and a mean annual rainfall of 680 mm. The soil is calcareous, with pH 8.2, and silt-loam texture, developed on Tertiary lutite/sandstone. The site lies within the natural distribution area of *T. melanosporum* (Garcia-Barreda et al. 2019).

A small, homogeneous surface of this plantation (ca. 400 m^2) was used as experimental plot in our study. This plot was planted in 2011 with *Quercus ilex* L. subsp. *ballota* (Desf.) Samp. seedlings inoculated with *T. melanosporum*. The seedlings were produced in a commercial nursery and the root colonization by *T. melanosporum* was evaluated following the INIA-Aragón method (Andrés-Alpuente et al. 2014), and checked to be at least 30% of the total short roots, with less than 1% of *Sphaerosporella brunnea* (Alb. & Schwein.) Svrcek & Kubicka. The seedlings were planted at a density of 278 trees ha^{-1} (6 × 6 m). The soil has been shallowly tilled once a year in early spring and afterwards the weeds have been controlled (when necessary) by hoeing around the trees in spring and autumn. Since the sixth year of the plantation, the trees have been biennially pruned.

In April 2021, the experimental plot was divided in two blocks. In the first block, 41 lavender (clone super) were planted. In the second block, 81 rosemary seedlings were initially planted, although a small area was discarded due the presence and digging activity of *Microtus arvalis* Pallas, thus leaving for the study 67 seedlings in the rosemary block. These two Mediterranean, perennial shrubs are amongst the most common MAPs cultivated in the calcareous, dry regions of Spain (More et al. 2010). They are commercially cultivated to obtain essential oil, fresh or dried sprigs. They are usually harvested by mechanically cutting flowering stems and thus crop yield is largely dependent on annual shoot growth (More et al. 2010). Following the common practice with these MAPs, the seedlings were planted in a row with 50 cm distance between plants (Fig. 1). The row of MAPs was 60 cm apart from the row of truffle trees, so that 36% of the MAP seedlings were located within a truffle *brûlé* (at distances ranging 0.5-1.3 m from the truffle tree trunk) and 64% outside *brûlés* (at distances ranging 1.6-3.1 m). The former represents a situation in which crops could potentially interfere with each other, whereas the latter represents a situation in which the probability of this happening is much lower (Fig. 1). The *brûlés* of the experimental trees presented mean radius 1.0–1.5 m around the host tree trunk.

Following the common practice, lavender was planted bare root, whereas rosemary was grown potted in the CIET nursery and then planted with its root ball. The MAP seedlings were drip watered once each 10-12 days (10 litres per seedling) during two months after plantation, to encourage their survival and rooting.

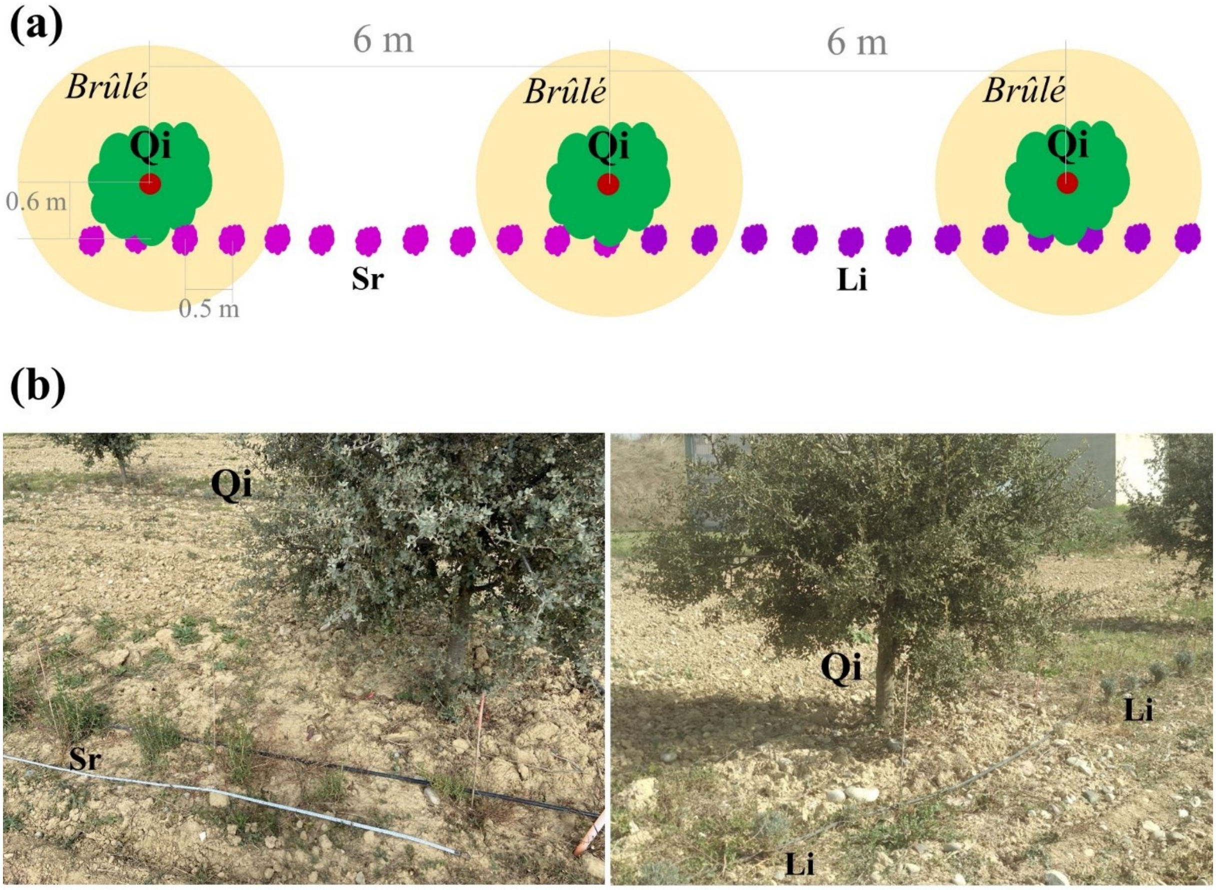

Figure 1. General layout diagram of the experimental design (a) and overall appearance of the experimental plot six months after plantation. Qi: *Quercus ilex*, Sr: *Salvia rosmarinus*, Li: *Lavandula* × *intermedia*.

Plant measurement and mycorrhizal sample collection

In October 2021, six months after planting, the survival and shoot height of all the MAPs (n = 108) were measured. Then, four truffle trees in the lavender block and four in the rosemary block were selected. For each tree, four MAPs were selected, two of them within the *brûlé* and two outside, to assess the effect of the *brûlé* on the MAPs (sample size: 32 MAPs). The plants were thoroughly uprooted and soil from the rhizosphere was collected by gently shaking the plant roots, for subsequent quantification of *T. melanosporum* extraradical mycelium. Then, the fine roots of each MAP were cut and kept in moist conditions at 4ºC, for subsequent quantification of AM fungi colonization. The sample size for AM fungi colonization was reduced to 12 MAPs (three truffle trees for each MAP species and two MAPs per tree). Finally, the shoots of the 32 MAPs were dried to constant weight at 80 ºC. In the eight truffle trees selected (four with lavender and four with rosemary) a bulk soil sample from the *brûlé* was taken. It consisted of a composed sample from three cylindrical cores (20 cm depth, 3.2 cm diameter), at 0.5-1 m distance from the truffle tree trunk. In each *brûlé*, two samples of soil from MAP rhizosphere were taken, as described above. Rhizosphere samples were compared to the bulk soil to assess the effect of MAP presence on the abundance of *T. melanosporum* extraradical mycelium (sample size: 24). For each tree, one bulk soil sample from outside the *brûlé* (at 2.5-3 m from the truffle tree trunk) was taken with the same methodology, as a control.

Finally, as a complementary analysis, we sampled dead MAPs in the *brûlés* to compare the abundance of *T. melanosporum* extraradical mycelium in their rhizosphere with that of living

MAPs in the same *brûlé*. Four dead MAPs were sampled, each one in a different *brûlé*, together with two living MAPs in each *brûlé*, for a total sample size of 12.

Extraradical mycelium of black truffle

The soil samples were air-dried at 30 °C and sieved through a 2-mm mesh. DNA extraction was performed on the fine soil fraction using the Soil DNA Isolation Plus Kit (Norgen Biotek Corp., Thorold, ON, Canada) following manufacturers' instructions. Specific quantification of soil mycelium was carried out with a StepOne™ Real-Time PCR System machine provided with the StepOne software v. 2.3 (Life Technologies, Carlsbad, CA). DNA samples and standards were prepared for real-time PCR using the 2× Takara Premix Ex Taq™ Perfect Real-Time (Takara Bio Europe, SAS, France), the TaqMan probe (200 nM) and primers (800 nM each) described in Parladé et al. (2013), 5 µL of the template DNA and HPLC water to a final reaction volume of 20 µL. Thermocycling profile was 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s and 60 °C for 34 s. The standard curve was generated from unripe *T. melanosporum* sporocarps as described in Parladé et al. (2013).

Root colonization by arbuscular mycorrhizal fungi

The extent of mycorrhizal colonization in the root systems of the MAPs was assessed under a dissecting microscope using the visual estimate of the percentage of cortex infected by the fungus as described by Giovannetti and Mosse (1980). Root samples were previously clarified and stained following the procedure described by Koske and Gemma (1989).

Essential oil yield and chemical characterization

In August 2022, one sample of plant shoots was taken for each of the following: lavenders growing within *brûlés*, lavenders outside *brûlés*, rosemaries within *brûlés* and rosemaries outside *brûles*. Each sample was composed of the shoots of five plants pooled together. The plants were randomly selected. Laboratory-scale hydro-distillation (three replicates of 100 g fresh plant material) was carried out in a Clevenger type apparatus for quality control, according to the European Pharmacopoeia (Navarro-Rocha et al. 2020). The essential oil yield was determined as the volume of oil (mL) extracted from a weight of distilled dry plant (kg). An aliquot of the oil sample was subjected to gas chromatography-mass spectrometry (GC-MS) to determine the essential oil composition. Essential oil constituents are key markers of the oil quality (Lafhal et al. 2016). A 6890 series chromatograph coupled with a 5973N serie mass selective detector (Agilent Technologies, California, USA) was used. The instrument was equipped with a capillary column HP-5MS (Agilent Technologies, California, USA) of 30 m, 0.25 mm i.d., 0.25 µm film thickness and a flow of 1 mL/min with helium as a carrier gas. The oven temperature was 60 ºC held for 1 min, then raised at 3 ºC/min to 246 ºC, for 1 min. The MS used the electron impact mode with an ionization potential of 70 eV and an ion source temperature of 230 ºC. The interface temperature was 240 ºC. The MS scanning was recorded in full scan mode (40–250 m/z). A ChemStation software was used for controlling the GC-MS system. The amounts of individual compounds were calculated in mode total ion chromatogram (TIC) as the percentage of area of the total GC peak area.

Data analysis

To assess the effect of intercropping on the MAPs, we analysed the survival of MAPs, the dry weight of their shoot, their shoot height, the percent root colonization by AM fungi and the abundance of *T. melanosporum* extraradical mycelium in the MAP rhizosphere. For each MAP species, the differences between the plants growing within and outside the *brûlé* were tested with a nested model, in which the MAP species was included as a main effects predictor and the *brûlé* / not *brulé* variable was nested within the MAP species. The shoot dry weight, shoot height and truffle mycelium abundance were tested with general linear models. None of them met all model assumptions (homogeneity of variance, normality and linearity). The shoot dry weight was log-transformed, whereas in the case of shoot height and truffle mycelium, a generalised (gamma) linear model was used instead. The MAP survival and the proportion of MAP roots colonized by AM fungi were analysed with generalised (binomial) linear models. In generalised linear models the fit of the error structure was assessed through overdispersion. Least-squares means tests were used for post-hoc comparisons, with a P = 0.05 threshold for statistical significance. The volatile compound profile of the essential oils was analysed with principal components analysis (PCA).

To assess the effect of intercropping on truffle, the abundance of *T. melanosporum* extraradical mycelium in the bulk soil of the *brûlé* was compared to the rhizosphere of MAPs planted in the *brûlé*, for each of the two MAPs studied. Data were analysed with a general linear model in which soil position (bulk soil / MAP rhizosphere) was nested within the MAP species. The response variable was log-transformed to meet model assumptions. All analyses were conducted with R and the emmeans package (Lenth 2021; R Core Team 2022).

Results

Effects on aromatic plants

Six months after planting, neither the survival of lavender nor that of rosemary was significantly affected by the *brûlé* (z = -0.01, P-value = 0.99 and z = 0.50, P-value = 0.62, respectively, n = 108, Fig. 2a). The shoot dry weight was significantly higher outside than inside the *brûlé* for both lavender and rosemary (t = -5.19, P-value < 0.001 and t = -2.78, P-value < 0.010, respectively, n = 32, Fig. 2b). The height of the MAP shoots followed a similar pattern, although the decrease of height in *brûlés* was only significant for lavender and not for rosemary (Table S1, Fig. S1). The height and the dry weight of MAP shoots showed a significant positive correlation (Spearman's $r = 0.74$, P-value < 0.001, $n = 32$).

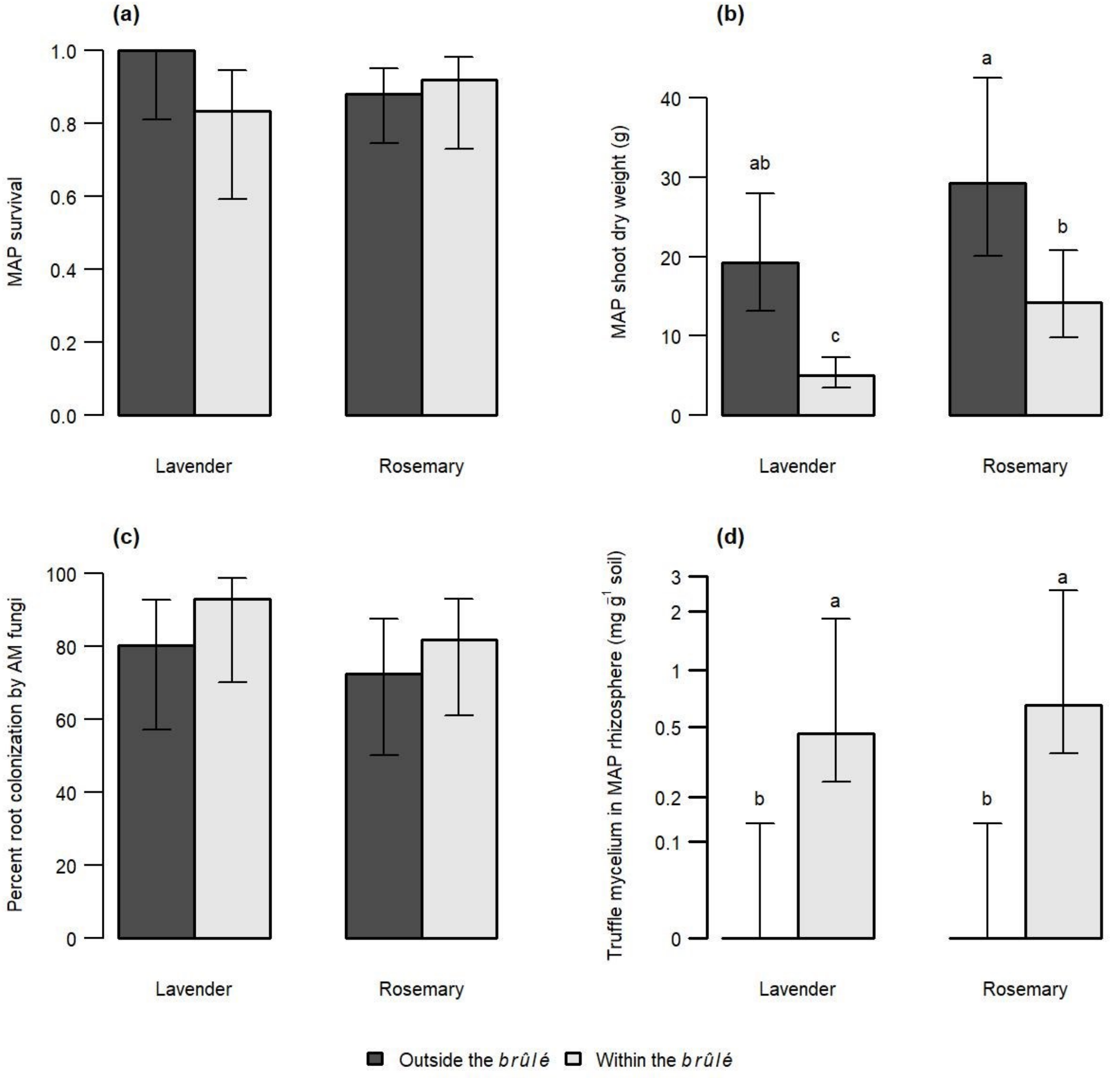

Figure 2. Effect of the truffle *brûlé* on the lavender and rosemary survival (a), shoot dry weight (b), percent root colonization by arbuscular mycorrhizal (AM) fungi (c), and abundance of *T. melanosporum* mycelium in the aromatic plant (MAP) rhizosphere (d), six months after planting the MAPs (predicted values and 95% confidence interval). Letters indicate significant differences among treatments according to least-squares means tests ($\alpha =$

0.05, n = 108 for the survival, n = 32 for shoot dry weight and truffle mycelium, and n = 12 for AM fungi colonization). The y-axis in (d) is represented in log scale.

The percent root colonization of the MAPs by AM fungi was not significantly affected by the *brûlé* either in lavender or rosemary (t = 1.1, P-value = 0.29 and t = 0.7, P-value = 0.48, respectively, n = 12, Fig. 2c). On the other hand, the abundance of *T. melanosporum* extraradical mycelium was significantly higher in the rhizosphere of the MAPs inside the *brûlé* than in those outside, for both lavender and rosemary (t = -2.4, P-value = 0.022 and t = -2.5, P-value = 0.019, respectively, n = 32, Fig. 2d). The dry weight of MAP shoots and the abundance of *T. melanosporum* extraradical mycelium in the rhizosphere of the MAPs showed a significant negative correlation (Spearman's r = -0.53, P-value = 0.001, n = 32). However, when the mycelium abundance in the rhizosphere of dead MAPs was compared with that of living MAPs in the same *brûlé*, no significant differences were found either for lavender or for rosemary (Table S2, Fig. S2). Finally, no significant correlation was found between AM fungi colonization of MAPs and *T. melanosporum* mycelium abundance in the rhizosphere of these MAPs (Spearman's r = 0.40, P-value = 0.20, n = 12).

The extraction of essential oils from the shoots of lavender yielded 10.0 mL kg^{-1} dry matter for the plants growing within the *brûlé* and 17.3 mL kg^{-1} for those growing outside it. For rosemary, shoots yielded 5.1 mL kg^{-1} for the plants growing within the *brûlé* and 9.9 mL kg^{-1} for those growing outside. The GC-MS allowed to identify a total of 43 volatile compounds in the lavender oil samples and 34 in the rosemary oil samples (Fig. 3). The most abundant compounds in lavender oil were linalool, 1,8-cineole, linalyl acetate and camphor. For rosemary the most abundant compounds were camphor, 1.8-cineole and α-pinene, and to a lesser degree borneol, camphene and limonene + β-phellandrene (Fig. 3, Table S3). The PCA for the scaled values of the percentage area explained 83.7% of the total variability with the first PCA component, which clearly separated the oil of lavender from that of rosemary (Fig. S3). An additional 11.7% of the variability was explained with the second component. The second component separated the lavender samples growing within and outside the *brûlé* based on 1,8-cineole and the less abundant β-pinene + 1-Octen-3-ol, which presented the more positive loadings with the second PCA component, in relation with their higher abundance within the *brûlé* (Fig. S3). However, the second PCA component could not separate the rosemary samples (Fig. S3).

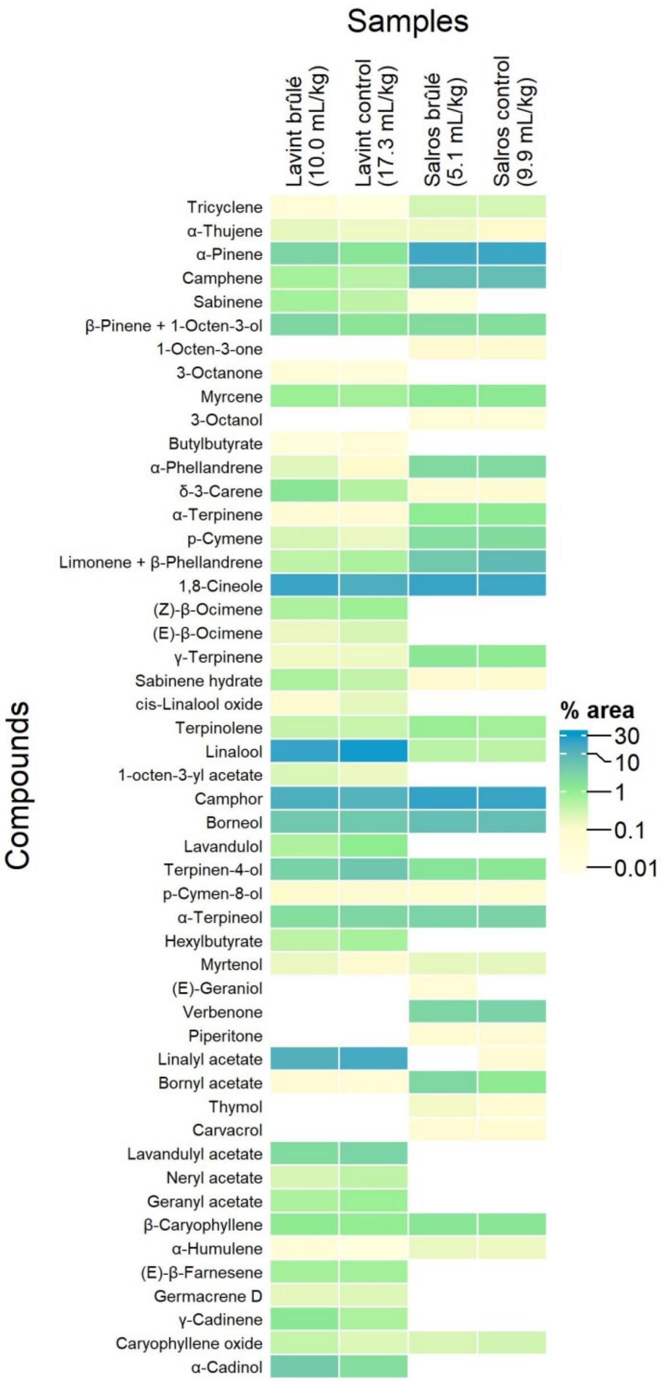

Figure 3. Heatmap for the relative frequency (percentage of area values, indicated by color) of the volatile compounds detected by GC-MS in the essential oil extracted from lavender (Lavint) and rosemary (Salros) cultivated within *brûlés* and outside them (control) (n = 4). The yield of the essential oil extraction is added next to each sample.

Effect on truffle extraradical mycelium

The effect of intercropping on truffle was assessed by comparing extraradical mycelium abundance in the bulk soil and in the MAP rhizosphere. Within the *brûlé*, the abundance of *T. melanosporum* extraradical mycelium was not significantly different in the bulk soil and in the rhizosphere of the MAPs, either for lavender or rosemary (t = 0.48, P-value = 0.64 and t = 0.42, P-value = 0.68, respectively, n = 24, Fig. 4). Outside the *brûlé*, no truffle extraradical mycelium was found in the bulk soil, whereas in the rhizosphere of MAPs it only appeared in one (6%) of the samples, with less than 0.001 mg g^{-1} soil.

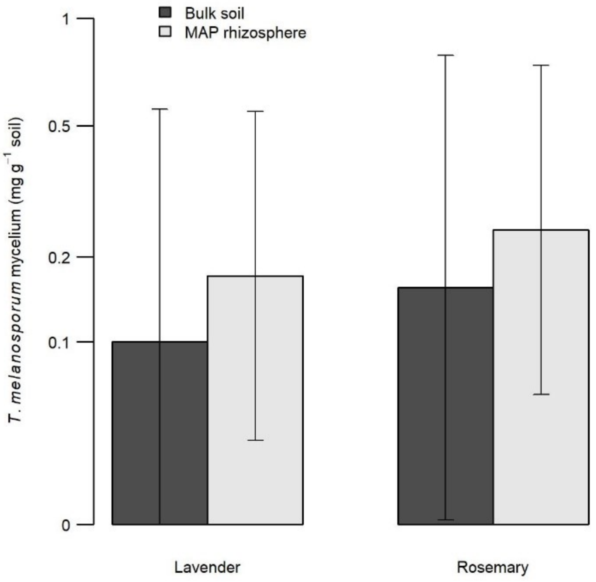

Figure 4. Effect of intercropping with lavender and rosemary on abundance of *T. melanosporum* extraradical mycelium: comparison between the bulk soil of the *brûlé* and the rhizosphere of the aromatic plants (MAPs) planted in the *brûlé*, six months after planting (predicted values of the linear model and 95% confidence interval, $\alpha = 0.05$, n = 24). The y-axis is represented in log scale.

Discussion

Intercropping with MAPs has the potential to promote ecosystem services in Mediterranean orchards if crop complementarity or facilitation is possible (Durán-Zuazo et al. 2008; Song et al. 2010). The sustained coexistence of the truffle – MAPs intercropping is potentially challenged in adult stages of the truffle plantation due to interference between crops stemming from *brûlé* spread (Martin-Chave 2019). Developing integrative practices that enhance crop complementarity beyond the early stage of the truffle plantation requires scientific information on the ecological relationships between truffle and MAPs (Geoffroy et al. 2018). In this study, we hypothesised that the truffle – MAPs intercropping would be associated with reduced survival and growth of MAPs in the *brûlé*, higher yield of essential oils of MAPs and altered volatile compounds profiles. We also hypothesised that the competition between *T. melanosporum* and VA fungi would result in lower colonization of MAP roots by AM fungi and lower abundance of truffle mycelium in the *brûlé*. The study showed a strong negative relation of *brûlé* soil with the shoot growth of lavender and rosemary during their first vegetative period in the field, although not with their survival. It also suggested a negative relation with the yield of essential oils. This could affect the productivity of lavender and rosemary because their shoots begin to be harvested from the second year (More et al. 2010). It would be interesting to assess how this growth decrease could have a negative influence on other ecosystem services of MAPs such as carbon sequestration or enhanced pollinator populations.

The *brûlé* of *T. melanosporum* heavily inhibits the germination and growth of many herbaceous and shrub species (González-Armada et al. 2010; Pacioni 1991; Taschen et al. 2020). This has generally been attributed to the allelopathic effect of truffle metabolites and the endophytic interaction of truffle mycelium with the roots of these plants (Schneider-Maunoury et al. 2020; Streiblová et al. 2012). In our study, *T. melanosporum* mycelium was practically absent outside the *brûlé*, and the shoot growth of MAPs was negatively correlated to the abundance of *T. melanosporum* mycelium in their rhizosphere. However, we cannot reject the hypothesis that MAP growth decrease is partly caused by root competition between MAPs and oaks, or by the competition between the fungi associated to their respective roots. Previous studies have frequently found a negative relationship between ECM and AM fungi (Chen et al. 2000; Knoblochová et al. 2017). However, we found no negative relationship of AM fungi colonization with *brûlé* occurrence or abundance of *T. melanosporum* mycelium, thus suggesting that MAP growth decrease is not related to deficient AM colonization.

Despite previous studies suggesting reduced survival of herbaceous and shrub species in *T. melanosporum brûlés* (González-Armada et al. 2010; Pacioni 1991), we did not find such an effect in the planted MAPs during their first vegetative period in the field. We hypothesise that this is due to the MAPs being introduced as plantlets, whereas weeds usually arrive at *brûlés* as seeds. Taschen et al. (2020) showed that truffle mycelium inhibited germination of weed seeds in rhizotron trials. The recently germinated seedlings are likely much more sensitive to the *brûlé* effect than the bareroot lavender or the containerised rosemary seedlings. This is supported by the lack of correlation between MAP mortality and abundance of *T. melanosporum* mycelium, which suggests that mortality was more related to the pre-planting vigour or physiological conditions of the seedlings.

With regard to the essential oils of the MAPs, we unexpectedly found lower yields in plants growing within *brûlés*, where the conditions for MAPs are apparently more stressful. In water stress experiments, it is frequent that *Lavandula* and *Salvia* species increase essential oils yield as a mean to protect themselves from water stress (Chrysargyris et al. 2016; Sarmoum et al. 2019). This would suggest more intense stress conditions in the *brûlé* or different biochemical/physiological mechanisms from those triggered by water stress (Kleinwächter and Selmar 2015). Previous studies also found that water stress altered the composition of essential oil, particularly in α-pinene, limonene, 1,8-cineole and camphor (Chrysargyris et al.

2016; Kulak 2020). Our study found an increased abundance of 1,8-cineole in lavenders growing within the *brûlé*, in agreement with Chrysargyris et al. (2016) and Kulak (2020) who found that water stress increased the abundance of this compound. Besides, the PCA shows that the *brûlé* involved a more substantial modification of the volatile compounds profile for lavender than for rosemary. This suggests a higher potential for influencing the essential oil commercial quality, although this should be confirmed in subsequent years (Kivrak 2018). Our results did not show that the mycelium of *T. melanosporum* was negatively affected by MAP presence in the *brûlé*, the soil where most truffle fruitbodies will be produced. The finding that truffle mycelium abundance was similar in the MAP rhizosphere and the bulk soil was unexpected, because MAP roots are colonised by AM fungi and previous studies have shown negative relationships between AM and ECM fungi (Chen et al. 2000; Knoblochová et al. 2017). This is all the more remarkable considering that MAPs show high levels of essential oils with biocidal properties (Garzoli et al. 2021; Valcárcel et al. 2021). Interestingly, Taschen et al. (2020) found that some non-ECM plants promoted truffle mycelium growth in rhizotron experiments, including *Thymus vulgaris* L., a MAP species. They hypothesised that this could be related to enhanced nutrition (through parasitism of non-ECM plants or thanks to root-associated microorganisms increasing soil nutrients availability). Another possible explanation would be a change in the distribution pattern of the oak fine roots in response to competition with non-ECM plants. Previous studies showed that oak fine roots tended to concentrate in soil layers where weed roots grew (Cubera et al. 2012; Gómez-Molina et al. 2020). Finally, we cannot rule out that truffle mycelium growth was boosted by the change in soil conditions: soil was locally disturbed and loosened to plant MAPs and then irrigated for a while. It would be interesting to monitor whether further growth of MAPs in subsequent years will affect *T. melanosporum* mycelium. Previous studies show that *T. melanosporum* mycelium is more abundant in productive than in non-productive trees, thus suggesting it influences truffle orchard productivity (Parladé et al. 2013; Queralt et al. 2017).

In conclusion, we found that the shoot growth and essential oil yield of lavender and rosemary seedlings was impaired during their first vegetative period growing in truffle *brûlé* soils, which are characterized by the presence of *T. melanosporum* as extraradical mycelium. On the other hand, the abundance of *T. melanosporum* extraradical mycelium was not affected by the occurrence of lavender and rosemary seedlings in the *brûlé*. We showed that the occurrence of *T. melanosporum* mycelium outside the *brûlé* was very limited, and the normal growth of the MAPs was not apparently disturbed, thus supporting the potential of truffle – MAPs intercropping during the early stage of the truffle plantation. Truffle orchards are typically planted at broad frameworks (e. g. 6 × 6 m), thus allowing three rows of lavender or rosemary to be installed between tree rows, separated at least two meters from trees (Martin-Chave 2019). During the first lavender or rosemary cultivation cycle (8-10 years), the contact between tree and MAP roots is expected to be very limited. The challenges will likely begin in a second MAP cultivation cycle due to the *brûlé* spread, with a MAP growth reduction that will reduce its essential oil production and may also affect oil quality and MAP ecosystem services such as carbon sequestration or enhancement of pollinator populations. It would be interesting to monitor and quantify the findings of this work throughout the entire cultivation cycle of lavender and rosemary, to exactly evaluate the economic viability of intercropping. In this monitoring, productive aspects should be addressed, such as the changes in the yield and quality of the MAP essential oils or the increase in truffle fruitbody yield and quality suggested by some MAP growers (Martin-Chave 2019). The latter could be related to changes in soil properties, and particularly in soil microbial populations (Geoffroy et al. 2018). It would also be interesting to investigate a potential role of MAPs (and their essential oils) in the control of the pest insect *Leiodes cinnamomeus* Panzer, which is the main problem nowadays in many truffle plantations in eastern Spain (Julià et al. 2023).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Conceptualization and methodology, S.G.-B., S.S., E.G.-M., J.P. and J.N.-R.; investigation, E.G.-M., V.B., J.P., M.A.S., J.N.-R. and S.S.; formal analysis, S.G.-B.; writing – original draft, S.G.-B., S.S:, J.P. and J.N.-R.; writing – review and editing, all authors.

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Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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