

# Soil microbial biomass responses to essential oils extracted from different Mediterranean herbs

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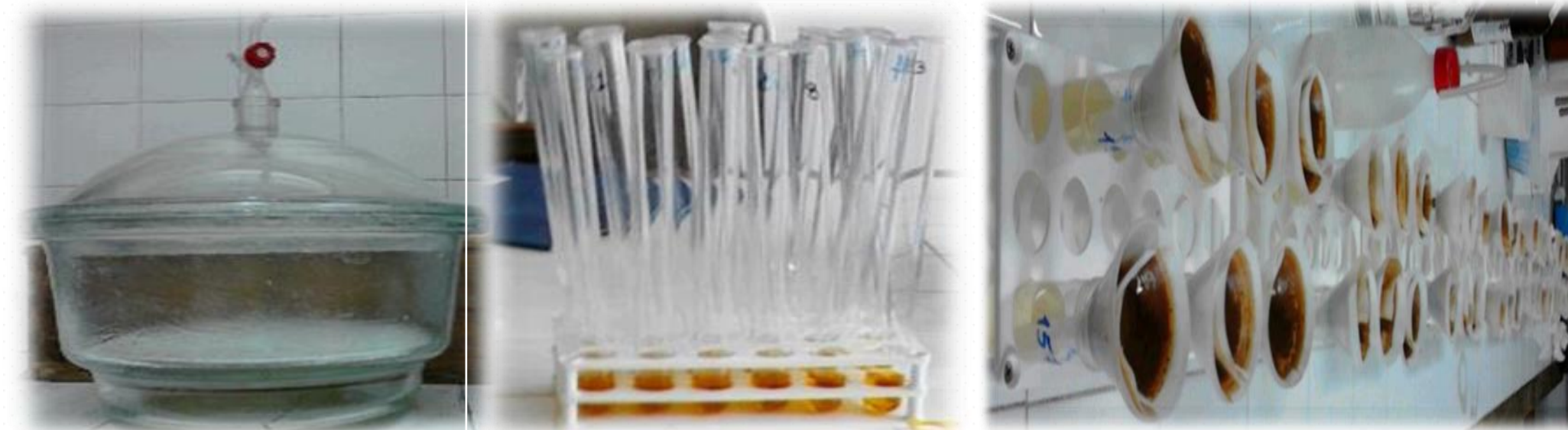
## INTRODUCTION

Previous *in vitro* and *in vivo* studies have found essential oils (EOs) extracted from *Thymbra capitata* (L.) Cav., *Mentha x piperita* L., and *Santolina chamaecyparissus* L., able to reduce seed germination and seedling emergence of many weeds. Such results suggest that EOs could potentially be used in the field for weed control. However, due to their possible antimicrobial activity, EOs could affect soil processes mediated by microorganisms. Therefore, *the aim of this work was to assess the impact of EOs extracted by hydrodistillation from T. capitata, M. piperita and S. chamaecyparissus on soil microbial biomass C and respiration, and on the relative abundance of main microbial groups.*

## MATERIALS AND METHODS

### Experimental design

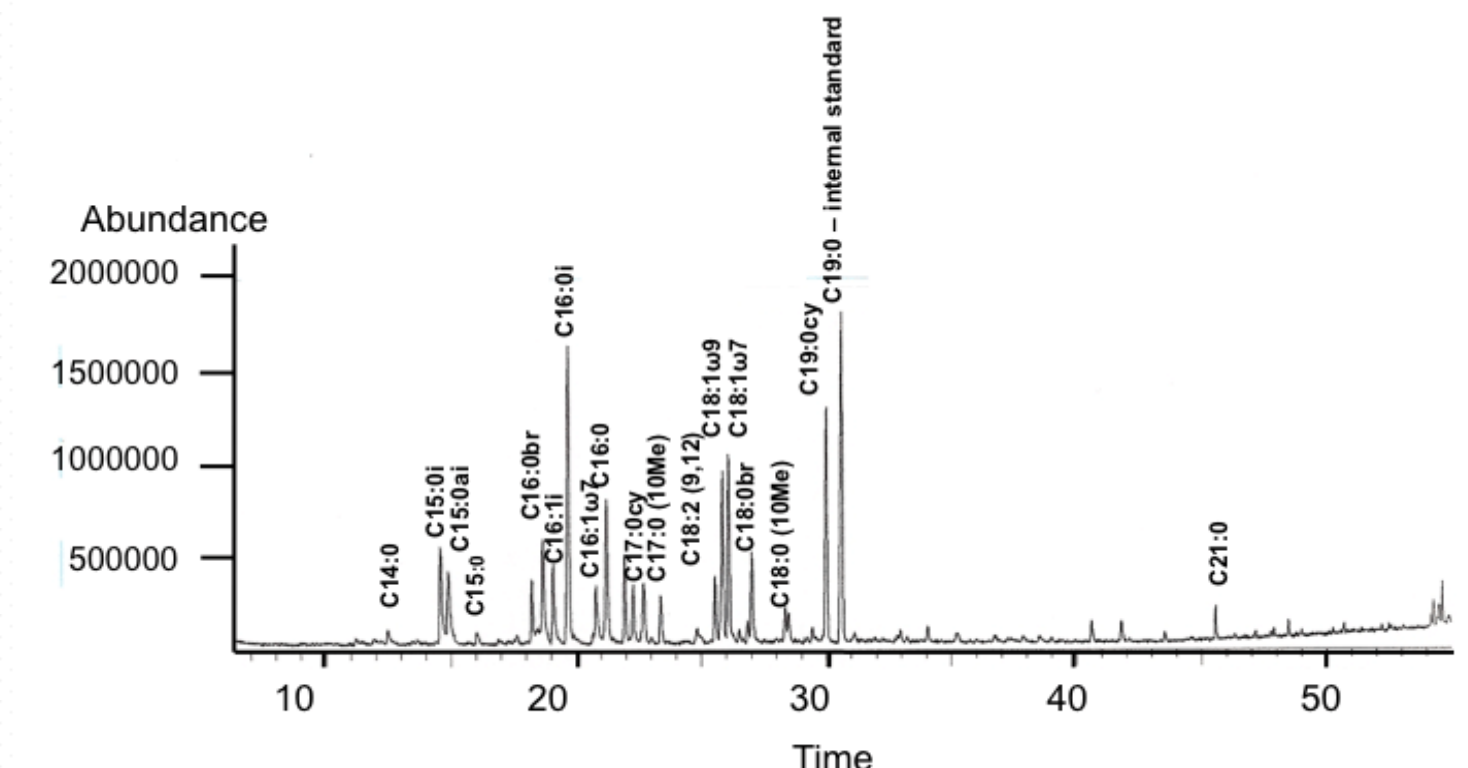
EOs were applied to 350 g aliquots of soil, disposed in 1L plastic bottles, previously moistened up to 2/3 of 50% of its water holding capacity (WHC). Then, soil aliquots were brought up to 50% of the WHC by adding a solution containing a given EO, so reaching a concentration of 31, 67, 93  $\mu$ l 100 g<sup>-1</sup> of soil for *T. capitata* EO (T1, T2, T3), and of 93, 123, 154  $\mu$ l 100 g<sup>-1</sup> of soil for *M. piperita* EO (M1, M2, M3) and *S. chamaecyparissus* EO (S1, S2, S3). Two controls were also prepared: one with water (Cw) and another with water and fitoil (Cf) at a concentration of 0.05% (v/v).



At days 7, 28 and 56 soil microbial biomass C was determined by the fumigation-extraction method.



The cumulative C mineralised at 7, 28 and 56 days of soil incubation (25 °C and 50% WHC) was determined in the headspace of glass jars by gas chromatography.



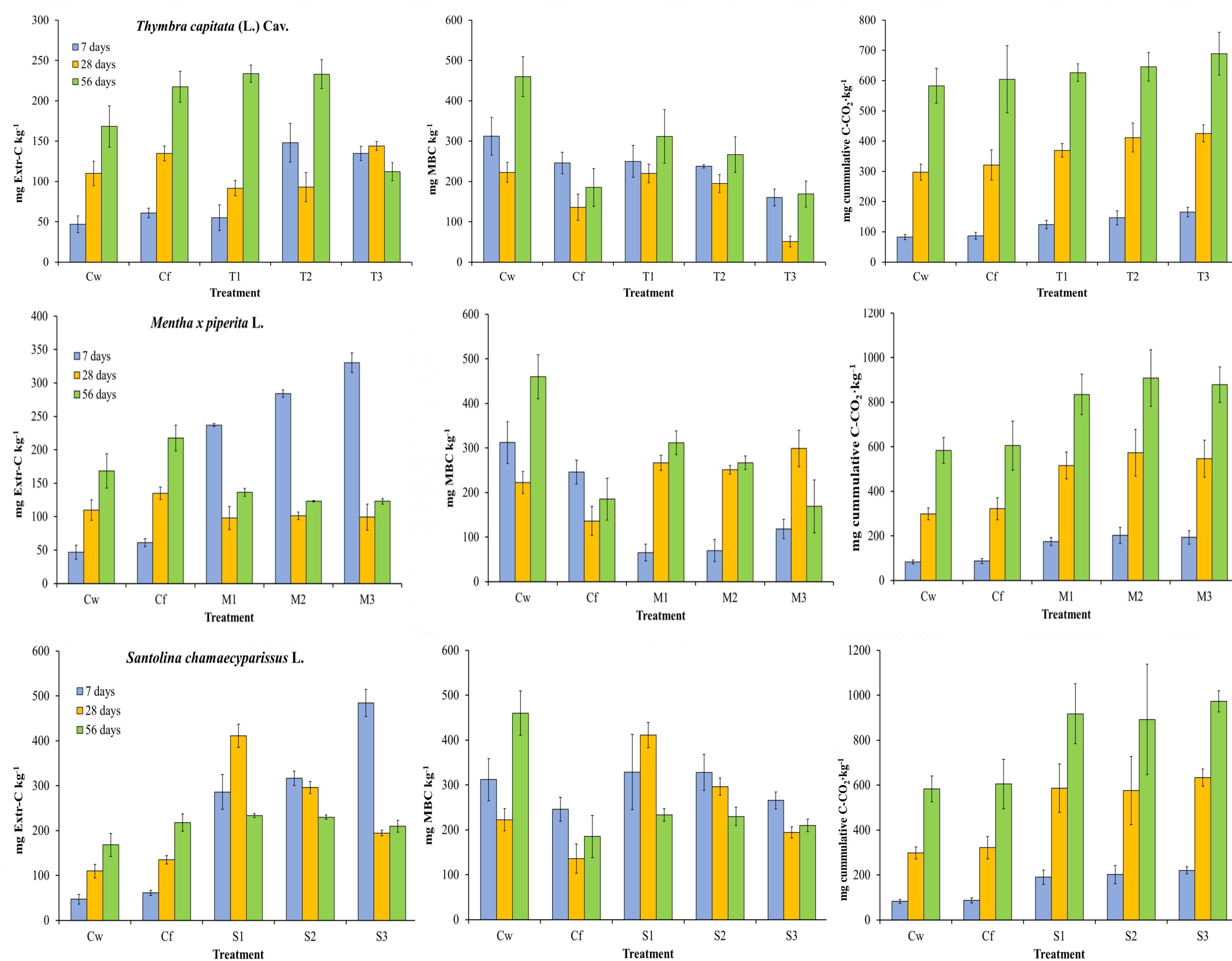
At days 7 and 28, the distribution of the main microbial groups was investigated by ELFAs method.

## RESULTS AND DISCUSSION

During the incubation, extractable C increased in both controls probably due to increased C availability following soil sieving and wetting; also MBC followed the same pattern but only in Cw, thus suggesting an inhibiting effect of fitoil. At day 7, *Thymbra* oil increased extractable C, compared to Cf, with T2 and T3 concentrations. Then, at day 28, extractable C decreased with T1 and T2 and at day 56 with T3. MBC increased with the two lowest concentrations at day 28 and 56 and decreased with the highest concentration at days 7 and 28, compared to Cf. The cumulative amount of C mineralised, compared to Cf, generally increased at days 7 and 28 at any concentration, but not at day 56. Overall, the above findings suggested that *Thymbra* oil at the highest concentration kills some microorganisms at day 28, whereas those surviving are anyway able to mineralize as organic C substrates both essential oil and killed microorganisms.

At day 7, *Mentha* oil increased extractable C and such an increase was proportional to the concentration applied. Thereafter, extractable C was always lower than Cf and no significant differences were observed among concentrations. MBC at day 7 strongly decreased at any oil concentration, compared to Cf, while slightly increased at 28 and 56 days. The cumulative C mineralisation was stimulated by any *Mentha* oil concentration at all observed days, compared to Cf.

*Santolina* EO increased extractable C only at day 7, compared to Cf, being the increase proportional to the applied concentrations, and at day 28, but with the increase inversely related to the concentrations. Also MBC was generally increased by *Santolina* EO at any observation day and any concentration, compared to Cf. The cumulative C mineralized was always stimulated by *Santolina* EO at the same extent by any concentration, being at the three observation days higher than Cf. The previous results provide insights that both *Mentha* and *Santolina* EOs stimulated both soil microbial biomass and activity.



At day 7, *Thymbra* EO at T3 concentration decreased the fungi to bacteria ratio (F/B) due to a concomitant slight increase of bacteria and a decrease of fungi. Also the bacteria gram positive to negative ratio (G+/G-) was negatively affected by *thymbra* EO: at day 7 by only T3 concentration, whereas at day 28 by all the applied concentrations. *Mentha* EO strongly affected the F/B ratio; indeed it was reduced by M1 concentration at day 7 and, at the same extent, by all concentrations at day 28. The reduction of the F/B ratio is to be ascribed mainly to a significant reduction of fungi. With regard to the G+/G- ratio, only M3 concentration slightly reduced it. *Santolina* EO affected the main microbial groups only after 28 days of incubation. Precisely, S2 and S3 concentrations decreased both F/B and G+/G- ratios, with the latter ratio even more reduced by the highest concentration.

## CONCLUSIONS

The carrier used to apply the EOs to soil (fitoil) decreased the microbial biomass, likely due to its liposoluble nature. However, the three investigated EOs extracted from wild Mediterranean plants, especially at the two lowest concentrations, stimulated both microbial biomass and activity. By contrast, at the highest concentration, both *Thymbra* and *Mentha* oils exerted a significant biocidal action. Our results are explainable in terms of interaction between native soil organo-mineral colloids and applied EOs. At lower concentration, EOs were trapped by soil colloids and made available to microbial mineralization/immobilization, while at the highest concentration some EOs molecules escaped colloids, thus keeping their biocidal action. The main effects of the tested EOs can be synthesized as follows: a) increase of the extractable C as a consequence of the killing of unresisting soil microorganisms, b) stimulation of soil microorganisms in terms of biomass and activity and c) alteration of the main microbial groups. Finally, our findings raise the alarm that caution must be used about the preservation of soil biological features if essential oils from plants are thought to be used for conservative agricultural practices, such as the weed control, especially in sandy soils or with low organic matter content.

