Leaflet No.

The Republic of the Union of Myanmar
Ministry of Natural Resources and Environmental Conservation

# Mediterranean Alley-Cropping Agroforestry with Walnut and Medicinal and Aromatic Plants: 14 Years Insights in Sagàs, northeastern Spain

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March, 2026

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## Abstract

In Mediterranean regions, agroforestry systems are increasingly being implemented to enhance the sustainability and resilience of land use in the face of climate change. However, long-term studies evaluating the performance of alley-cropping systems remain scarce. This study reports on a 14-year alley-cropping field experiment in Sagàs (northeastern Spain), which combined *Juglans × intermedia* with *Salvia officinalis* (years 1-7) and with *Melissa officinalis* (years 8-14). The objectives were to investigate the performance of medicinal and aromatic plants (MAPs) and trees, both alone and in combination. The results indicate that in the first crop cycle (*Salvia officinalis*), the trees had no significant impact on the MAPs, either in terms of microclimate or crop performance. However, in the second crop cycle (*Melissa officinalis*), trees reduced light availability up to 50% in mid-summer and buffered extreme temperatures by 2-3ºC. Under these conditions, all crop performance indicators showed a consistent decline, with overall dry biomass reduced by approximately 67% under agroforestry compared to the control, especially in summer and autumn. Generally, trees were unaffected by the presence of MAPs throughout the study. Overall, our findings underscore the dynamic trade-offs in crop performance between monoculture and agroforestry conditions. This highlights the importance of optimizing design, species selection, and management in Mediterranean agroforestry systems.

**Keywords:** *Juglans, Melissa, Salvia, long-term experiment, crop yield, biomass, silvoarable*

# သမပိုင်းရာသီဥတုရှိ စပိန်နိုင်ငံအရှေ့မြောက်ပိုင်း၊ ဘာစီလိုးနားခရိုင်၊ ဆာဂါ့စ်ဒေသတွင် ၁၄ နှစ်ကြာ သစ်ကြားမျိုးနှင့် ဆေးဖက်ဝင်၊ ရနံ့ပါဝင်သောအပင်မျိုးများဖြင့် တန်းကျော် တန်းကြား ပေါင်းစပ်စိုက်ပျိုးခြင်း နည်းစနစ်၏ ဇီဝကမ္မဆိုင်ရာ ရလဒ်များအပေါ် သုတေသနပြု ဆန်းစစ်လေ့လာခြင်း

ဖွဲ့ညီညီအောင်၊ တောအုပ်ကြီး
သစ်တောဦးစီးဌာန

**စာတမ်းအကျဉ်း**

ယခုသုတေသနစာတမ်းသည် ၂၀၁၁ ခုနှစ်မှ ၂၀၂၄ ခုနှစ်ထိ၊ စပိန်နိုင်ငံ၊ အနောက်မြောက်ပိုင်း၊ ဘာစီလိုးနားခရိုင်၊ ကုန်းတွင်းပိုင်းဒေသတစ်ခုဖြစ်သည့် Sagàs ဒေသတွင် အဖိုးတန် သစ်မျိုးတစ်ခုဖြစ်သည့် *Juglans x intermedia* နှင့် ဆေးဖက်ဝင်ဒေသ ဈေးကွက်ရှိ အပင် မျိုးများဖြစ်သော *Salvia officinalis* (ပထမ ၇ နှစ်)နှင့် *Melissa officinalis* (ဒုတိယ ၇ နှစ်) တို့အား ပေါင်းစပ်၍ သုတေသနပြု အကောင်အထည်ဖော်ဆောင်ရွက်ခဲ့သည့် ၁၄ နှစ် သက်တမ်းရှိသည့် သီးညှပ်စိုက်ပျိုးခြင်းစနစ်၏ ရလဒ်များအား စနစ်တကျသုတေသနပြု ဆန်းစစ်လေ့လာထားခြင်း ဖြစ်ပါသည်။ ဤသုတေသနတွင် ကမ္ဘာပေါ်ရှိ သမပိုင်းဇုံများနှင့် အလားတူ ရာသီဥတုအမျိုးအစားရှိ နေရာဒေသများ၊ ဥရောပနိုင်ငံများတွင် ရေရှည်တည်တံ့ စေမည့် မြေအသုံးချနည်းလမ်းများကို အထောက်အပံ့ဖြစ်စေရန်အလို့ငှာ သစ်နှင့်ဆေးဘက် ဝင်သီးနှံပင်များ သီးညှပ်စိုက်ပျိုးခြင်း နည်းစနစ်တစ်ခုကို အကောင်အထည်ဖော်ကာ ၎င်းပါ ဝင်အပင်များ၏ ဇီဝဒြပ်ထုအထွက်နှုန်း၊ ဇီဝဒြပ်ထုအရည်အသွေးများ၊ ဇီဝကမ္မဆိုင်ရာ အရည် အသွေးများကို ဆန်းစစ်လေ့လာရန် အဓိကရည်ရွယ်၍ ၁၄ နှစ်ကြာ သုတေသနပြု ဆောင်ရွက် ခဲ့ခြင်းဖြစ်ပါသည်။ တစ်ဖက်တွင်လည်း ထိုကဲ့သို့ သီးညှပ်စိုက်ပျိုးသည့် နည်းစနစ်များနှင့် ၎င်းတို့၏ နှစ်ပေါင်းများစွာ အကျိုးဆက်စပ်ပက်သတ်မှုများ၊ ဇီဝကမ္မဆိုင်ရာ သဘောတရားများ ကို ကောင်းစွာနားလည်သဘောပေါက်ခြင်းအားဖြင့် ပိုမိုကောင်းမွန်သည့် သီးနှံသစ်တော ရောနှော စိုက်ပျိုးသည့်နည်းစနစ်များ၊ ၎င်းတို့၏ အထွက်နှုန်းများနှင့် ထာဝစဥ်တည့်တံ့ စေမည့် မြေအသုံးချမှုနည်းလမ်းများကို ကောင်းစွာပံ့ပိုးအကြံပြုနိုင်ရန်ရည်ရွယ်ပါသည်။အဆိုပါ သုတေ သနကို အကောင်အထည်ဖော် ဆောင်ရွက်ရန် Experimental Design တစ်ခုတည်ဆောက်၍ trials များကို plots (Control Vs Agroforestry) အကွက်များ အဖြစ် ဖော်ဆောင်ကာ ၎င်းတို့ အကြား Interested Variables များ (MAPs yield, MAPs quality, MAPs physiological features, Tree vegetative status, Tree growth and Tree physiological features) များ ကို တိုင်းတာပြီး၊ replicated field trials များအကြား နှိုင်းယှဥ်ဆန်းစစ်လေ့လာ၍တင်ပြ ထားပါသည်။

# Mediterranean Alley-Cropping Agroforestry with Walnut and Medicinal and Aromatic Plants: 14 Years Insights in Sagàs, northeastern Spain

## 1. Introduction

Agroforestry systems, defined as the deliberate combination of trees, crops, and/or livestock on the same area of land to benefit from ecological or economic interactions (Quinkenstein et al. 2009; Burgess and Rosati 2018), have been recognized for their ability to provide multiple ecosystem services enhancing sustainable land use. Alley-cropping agroforestry, which alternates rows of trees with alleys of crops, shows promising potential in mitigating the economic, environmental, and social limitations of contemporary agriculture (Quinkenstein et al. 2009). This is particularly applicable in Mediterranean regions, where land-use practices must strike an appropriate balance between agricultural productivity and ecological resilience during the hot and dry summers (Reyes et al. 2021). These climatic conditions exacerbate the risks associated with soil degradation, water scarcity, and climate change impacts, to which Mediterranean areas are particularly vulnerable (Rodrigues et al. 2024).

In response to these climatic and environmental barriers, Mediterranean alley-cropping systems have been shown to mitigate desertification, control erosion, and increase soil organic carbon retention when compared to traditional crop systems (Cardinael et al. 2015; Ivezić et al. 2022). Additional co-benefits of these systems include improved land-use efficiency, enhanced biodiversity, and microclimate regulation (Acosta et al. 2024; Koch et al. 2025). These systems are also characterized by complex and dynamic interactions between trees and crops, which can lead to competition for light, water, and nutrients between the system components (Majaura et al. 2024; Swieter et al. 2022), so their design and management must be based on a deep understanding of the interactions between trees and crops.

Among the various options available, alley-cropping systems that combine high-value timber trees with MAPs (Medicinal and Aromatic Plants) can be both ecologically and economically beneficial (Martin-Gorriz et al. 2022; Pan et al. 2025). *Juglans x intermedia* (hybrid walnut) is one of the most valued timber species in temperate areas, with rapid growth, drought tolerance, and good quality wood, suitable for veneer and premium sawn wood markets (Pelleri et al. 2020). In addition, its late flushing (late May to early June) makes it particularly suitable for agroforestry. In return, MAPs generate valuable biomass and secondary metabolites for the pharmaceutical, cosmetic, and food industries (Coşge Şenkal 2020; Rao et al. 2004), which are in-demand market products. *Salvia officinalis* (sage) is a valuable crop, adapted to drought and moderately tolerant to shade, making it suitable for agroforestry (Mohammadi-Cheraghabadi et al. 2021; Şeker et al. 2023). Conversely, another valuable MAP species, *Melissa officinalis* (lemon balm), is more sensitive to shade and requires a higher water supply. While mild shade (30%) can mitigate drought stress and increase yields in dry conditions, its higher overall water demand raises questions about its ability to consistently adapt to agroforestry systems (Zubay et al. 2021).

Despite their valuable contributions, not many mid to long-term studies have examined the performance of these species combined within Mediterranean alley-cropping systems in terms of yield, quality, and physiological responses. This reflects the broader lack of long-term evaluations of agroforestry systems in Europe (Ivezić et al. 2022; Rigueiro-Rodríguez et al. 2008). This study aims to contribute to filling this gap by evaluating the effects of combining *Juglans x intermedia* (hybrid walnut) with *Salvia officinalis* (sage) and *Melissa officinalis* (lemon balm) during a 14-year alley cropping field experiment, compared to monocrop systems where MAPs and trees are grown separately. The experiment was carried out in Sagàs, a continental Mediterranean area within the province of Barcelona (Catalonia, northeastern Spain). This area is traditionally characterized by rainfed winter cereal cropping and is affected by declining crop profitability and soil fertility levels, as well as soil erosion, drought impacts, and nitrate pollution associated with intensive farming (Loureiro et al. 2017; Blanc et al. 2019). To address these challenges, the alley cropping system implemented in this study was developed to diversify farm income, increase annual productivity, and improve environmental performance, with special regard to soil and water protection.

The objectives of this study are to assess: (i) the yield and quality of sage and lemon balm grown in monocrop and in agroforestry systems throughout a full rotation (7 years) of each species; (ii) the buffering of micro-site conditions (soil temperature, light availability) induced by trees on MAPs over time; and (iii) walnut performance (survival, growth, physiology) grown in monocrop and combined with MAPs. Based on existing literature, we hypothesized that trees would affect crop performance dynamically over time, resulting in buffered temperatures, higher humidity, and reduced light availability. Understanding tree-crop interactions provides valuable insights into optimizing agroforestry designs and management to maximize productivity and sustainability (Gonçalves et al. 2021; Grigoriadou et al. 2019; Jose et al. 2015).

## 2. Materials and Methods

### 2.1 Study site

The study site was established in spring 2011 in Sagàs (inland Barcelona province, 42.0455ºN, 1.9618ºE), in a flat field standing at an altitude of 690 m, and traditionally devoted to rain-fed winter cereal production. The Köppen climate type (1981-2010) ranges between Cfa and Cfb, i.e., temperate, with no dry season, and a hot or warm summer. From 1991-2020, the mean annual temperature was 13.1 °C and the mean annual rainfall amounts were 715 mm, with 220 mm in summer (SMC, 2023). The soil is a leptic regosol, characterized by a loamy texture (44% sand, 34% silt, 22% clay). The pH is 8.1, with 2.4% organic matter, 18% carbonates, 4% active limestone, and an electrical conductivity of 0.18 dS/m.

The mean annual temperature and precipitation, as well as the summer precipitation during the study period (2011-2024) were in line with the reference values for the area, despite the typically contrasting annual and seasonal variations of Mediterranean areas (Table S1 in Supplementary Material). Monthly temperature and precipitation data were obtained from the nearest weather station (La Quar) of the Catalan Meteorological Service, located less than 4 km away.

### 2.2 Field trial implementation, management and experimental design

#### 2.2.1 Medicinal and aromatic plants (MAPs) component

At the start of the experiment, the MAP species was sage (*Salvia officinalis*) variety "Regula", grown from seed and planted at 4 months old using a "Super-prefer" planter in early April 2011. Sage was arranged in 8 simple rows, each with a 30 x 140 cm frame and with 580 cm between two double rows (Figure 1). This species was managed extensively, without irrigation or fertilization, and with interventions limited to annual manual weeding and two annual harvesting cuts: one in late spring (blooming time) to stimulate leaf production, and a commercial harvest (leaves) between late summer and early autumn. In January 2018, the sage plants were pulled up as they showed symptoms of senescence.

In April 2018, lemon balm (*Melissa officinalis*) variety "Lorelei" was established. It was grown from seed and planted at 4 months old using the same equipment and arrangement as sage; except for the within-row spacing extended to 35 cm. This species was managed with support irrigation consisting of fortnightly irrigation in March, 1-2 weekly irrigations in April, May, and September, and 2-3 weekly irrigations in June, July, and August, with an average water dose of 10 L/m² per irrigation. Fertiliser was applied in diluted form in the irrigation water three times per year: in March, after the first cut in late May, and after the second cut in July, using 250 kg/ha of NPK 7-4-10. In 2023, irrigation and fertilization were reduced by 50% due to water shortages, and in 2024, no irrigation or fertilization was applied. The crop was mechanically harvested 2-4 times annually, depending on productivity, between April and November.

### 2.2.2 Tree component

The tree species is a hybrid walnut (*Juglans x intermedia*) progeny, Mj-209xRa, developed for valuable timber production. In March 2011, we planted 1-year-old seedlings, 40-60 cm high, bare-rooted, on a 7 x 6 m frame with 7 m between rows, and therefore with 2.8 m between the walnut row and the closest MAPs row. Soil preparation consisted of deep (50 cm) linear subsoiling, and planting pits (30 cm x 30 cm x 30 cm) were opened manually just before manual tree planting. Immediately after planting, a 1x1 m ground cover made of black, UV-treated polyethylene 80 μm thick was installed, and a 60 cm high black plastic mesh shelter, fixed with a steel bar. Tree management was limited to annual or semi-annual manual mechanical weeding within the rows in late spring and annual pruning in mid-July, aimed at achieving a 4- to 5-m high straight bole, removing up to 40% of the leaves in a single operation.

### 2.2.3 Arrangement of experimental MAPs and trees

The experimental MAPs are those located in the 4 central rows (3, 4, 5, and 6 in Figure 1), while the rows 1, 2, 7, and 8 are considered as buffers. We deployed four experimental conditions as follows:

- MAPs without effect from trees (MAPs-Control); 400 experimental plants located in rows 3 to 6, far from the trees
- MAPs combined with trees (MAPs-AF): 400 experimental plants located in rows 3 to 6, with trees at both sides
- Trees without effect from the MAPs (Trees-Control); 14 experimental trees
- Trees combined with MAPs at both sides (Trees-AF): 21 experimental trees.

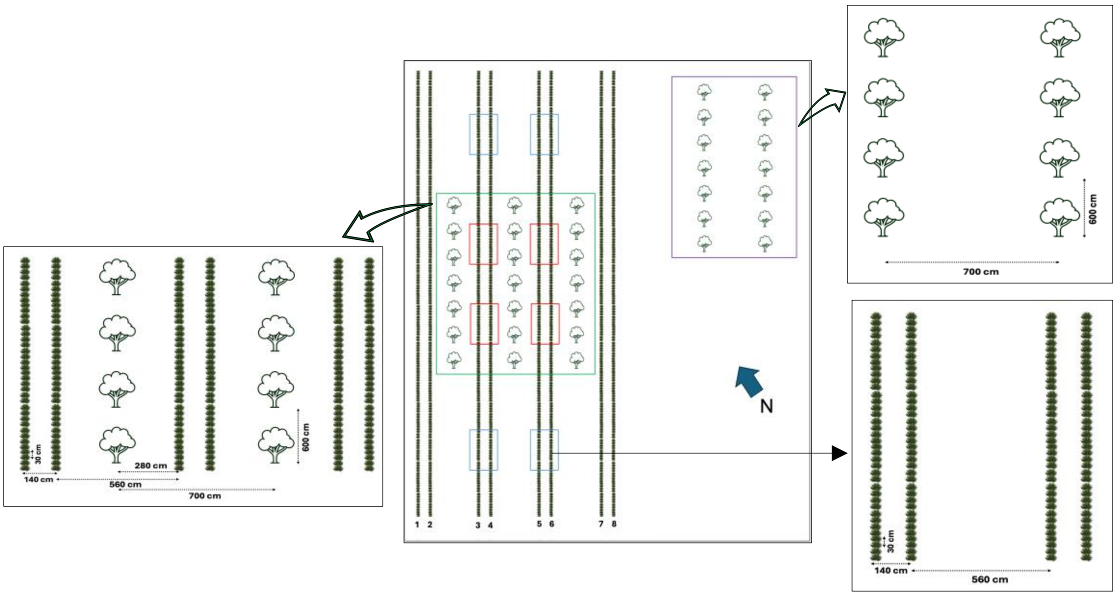


Figure 1: Arrangement of the experimental MAPs and trees

### 2.3 MAPs monitoring

To assess MAPs yield, manual samplings of sage fresh biomass (48 plants per treatment) were taken right before each commercial harvest, annually from year 1 (2011) to 7. For lemon balm, the manual fresh biomass samplings covered 50 cm long transects, as individual plant identification was not possible due to vegetative propagation resulting in complete row coverage after the first few months. Therefore we established 32 sampling points per treatment, in years 12 (spring and autumn, plants aged 5), 13 (spring, summer, and autumn), and 14 (spring and autumn). Right after each sage and lemon balm sampling, the fresh biomass collected was dried in an oven (35ºC for 96 h) to ascertain plant moisture and dry biomass. This was measured in all sage samples and 8 lemon balm samples per treatment and measuring time.

The MAPs quality was assessed using 6-8 stems per treatment from the dried samples to calculate the leaf: plant biomass ratio. Moreover, in years 2 (autumn), 3 (autumn and spring), 4 (autumn), and 6 (spring), sage essential oil concentration was determined from dried leaves (40 to 60 g per treatment), through hydro-distillation with Clevenger equipment, following the protocol of the Royal Spanish Pharmacopoeia (Real Farmacopea Española 2015). For lemon balm, the rosmarinic acid content was measured at the spring, summer, and autumn harvests of year 13 (plants aged 6), from dried leaves (40 to 60 g per treatment), using liquid chromatography (HPLC-DAD).

Several indicators were used to assess the physiological status of MAPs. Leaf chlorophyll content was estimated in years 2 (2 times), 3 (1), 4 (1), 5 (2), and 6 (1) for sage, and in year 12 (4 times), 13 (4) and 14 (2) for lemon balm using SPAD (Soil Plant Analysis Development); a relative indicator of leaf greenness, using a handheld Minolta SPAD-502 instrument (Minolta Camera Co. Osaka, Japan). Each sampling consisted of 16 plants per treatment, and the average SPAD value was calculated from 3 fully elongated, sun-exposed leaves. Moreover, in years 12 (once), 13 (three times) and 14 (twice), the NDVI was measured between late spring and early autumn using a TRIMBLE Greenseeker handheld device (TRIMBLE Inc., Westminster, USA). In this case, we conducted 12 measurements per treatment and date.

The effect of trees on MAPs micro-site conditions was assessed by monitoring soil moisture, soil temperature, and light availability. Soil moisture was measured in years 2 (once), 12 (three times), 13 (three times), and 14 (twice) at the 0-10 cm layer in 12-16 points per treatment. We used gravimetry (year 2) and a handheld HOBO MX2306 moisture logger (Onset Computer Corporation, Bourne, USA) afterwards.

From April 2022 to June 2024, the soil temperature was monitored on an hourly basis using HOBO Pendant temperature loggers (UA-001-64 model, Onset Computer Corporation, Bourne, USA), with five sensors per treatment located at 5 cm depth. Light availability was measured hourly in lux at a height of 1.5 m using a HOBO Pendant temperature/light sensor (UA-002-64, same manufacturer) at two points per treatment, from May 1st to August 31st, 2022. Due to the failure of some devices, only data from two temperature sensors and one light sensor in the case of MAPs-Control treatment could be used.

### 2.4 Tree monitoring

At the end of each growing season (2011 to 2024), we visually assigned each tree to three possible categories of vegetative status: (1) alive and healthy; (2) alive with vegetative problems (basal sprouting, loss of apical shoot, wilted leaves); (3) dead. We also registered damages caused by wildlife. In parallel to tree vegetative status, we measured basal diameter with a digital caliper in two perpendicular directions at a constant point marked on each seedling. Total height was also considered using either a measuring tape (trees up to 150 cm high), a telescopic measuring tape (trees 150 - 400 cm high) or a digital hypsometer Vertex IV

(Haglöf Sweden AB, Långsele, Sweden) for trees over 350 cm high. Annual diameter and height growth were calculated as the difference between two consecutive measurements.

Tree physiological status was also characterised by measuring (i) NDVI in 10 trees per treatment, using the same procedure and timing as the MAPs; (ii) leaf chlorophyll content using the same procedure as the MAPs in years 2 (three times), 3 (once), 4 (twice) 12 (three times), 13 (four times) and 14 (twice) in 10 trees per treatment; and (iii) midday leaf water potential using a pressure chamber (Solfranc technologies, Vila-Seca, Spain) with ten leaves per treatment and measurement date, in summer days of years 2 (once), 3 (once) and 4 (twice).

### 2.5 Data analyses

Annual tree survival and vegetative status were analyzed using descriptive statistics. For the rest of the MAP and tree variables, differences between MAPs-Control and MAPs-AF, and between Trees-Control and Trees-AF, were assessed using a Welch t-test. The data were transformed (inverse, square root, or log) when necessary to meet the assumptions of normality and homoscedasticity, while tables and figures in this document show the untransformed data. When a normal distribution could not be assumed, the non-parametric Mann-Whitney test was conducted. To control the family-wise error rate arising from the large number of pairwise comparisons, Holm's method was applied for p-value correction.

In the case of micro-site variables, we focused on studying soil temperature during the coldest and hottest spells of the monitoring period (4-6 January and 25-26 August 2023, respectively), and conducted a Welch t-test using the mean hourly values. Light availability data were analyzed, discarding the night hours (value zero) and the first and last positive values of each day (dusk / dawn). The resulting dataset was analyzed using the Mann-Whitney test independently each fortnight (May to August 2022). All the analyses were conducted with R 4.4.1.

## 3. Results

### 3.1 MAPs yield

Table 1 presents dry plant and leaf biomass (g) of sage (2012-2017) and lemon balm (2022-2024) under monocrop (MAPs-Control) and agroforestry (MAPs-AF) conditions.

Table 1. Dry plant and leaf biomass (g) of sage (2012-2017) and lemon balm (2022-2024). Values are means ± SE. Significant differences between treatments according to Mann-Whitney tests are indicated as: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$. Exact p-values and Mann-Whitney W statistics are provided in Supplementary Material (Table S3).

| Species | Date | Dry plant biomass (g) | | Dry leaves biomass (g) | |
|---|---|---|---|---|---|
| | | MAPs-Control (Mean ± SE) | MAPs-AF (Mean ± SE) | MAPs-Control (Mean ± SE) | MAPs-AF (Mean ± SE) |
| Sage | 10-Oct-12 | **65.5 ± 5.17** | **44.0 ± 3.48 *** ** | **22.6 ± 1.89** | **17.9 ± 1.62 *** |
| | 21-May-13 | 31.1 ± 3.41 | 23.2 ± 2.03 | 5.12 ± 0.59 | 4.04 ± 0.37 |
| | 18-Sep-13 | 65.8 ± 7.54 | 56.3 ± 4.15 | 19.5 ± 2.48 | 16.9 ± 1.27 |
| | 07-May-14 | 39.7 ± 4.95 | 36.1 ± 4.74 | 7.68 ± 0.93 | 7.17 ± 0.87 |

| | | | | | |
|---|---|---|---|---|---|
| | 15-Oct-14 | 152.0 ± 13.9 | 136.0 ± 9.69 | 47.3 ± 4.47 | 43.3 ± 2.97 |
| | 30-Sep-15 | 110.0 ± 7.24 | 125.0 ± 7.12 | 40.7 ± 2.74 | 43.4 ± 2.51 |
| | 03-Oct-16 | 77.8 ± 1.42 | 74.0 ± 2.14 | 23.6 ± 0.57 | 22.5 ± 0.87 |
| | 16-Oct-17 | **125.0 ± 11.2** | **96.2 ± 7.98 *** | **52.4 ± 4.90** | **39.2 ± 3.56 *** |
| Lemon balm | 18-May-22 | **437.0 ± 31.2** | **216.0 ± 32.0 ***** | **288.0 ± 20.6** | **143.0 ± 21.1 ***** |
| | 25-Aug-22 | **283.0 ± 72.8** | **61.7 ± 17.7 **** | **187.0 ± 48.1** | **40.7 ± 11.7 **** |
| | 31-May-23 | **327.0 ± 35.1** | **165.0 ± 23.1 **** | **226.0 ± 24.3** | **114.0 ± 16.0 **** |
| | 22-Aug-23 | **240.0 ± 55.6** | **33.0 ± 8.56 **** | **166.0 ± 38.5** | **22.8 ± 5.92. **** |
| | 26-Oct-23 | **170.0 ± 40.2** | **27.2 ± 7.91 **** | **118.0 ± 27.8** | **18.8 ± 5.48 **** |
| | 05-Jun-24 | **183.0 ± 31.2** | **39.4 ± 15.5 **** | **130.0 ± 22.2** | **28.0 ± 11.0 **** |

Statistically significant differences were observed between treatments in sage dry biomass (either per whole plant or considering only the leaves), only in the first and last measurement dates. On those dates, the MAPs-Control exhibited higher plant and leaf biomass compared to MAPs-AF. In most remaining measurements, MAPs-Control tended to yield higher values than MAPs-AF, although the difference was not statistically significant. Differences across measurement dates were more evident than the differences between treatments on a given date.

As for lemon balm, the dry biomass (either the whole plant or the leaf) revealed significant differences between treatments, favouring MAPs-Control, across all measurement dates, particularly with the summer and autumn measurements.

### 3.2 MAPs quality

The sage essential oil yield analysis revealed no significant differences between treatments on any of the measurement dates or for the entire period, and no consistent trend was observed (Table 2). However, in lemon balm, MAPs-Control consistently exhibited a higher rosmarinic acid content compared to MAPs-AF, with significant differences in two out of three treatments, and in the overall value as well.

Table 2: Yield in essential oil (sage) and in rosmarinic acid (lemon balm) under monocrop (MAPs-Control) and agroforestry (MAPs-AF) conditions. Values are means ± SE. Significant differences between treatments according to Mann-Whitney tests are indicated as: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$. Exact p-values and Mann-Whitney W statistics are provided in the Supplementary Material (Table S4).

| Variable | Date | MAPs-Control (Mean ± SE) | MAPs-AF (Mean ± SE) |
|---|---|---|---|
| Sage essential oil yield (ml/kg) | 21-May-13 | 26.50 ± 0.55 | 28.50 ± 0.42 |
| | 18-Sep-13 | 27.60 ± 0.48 | 25.40 ± 0.76 |
| | 15-Oct-14 | 27.10 ± 0.29 | 27.60 ± 0.56 |

| Variable | Date | MAPs-Control (Mean ± SE) | MAPs-AF (Mean ± SE) |
|---|---|---|---|
| | 21-May-13 to 15-Oct-14 | 24.50 ± 0.21 | 21.10 ± 0.34 |
| Lemon balm rosmarinic acid (mg/g) | 31-May-23 | 59.88 ± 1.74 | 60.40 ± 1.07 |
| | 22-Aug-23 | **44.11 ± 2.84** | **22.98 ± 1.74 ***** |
| | 26-Oct-23 | **49.30 ± 2.24** | **34.90 ± 2.01 ***** |
| | 31-May-23 to 26-Oct-23 | **51.09 ± 1.03** | **39.40 ± 1.66 *** |

### 3.3 MAPs physiological indicators

Table 3 presents the SPAD and NDVI (used as indicators of vegetative vigor) values of sage (2012-2015) and lemon balm (2022-2024) under monocrop (MAPs-Control) and agroforestry (MAPs-AF) treatments.

Table 3: SPAD and NDVI values of sage and lemon balm 2012-2024. Significant differences between treatments according to Welch t-tests are indicated as: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$. Exact p-values and test statistics are provided in the Supplementary Material (Table S5 and S6).

| Species | Variables | Date | MAPs-Control (Mean ± SE) | MAPs-AF (Mean ± SE) |
|---|---|---|---|---|
| Sage | SPAD | 24-Jul-12 | 46.7 ± 0.4 | 46.8 ± 0.7 |
| | | 10-Jul-13 | 41.0 ± 0.7 | 39.9 ± 1.0 |
| | | 30-Sep-15 | 42.3 ± 0.5 | 41.8 ± 0.3 |
| | | 24-Jul-12 to 30-Sep-15 | 43.1 ± 0.2 | 43.3 ± 0.3 |
| Lemon balm | SPAD | 12-Apr-22 | 25.0 ± 0.5 | 25.0 ± 0.6 |
| | | 18-May-22 | **28.8 ± 0.6** | **27.2 ± 0.3 **** |
| | | 20-Jul-22 | **23.6 ± 0.5** | **26.8 ± 0.6 ***** |
| | | 25-Aug-22 | 28.8 ± 0.6 | 30.2 ± 0.5 |
| | | 31-May-23 | 24.2 ± 0.4 | 23.6 ± 0.3 |
| | | 19-Jul-23 | **30.4 ± 0.9** | **27.4 ± 0.9 *** |
| | | 22-Aug-23 | **25.6 ± 0.6** | **22.2 ± 0.8 *** |
| | | 26-Oct-23 | 23.4 ± 0.3 | 23.5 ± 0.5 |
| | | 05-Jun-24 | 20.6 ± 0.5 | 21.0 ± 0.3 |
| | | 20-Aug-24 | **24.4 ± 0.9** | **32.1 ± 0.7 ***** |

| Species | Variables | Date | MAPs-Control (Mean ± SE) | MAPs-AF (Mean ± SE) |
|---|---|---|---|---|
| | | 12-Apr-22 to 20-Aug-24 | 25.7 ± 0.2 | 26.0 ± 0.2 |
| | NDVI | 20-Jul-22 | **0.40 ± 0.03** | **0.29 ± 0.01 **** |
| | | 19-Jul-23 | **0.65 ± 0.04** | **0.50 ± 0.04 *** |
| | | 22-Aug-23 | **0.85 ± 0.01** | **0.62 ± 0.02 ***** |
| | | 26-Oct-23 | **0.82 ± 0.01** | **0.69 ± 0.01 ***** |
| | | 05-Jun-24 | **0.79 ± 0.01** | **0.72 ± 0.01 **** |
| | | 20-Aug-24 | **0.71 ± 0.01** | **0.58 ± 0.02 ***** |
| | | 20-Jul-22 to 20-Aug-24 | **0.66 ± 0.01** | **0.54 ± 0.01 ***** |

Sage SPAD values showed no significant differences between treatments, indicating that the sage chlorophyll content was not affected by the presence of the tree. In contrast, lemon balm exhibited significant differences on five out of ten measurement dates, with an unclear trend: on three of these dates, MAPs-Control had higher SPAD values than MAPs-AF, with the trend reversing on two other dates. The overall mean SPAD values for the entire study period were similar between treatments.

In the case of NDVI for lemon balm, all measurement dates, as well as the overall results, consistently showed higher NDVI values for MAPs-Control than for MAPs-AF.

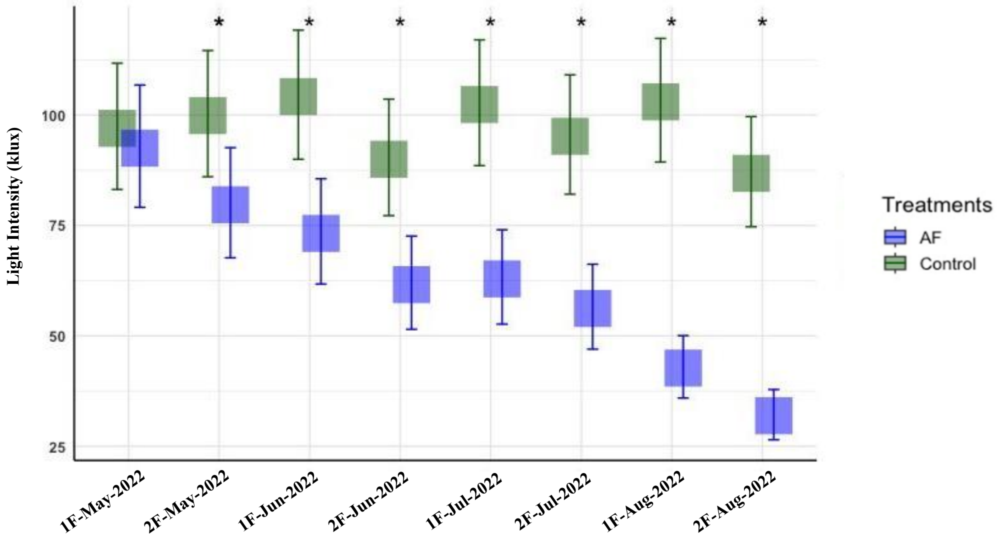


Figure 2: Fortnight light intensity in MAPs-control and MAPs-agroforestry conditions, in May to August 2022. 1F and 2F correspond to the first and second fortnight of each month, respectively. Asterisks indicate significant differences between treatments according to Mann-Whitney test. Exact p-values and Mann-Whitney W statistics are provided in Supplementary Material (Table S11).

### 3.4 Tree-induced micro-site features

Figure 2 shows comparison of light intensity (klux) available for MAPs in control and agroforestry conditions, measured fortnightly from May to August 2022. MAPs-AF experienced a significant reduction in light intensity on all measurement dates except the first (1-15 May), right before walnut flushing (visual observation). Subsequently, in contrast light availability in MAPs-Control remained stable. With the MAPs-AF light availability declined steadily (with a minor increase in early July caused by tree pruning), and reduced to 50% compared to MAPs-Control by early August, and to 33% by late August.

Figure 3 shows the comparison of soil temperature (°C) in MAPs-Control and MAPs-AF treatments during the coldest (January 2023) and hottest (August 2023) spells.

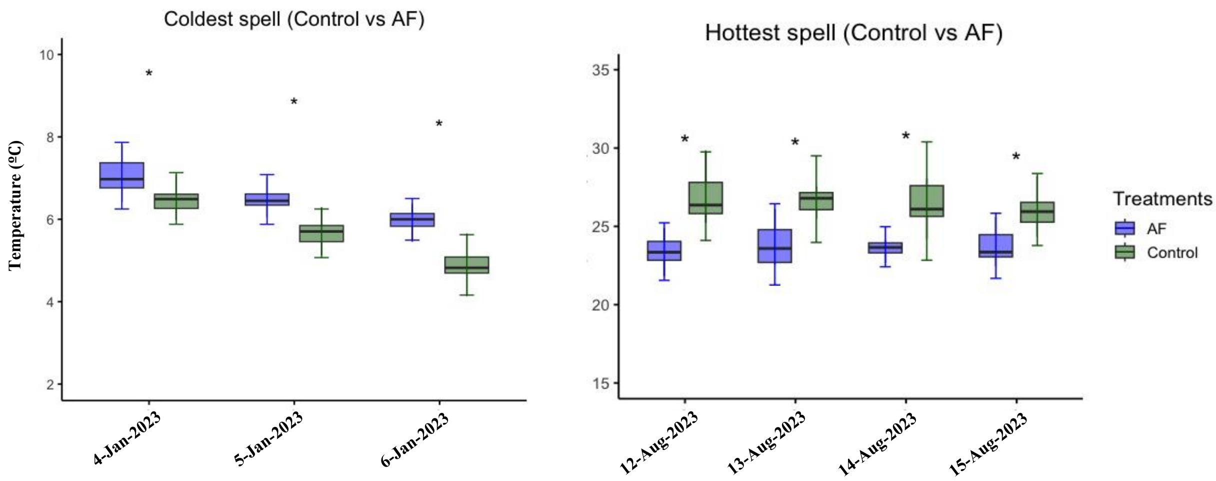


Figure 3: Comparison of soil temperature (°C) in MAPs-Control and MAPs-AF treatments during the coldest spell (January 2023) and the hottest one (August 2023). Asterisks indicate significant differences between treatments according to Welch t-tests. Exact p-values and test statistics are provided in the Supplementary Material (Table S12).

soil MAPs-Control were exposed to significantly higher soil temperatures compared to MAPs-AF on all dates, with temperature differences reaching up to 2-3ºC.

### 3.5 Tree growth

Figure 4 shows the annual evolution of cumulative basal diameter (up) and total height (down) of walnut Trees-Control and Trees-AF over the study period.

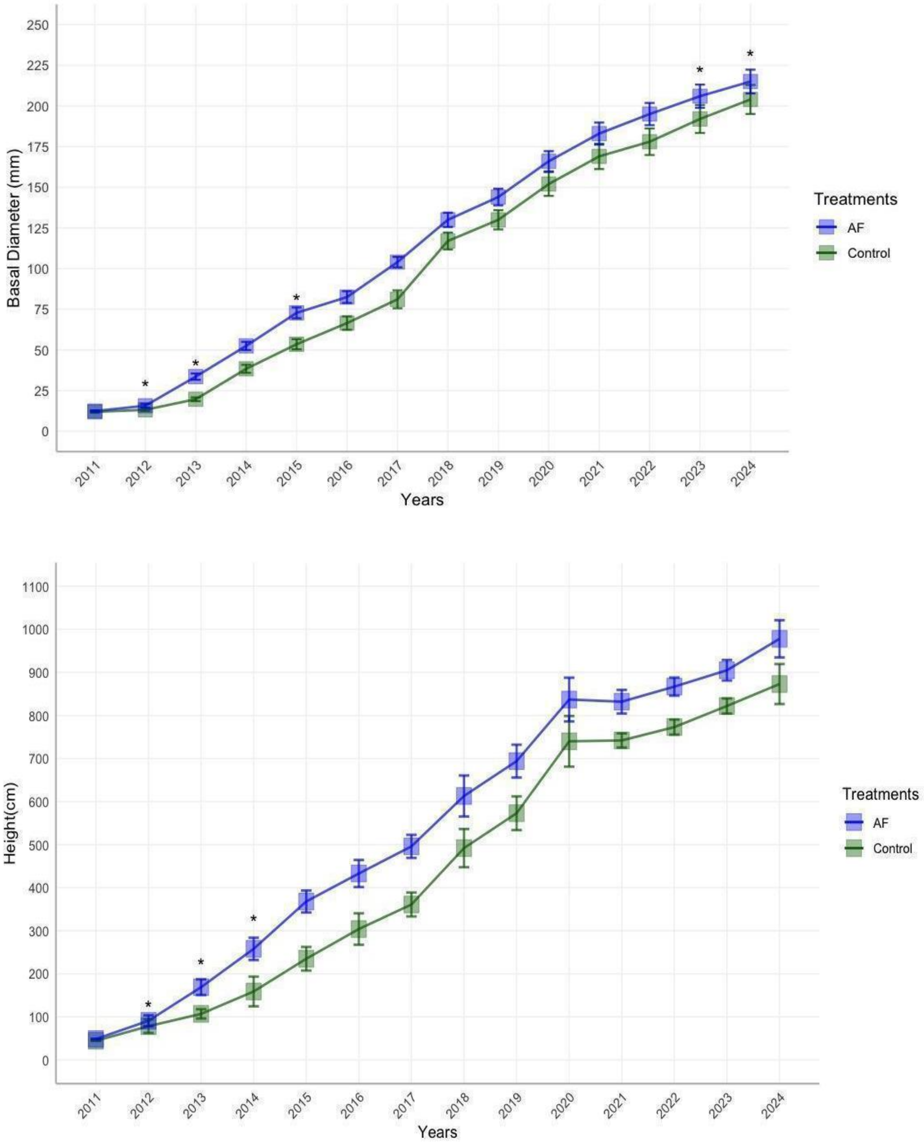


Figure 4: Evolution of cumulative basal diameter (up) and total height (down) over the study years. Asterisks indicate significant differences in annual growth between Trees-Control and Trees-AF according to Welch t-tests. Exact p-values and test statistics are provided in the Supplementary Material (Table S7).

Annual walnut diameter growth revealed significant differences between treatments favorable to Trees-AF during the early establishment phase, particularly in 2012, 2013, and 2015. However, from 2016 to 2022, annual growth rates remained similar between the two treatments, with no statistically significant differences. Interestingly, significant differences re-emerged in the final two years of the study (2023 and 2024), but with an inverse pattern compared to the early years, i.e., Trees-Control grew faster than Trees-AF. Overall, across the study, Trees-AF individuals exhibited diameters approximately 10% larger than those of Trees-Control.

The annual height growth of walnut trees followed a similar pattern, with significant differences favoring Trees-AF during the early years (2012-2014). However, from 2015 onwards, no significant differences were observed between treatments. Despite inter-annual fluctuations, Trees-AF individuals maintained a higher cumulative height (+14%) at the end of the study.

### 3.6 Tree physiological indicators

Table 10 summarizes SPAD values for walnut trees under both control and agroforestry system from 2012 to 2024.

Table 4: SPAD values of walnut trees (2012-2024). Significant differences between treatments according to Welch t-tests are indicated as: *** p < 0.001; ** p < 0.01; * p < 0.05. Exact p-values and test statistics are provided in the Supplementary Material (Table S10).

| Date | Trees-Control (Mean ± SE) | Trees-AF (Mean ± SE) |
|---|---|---|
| 22-Jun-12 | 30.5 ± 0.6 | 28.3 ± 0.5 |
| 24-Jul-12 | 38.3 ± 0.6 | 37.1± 0.6 |
| 21-Sep-12 | **45.2 ± 0.7** | **41.4 ± 0.8 **** |
| 10-Jul-13 | 36.1 ± 0.6 | 34.2 ± 1.1 |
| 16-Jul-14 | **35.1 ± 1.8** | **41.9 ± 0.9 **** |
| 03-Sep-14 | 36.1 ± 2.1 | 35.0 ± 1.9 |
| 18-May-22 | **33.5 ± 1.9** | **26.1 ± 1.0 **** |
| 20-Jul-22 | 39.2 ± 1.1 | 39.1 ± 0.7 |
| 25-Aug-22 | 40.8 ± 0.7 | 41.3 ± 0.5 |
| 19-Jul-23 | 36.8 ± 1.6 | 38.6 ± 1.1 |
| 22-Aug-23 | 41.5 ± 1.6 | 42.1 ± 2.0 |
| 26-Oct-23 | 38.2 ± 3.5 | 36.1 ± 1.5 |
| 05-Jun-24 | 30.9 ± 1.0 | 29.0 ± 1.3 |
| 20.Aug.24 | 45.6 ± 1.7 | 43.9 ± 1.8 |
| 22-Jun-12 to 20.Aug.24 | 37.6 ± 0.4 | 36.4 ± 0.4 |

Walnut SPAD revealed significant differences between treatments on three out of fourteen measurement dates, where Walnut-Control showed higher SPAD values twice, while the opposite trend occurred once. Across the entire study period, the overall mean SPAD values showed no significant treatment effect. Temporal variability in SPAD values was evident, with fluctuations across years and measurement dates rather than treatment effect.

NDVI and leaf water potential measurements did not indicate a significant treatment effect (data provided in Tables S8 and S9 of the Supplementary Materials).

## 4. Discussion

Sage and lemon balm exhibited contrasting responses to the presence of walnut trees in an agroforestry system, highlighting the need for species-specific assessments of the dynamic and complex interactions between trees and medicinal and aromatic plants. Our study incorporates 14 years of data on several performance variables, and can provide a basis for selecting the optimal crop for Mediterranean alley-cropping systems, while also providing valuable insights into the necessity and timing of management interventions to enhance system sustainability over time.

### 4.1 Sage performance

In general, agroforestry had no relevant effects on the various measured parameters of sage performance. Sage yield was generally lower in the agroforestry system than in the control, but this difference was statistically significant on only two out of eight measurement dates, suggesting that tree competition did not significantly constrain sage productivity. This hints that either the trees were too small to exert strong competition or that sage gradually adapted to agroforestry conditions. Along the same lines, other studies suggest that shading only influences sage yield marginally, with no or limited consistent reductions reported across agroforestry or intercropping systems, as sage reveals strong plasticity and adaptability to different light conditions (Zervoudakis et al. 2012; Şeker et al. 2023).

There were no significant differences in sage essential oil production between the agroforestry and control treatments on any measurement date, suggesting that the presence of trees did not affect secondary metabolite production. These results align with studies showing that shading has limited or species-dependent effects on the essential oil content and composition of aromatic herbs (Milenković et al. 2019, 2021; Zubay et al. 2021). Similarly, sage SPAD values showed no significant difference between treatments, suggesting that the relatively small size of the trees during the sage cultivation phase did not produce enough shade to impact chlorophyll content. Similar results were observed in sage by (Zervoudakis et al. 2012), who reported that leaf chlorophyll and photosynthetic pigments remained stable under low-light conditions, indicating the species' adaptability to varied light environments.

### 4.2 Lemon balm performance

Both lemon balm yield and rosmarinic acid content were consistently lower in the agroforestry system than in the control, suggesting a strong treatment effect. Compared to sage (combined with trees aged 1-7, with an average height of 550 cm at the end of the crop cycle), lemon balm was grown under larger trees, aged 8-15, with an average height of 900 cm and canopy closure at the end of the crop cycle. Moreover, lemon balm is known to be particularly sensitive to shade (Oliveira et al. 2016), which is a limitation for many crops used in agroforestry systems (Tmušić et al. 2021; Lalević et al. 2023). Differences between treatments were particularly pronounced in mid-to-late season measurements (from June onwards), in line with the measured decline of light availability (Figure 2). Light availability is known to reduce rosmarinic acid accumulation, with studies showing reductions under shaded conditions in some years (Zubay et al. 2021).

Unlike sage, which was cultivated under rain-fed conditions, lemon balm was irrigated. However, irrigation did not prevent the yield reduction under agroforestry, suggesting that light availability was a major limiting factor, as lemon balm is known to reduce photosynthesis performance and yield under shade conditions (Nurzyńska-Wierdak et al. 2023; Oliveira et al. 2016). Root competition for water and nutrients is a potential below-ground interaction that may have also contributed, although this was not investigated in the current study. Kuyah et al. (2019) showed that tree-crop root competition for water and nutrients can sometimes outweigh the benefits of irrigation in agroforestry systems.

Similar to sage, lemon balm SPAD values showed no consistent pattern related to treatment or annual or seasonal precipitation, suggesting that this variable is not clearly affected by shade or weather. Previous studies on lemon balm suggest that SPAD-related variables such as photosynthetic pigments, including chlorophyll a and b, are primarily nutrient-driven, particularly in response to nitrogen availability (Fallah et al. 2024; Shoor et al. 2012). This supports the hypothesis that the effect of tree competition on lemon balm is related less to nutrients (belowground) than light deprivation.

NDVI values for lemon balm were consistently higher in MAPs-Control compared to MAPs-AF across all measurement dates, indicating a clear reduction in vegetative activity under agroforestry conditions. The differences peaked during the drier and warmer measurement periods, such as in July and August, which corresponded with both low precipitation and high canopy shading. In contrast, NDVI differences between treatments were less evident in early June and late October, coinciding with the onset of walnut flushing and wetter conditions, respectively. These seasonal contrasts underscore the combined influence of light availability and water status in shaping NDVI response. Consistent with our findings, Rihan et al. (2022) reported that light spectra significantly influenced NDVI in lemon balm, since blue-rich wavelengths (particularly 435 nm) caused marked reductions, thereby confirming the NDVI sensitivity to light conditions.

These outcomes emphasize the importance of adapting crop species to the developmental stage (i.e.,light availability) of the agroforestry system. Other factors that could mitigate the detrimental effect of shading include adjusting the design (especially row spacing and orientation) and management practices, such as strategic pruning and/or early thinning (Honfy et al. 2023).

### 4.3 Overall tree performance

The growth dynamics of walnut trees in agroforestry and control systems followed a biphasic pattern, with agroforestry trees exhibiting a growth advantage in both diameter and height during the early years (2012-2014). This initial benefit is unlikely to be the result of facilitative effects from sage, which were minimal at that time. Instead, it may be attributed to indirect effects of MAPs management, particularly weeding around sage rows, which might have reduced early-stage weed competition for trees. Despite the 1 $m^2$ groundcover in all trees, this intervention may have improved the availability of water and soil nutrients during a period when trees are most vulnerable to weed competition, a factor to which young walnut trees are known to be highly sensitive (Coello et al. 2017; Paris et al. 2005).

From 2015 to 2022, tree growth in both treatments progressed in parallel, indicating that agroforestry (either directly or indirectly, as mentioned above) had no significant positive or negative impact on walnut performance. However, in 2023-2024, AF trees exhibited reduced diameter growth compared to control trees. This may be explained by the earlier onset of intraspecific competition within Trees-AF, which are larger in diameter than Trees-Control. Therefore, the former might have entered the crown competition stage (canopy closure stage) earlier, reducing stem radial growth. This suggests that an urgent thinning intervention in AF trees may be needed to sustain growth and timber quality (Honfy et al. 2023). There is no evidence of a potential benefit from lemon balm fertigation (2018-2022) on walnut trees. These results highlight the dynamic nature of tree-crop interactions: whereas initial growth gains under agroforestry were probably due to indirect effects of MAPs management (i.e., weeding), there is no evidence of any direct effect of MAPs on trees.

As in the case of MAPs, walnut SPAD showed no clear treatment effect. SPAD values fluctuated in response to the seasonal dynamics of chlorophyll concentration, typically rising from spring to late summer, and then declining at the end of the growing season (Gauthier et al. 2011; Montpied et al. 2009).

Beyond species-specific responses, our results highlight that walnut trees moderated microclimatic extremes by reducing peak summer soil temperatures and increasing minimum winter temperatures by up to 2-3 °C. This buffering effect is particularly relevant in continental Mediterranean regions, where agricultural systems are increasingly exposed to heatwaves, cold spells, and greater climatic variability. Such microclimate regulation suggests that agroforestry can contribute not only to crop-tree interactions but also to enhancing the resilience of farming systems under scenarios of more frequent and intense extreme weather events (Acosta et al. 2024; Koch et al. 2025).

## 4. Conclusions

This 14-year study shows that tree-MAPs interactions in Mediterranean alley-cropping systems are highly dynamic over time and species. Sage, grown in years 1-7, was negligibly affected by trees, suggesting a minimal shadowing effect and/or certain shade tolerance. In contrast, lemon balm was strongly and negatively affected by trees (aged 8-15) in terms of yield, NDVI, and rosmarinic acid content, particularly in the periods of the year when walnuts were fully leafed.

Walnut trees initially appeared to benefit from their combination with sage, exhibiting greater height and diameter growth during the first years, probably due to more intensive weed control in the agroforestry system. After a few years, however, this advantage disappeared. Over the last two years, diameter growth declined relative to the control, likely in response to early canopy closure in agroforestry trees due to their large size.

Overall, these findings illustrate the dynamic interactions between crops and trees over time. In Mediterranean agroforestry systems, selecting the right crop and tree species, and managing light availability (through tree spacing, pruning, and thinning) are crucial to enhancing system productivity and sustainability over time.

**Acknowledgements**

This study was conducted as part of the LIFE AgroForAdapt project (LIFE20 CCA/ES/001682), which is co-funded by the European Union's LIFE Program. The field trial establishment and early measurements were part of the "Sistemes Agroforestals a Catalunya" project (2011-2020), which was financed by the Deputy Directorate General for Rural Planning of the Catalan Department of Agriculture, Livestock, Fisheries, and Food. The authors are grateful to Ramon Comellas and to all the staff involved in site management and data gathering, including but not limited to Guillem Llena, Eduard Busquets, Henriette Gercken, Hsiao-Fan Wu, Martín Müller, Leah Martin, Sónia Navarro, and Helena Sarri. Aitor Ameztegui acknowledges the support he received from the Serra Húnter Program of the Generalitat de Catalunya and the Department of Agricultural and Forest Sciences and Engineering (DCEFA) at the University of Lleida. Zwe Nyi Nyi Aung is grateful to the MSc European Forestry Erasmus Mundus Joint Master Programme, co-funded by the European Union.

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# Supplementary materials

Table S1: Mean annual and seasonal temperature (ºC) and precipitation (mm) over the study period

| Year | Mean annual temperature (ºC) | Precipitation (mm) | | | |
|---|---|---|---|---|---|
| | | *Annual* | *Spring* | *Summer* | *Growing season (April-October)* |
| 2011 | 12.2 | 866 | 274 | 289 | 552 |
| 2012 | 11.9 | 699 | 265 | 193 | 575 |
| 2013 | 11.1 | 774 | 312 | 256 | 565 |
| 2014 | 11.9 | 990 | 207 | 465 | 776 |
| 2015 | 12.5 | 581 | 167 | 257 | 470 |
| 2016 | 12.1 | 620 | 211 | 130 | 446 |
| 2017 | 12.4 | 494 | 132 | 105 | 287 |
| 2018 | 11.6 | 1,303 | 367 | 349 | 932 |
| 2019 | 12.3 | 597 | 167 | 156 | 476 |
| 2020 | 12.1 | 1,139 | 345 | 476 | 895 |
| 2021 | 11.8 | 612 | 204 | 187 | 480 |
| 2022 | 13.4 | 708 | 193 | 315 | 532 |
| 2023 | 12.9 | 566 | 262 | 151 | 494 |
| 2024 | 12.6 | 948 | 341 | 257 | 708 |
| *Average 2011-2024* | ***12.2*** | ***778*** | ***246*** | ***256*** | ***585*** |

Table S2: Total precipitation (mm) before each physiological measurement date (2012-2024)

| Dates | Total precipitation (mm) – before measurement date | | | |
|---|---|---|---|---|
| | 1 weeks | 2 weeks | 4 weeks | 8 weeks |
| 22-Jun-12 | 2 | 2 | 97 | 197 |
| 24-Jul-12 | 3 | 3 | 53 | 150 |
| 21-Sep-12 | 15 | 66 | 121 | 131 |
| 10-Oct-12 | 0 | 50 | 83 | 172 |
| 21-May-13 | 78 | 79 | 193 | 276 |
| 10-Jul-13 | 3 | 3 | 20 | 128 |
| 18-Sep-13 | 7 | 46 | 118 | 208 |
| 07-May-14 | 2 | 10 | 75 | 94 |
| 16-Jul-14 | 11 | 54 | 139 | 217 |
| 03-Sep-14 | 3 | 65 | 184 | 332 |
| 15-Oct-14 | 17 | 38 | 105 | 255 |
| 15-Jul-15 | 0 | 0 | 31 | 133 |
| 30-Sep-15 | 11 | 12 | 69 | 216 |
| 03-Oct-16 | 25 | 48 | 92 | 113 |
| 16-Oct-17 | 0 | 1 | 38 | 116 |
| 12-Apr-22 | 0 | 32 | 78 | 120 |
| 18-May-22 | 0 | 2 | 59 | 108 |
| 20-Jul-22 | 0 | 6 | 59 | 124 |
| 25-Aug-22 | 42 | 45 | 146 | 157 |
| 31-May-23 | 52 | 71 | 169 | 209 |
| 19-Jul-23 | 0 | 6 | 44 | 185 |
| 22-Aug-23 | 10 | 10 | 16 | 54 |
| 26-Oct-23 | 28 | 59 | 59 | 138 |
| 05-Jun-24 | 57 | 65 | 108 | 197 |
| 20-Aug-24 | 21 | 21 | 74 | 163 |

Table S3: MAPs dry plant and leaves biomass 2012-2024

| Species | Variable | Date | MAPs-Control (Mean ± SE) | MAPs-AF (Mean ± SE) | Mann Whitney test | |
|---|---|---|---|---|---|---|
| | | | | | W | p |
| Sage | Dry plant biomass (g) | 10-Oct-12 | **65.5 ± 5.17** | **44.0 ± 3.48** | **713** | **0.001** |
| | | 21-May-13 | 31.1 ± 3.41 | 23.2 ± 2.03 | 923 | 0.094 |
| | | 18-Sep-13 | 65.8 ± 7.54 | 56.3 ± 4.15 | 2129 | 0.459 |
| | | 07-May-14 | 39.7 ± 4.95 | 36.1 ± 4.74 | 484 | 0.276 |
| | | 15-Oct-14 | 152.0 ± 13.9 | 136.0 ± 9.69 | 2007 | 0.208 |
| | | 30-Sep-15 | 110.0 ± 7.24 | 125.0 ± 7.12 | 41 | 0.682 |
| | | 03-Oct-16 | 77.8 ± 1.42 | 74.0 ± 2.14 | 229 | 0.230 |
| | | 16-Oct-17 | **125.0 ± 11.2** | **96.2 ± 7.98** | **859** | **0.032** |
| | Dry leaves biomass per plant (g) | 10-Oct-12 | **22.6 ± 1.89** | **17.9 ± 1.62** | **882** | **0.047** |
| | | 21-May-13 | 5.12 ± 0.59 | 4.04 ± 0.37 | 999 | 0.265 |
| | | 18-Sep-13 | 19.5 ± 2.48 | 16.9 ± 1.27 | 2218 | 0.717 |
| | | 07-May-14 | 7.68 ± 0.93 | 7.17 ± 0.87 | 490 | 0.309 |
| | | 15-Oct-14 | 47.3 ± 4.47 | 43.3 ± 2.97 | 2099 | 0.386 |
| | | 30-Sep-15 | 40.7 ± 2.74 | 43.4 ± 2.51 | 42 | 0.616 |
| | | 03-Oct-16 | 23.6 ± 0.57 | 22.5 ± 0.87 | 216 | 0.141 |
| | | 16-Oct-17 | **52.4 ± 4.90** | **39.2 ± 3.56** | **830** | **0.018** |
| Lemon balm | Dry plant biomass per lm (g) | 18-May-22 | **437.0 ± 31.2** | **216.0 ± 32.0** | **0** | **0.000** |
| | | 25-Aug-22 | **283.0 ± 72.8** | **61.7 ± 17.7** | **5** | **0.002** |
| | | 31-May-23 | **327.0 ± 35.1** | **165.0 ± 23.1** | **5** | **0.003** |
| | | 22-Aug-23 | **240.0 ± 55.6** | **33.0 ± 8.56** | **5** | **0.002** |
| | | 26-Oct-23 | **170.0 ± 40.2** | **27.2 ± 7.91** | **6** | **0.004** |
| | | 05-Jun-24 | **183.0 ± 31.2** | **39.4 ± 15.5** | **0** | **0.004** |
| | Dry leaves biomass per lm (g) | 18-May-22 | **288.0 ± 20.6** | **143.0 ± 21.1** | **0** | **0.000** |
| | | 25-Aug-22 | **187.0 ± 48.1** | **40.7 ± 11.7** | **5** | **0.002** |
| | | 31-May-23 | **226.0 ± 24.3** | **114.0 ± 16.0** | **5** | **0.002** |
| | | 22-Aug-23 | **166.0 ± 38.5** | **22.8 ± 5.92** | **5** | **0.003** |
| | | 26-Oct-23 | **118.0 ± 27.8** | **18.8 ± 5.48** | **6** | **0.005** |
| | | 05-Jun-24 | **130.0 ± 22.2** | **28.0 ± 11.0** | **0** | **0.004** |

Table S4: Yield in essential oil (sage) and in rosmarinic acid (lemon balm)

| Variable | Date | MAPs -Control (Mean ± SE) | MAPs -AF (Mean ± SE) | Mann Whitney test | |
|---|---|---|---|---|---|
| | | | | W | p (adjusted) |
| Essential oil yield (ml/kg) | 21-May-13 | 26.50 ± 0.55 | 28.50 ± 0.42 | 11 | 0.928 |
| | 18-Sep-13 | 27.60 ± 0.48 | 25.40 ± 0.76 | 21 | 0.929 |
| | 15-Oct-14 | 27.10 ± 0.29 | 27.60 ± 0.56 | 58 | 0.928 |
| | 21-May-13 to 15-Oct-14 | 24.50 ± 0.21 | 21.10 ± 0.34 | 586 | 0.558 |
| Rosmarinic acid (mg/g) | 31-May-23 | 59.88 ± 1.74 | 60.40 ± 1.07 | 32 | 1.000 |
| | 22-Aug-23 | 44.11 ± 2.84 | 22.98 ± 1.74 | 0 | **0.000** |
| | 26-Oct-23 | 49.30 ± 2.24 | 34.90 ± 2.01 | 2 | **0.000** |
| | 31-May-23 to 26-Oct-23 | 51.09 ± 1.03 | 39.40 ± 1.66 | 167 | 0.012 |

Table S5: SPAD values of Sage (2012-2015) and Lemon balm (2022-2024)

| Species | Date | MAPs-Control (Mean ± SE) | MAPs-AF (Mean ± SE) | Welch t-test | | |
|---|---|---|---|---|---|---|
| | | | | df | t | p |
| Sage | 24-Jul-12 | 46.7 ± 0.4 | 46.8 ± 0.7 | 22 | 0.11 | 0.908 |
| | 10-Jul-13 | 41.0 ± 0.7 | 39.9 ± 1.0 | 25 | -0.84 | 0.408 |
| | 30-Sep-15 | 42.3 ± 0.5 | 41.8 ± 0.3 | 27 | -0.36 | 0.720 |
| | 24-Jul-12 to 30-Sep-15 | 43.1 ± 0.2 | 43.3 ± 0.3 | 85 | 1.34 | 0.182 |
| Lemon balm | 12-Apr-22 | 25.0 ± 0.5 | 25.0 ± 0.6 | 45 | 0.01 | 0.992 |
| | 18-May-22 | 28.8 ± 0.6 | 27.2 ± 0.3 | 55 | -2.63 | **0.010** |
| | 20-Jul-22 | 23.6 ± 0.5 | 26.8 ± 0.6 | 45 | 3.97 | **0.000** |
| | 25-Aug-22 | 28.8 ± 0.6 | 30.2 ± 0.5 | 45 | 1.55 | 0.127 |
| | 31-May-23 | 24.2 ± 0.4 | 23.6 ± 0.3 | 45 | -1.07 | 0.288 |
| | 19-Jul-23 | 30.4 ± 0.9 | 27.4 ± 0.9 | 15 | -2.21 | **0.043** |
| | 22-Aug-23 | 25.6 ± 0.6 | 22.2 ± 0.8 | 26 | -2.31 | **0.029** |
| | 26-Oct-23 | 23.4 ± 0.3 | 23.5 ± 0.5 | 15 | 0.03 | 0.976 |
| | 05-Jun-24 | 20.6 ± 0.5 | 21.0 ± 0.3 | 20 | 0.37 | 0.708 |
| | 20-Aug-24 | 24.4 ± 0.9 | 32.1 ± 0.7 | 24 | 6.66 | **0.000** |
| | 12-Apr-22 to 20-Aug-24 | 25.7 ± 0.2 | 26.0 ± 0.2 | 385 | 0.59 | 0.551 |

Table S6: NDVI values 2022-2024 (species: lemon balm)

| Dates | MAPs-Control (Mean ± SE) | MAPs-AF (Mean ± SE) | Welch t-test | | |
|---|---|---|---|---|---|
| | | | df | t | p |
| 20-Jul-22 | 0.40 ± 0.03 | 0.29 ± 0.01 | 33 | -3.03 | **0.004** |
| 19-Jul-23 | 0.65 ± 0.04 | 0.50 ± 0.04 | 15 | -2.70 | **0.016** |
| 22-Aug-23 | 0.85 ± 0.01 | 0.62 ± 0.02 | 20 | -7.38 | **0.000** |
| 26-Oct-23 | 0.82 ± 0.01 | 0.69 ± 0.01 | 18 | -5.19 | **0.000** |
| 05-Jun-24 | 0.79 ± 0.01 | 0.72 ± 0.01 | 26 | -3.11 | **0.004** |
| 20-Aug-24 | 0.71 ± 0.01 | 0.58 ± 0.02 | 22 | -4.88 | **0.000** |

| Dates | MAPs-Control (Mean ± SE) | MAPs-AF (Mean ± SE) | Welch t-test | | |
|---|---|---|---|---|---|
| | | | df | t | p |
| 20-Jul-22 to 20-Aug-24 | 0.66 ± 0.01 | 0.54 ± 0.01 | 156 | -3.82 | **0.000** |

## Tree vegetative status


Figure S1: Tree vegetative status over the study years

Figure S1 illustrates the annual vegetative status of walnut trees from 2011 to 2024 under both control and agroforestry treatments, categorized as healthy, alive but with vegetative problems, wildlife-damaged, or dead.

Walnut tree survival was nearly complete throughout the study, with all Trees-AF remaining alive. Trees-Control mortality was limited to a single tree in 2019 and another one in 2021 (totaling 14% mortality). Tree health was predominantly good, although some years showed notable vegetative problems: in 2014 and 2015, 29% and 14% of the control trees, respectively, exhibited vegetative issues, while for the agroforestry trees the values were 10% in both years, respectively. Wildlife damage peaked in 2018, affecting 29% of control trees and 10% of agroforestry trees. Despite these problems, both types of trees showed recovery in the later years. By 2024, 86% of control trees and 95% of agroforestry trees were identified as healthy.

Table S7: Walnut annual diameter and height growth (2012-2024)

| Year | Diameter growth (mm year$^{-1}$) & Height growth (cm year$^{-1}$) | Trees-Control (Mean ± SE) | Trees-AF (Mean ± SE) | Welch t-test | | |
|---|---|---|---|---|---|---|
| | | | | df | t | p |
| 2012 | Diameter | 1.3 ± 0.2 | 4.9 ± 0.6 | 25 | 5.42 | **0.000** |
| | Height | 33.5 ± 3.9 | 44.6 ± 3.2 | 19 | 2.24 | **0.036** |
| 2013 | Diameter | 6.8 ± 0.6 | 16.7 ±1.4 | 32 | 6.89 | **0.000** |
| | Height | 29.5 ± 2.7 | 77.4 ± 4.5 | 20 | 8.38 | **0.000** |
| 2014 | Diameter | 18.2 ± 1.8 | 18.9 ± 1.2 | 22 | 0.31 | 0.754 |
| | Height | 52.4 ± 8.6 | 95.9 ± 6.5 | 15 | 3.41 | **0.003** |
| 2015 | Diameter | 15.8 ± 0.9 | 21.5 ± 0.7 | 19 | 4.28 | **0.000** |
| | Height | 84.7 ± 6.8 | 103.0 ± 6.4 | 23 | 1.83 | 0.080 |
| 2016 | Diameter | 12.4 ± 1.5 | 14.0 ± 1.1 | 19 | 0.83 | 0.414 |
| | Height | 74.2 ± 9.1 | 73.9 ± 7.8 | 27 | -0.03 | 0.976 |
| 2017 | Diameter | 20.2 ± 2.2 | 19.0 ± 1.0 | 18 | -0.30 | 0.763 |
| | Height | 62.3 ± 6.9 | 62.8 ± 6.7 | 29 | 0.04 | 0.963 |
| 2018 | Diameter | 27.0 ± 1.4 | 26.8 ± 1.3 | 19 | -0.11 | 0.914 |
| | Height | 112.0 ± 11.1 | 117.0 ± 11.9 | 26 | 0.25 | 0.799 |
| 2019 | Diameter | 14.4 ± 1.1 | 14.8 ± 0.8 | 20 | 0.33 | 0.743 |
| | Height | 81.2 ± 9.7 | 81.3 ± 9.5 | 29 | 0.00 | 0.996 |
| 2020 | Diameter | 21.3 ± 1.8 | 21.5 ± 1.4 | 24 | 0.08 | 0.929 |
| | Height | 167.0 ± 14.7 | 143.0 ± 12.7 | 29 | -1.34 | 0.190 |
| 2021 | Diameter | 17.9 ± 1.2 | 18.4 ± 0.5 | 15 | 0.35 | 0.726 |
| | Height | 30.7 ± 4.1 | 45.5 ± 6.8 | 29 | 1.82 | 0.079 |
| 2022 | Diameter | 9.5 ± 1.1 | 12.3 ± 0.7 | 21 | 2.02 | 0.056 |
| | Height | 27.7 ± 4.4 | 34.7 ± 5.3 | 29 | 0.89 | 0.377 |
| 2023 | Diameter | 14.0 ± 0.9 | 10.8 ± 0.6 | 22 | -2.83 | **0.009** |
| | Height | 32.3 ± 4.3 | 36.3 ± 5.9 | 29 | 0.03 | 0.974 |
| 2024 | Diameter | 11.6 ± 0.8 | 9.1 ± 0.7 | 26 | -2.18 | **0.038** |
| | Height | 80.0 ± 11.6 | 74.5 ± 10.8 | 28 | -0.33 | 0.739 |

Table S8: NDVI values of walnut trees (2022-2024)

| Date | Trees-Control (Mean ± SE) | Trees-AF (Mean ± SE) | Welch t-test | | |
|---|---|---|---|---|---|
| | | | df | t | p |
| 20-Jul-22 | 0.68 ± 0.01 | 0.69 ± 0.01 | 27 | 0.07 | 0.942 |
| 19-Jul-23 | 0.69 ± 0.01 | 0.72 ± 0.01 | 15 | 1.22 | 0.241 |
| 22-Aug-23 | 0.76 ± 0.02 | 0.71 ± 0.01 | 8 | -1.87 | 0.099 |
| 26-Oct-23 | 0.56 ± 0.02 | 0.56 ± 0.01 | 6 | 0.14 | 0.889 |
| 05-Jun-24 | 0.69 ± 0.01 | 0.74 ± 0.01 | 28 | 3.01 | **0.005** |
| 20-Aug-24 | 0.71 ± 0.02 | 0.71 ± 0.01 | 21 | 0.29 | 0.776 |
| 20-July-2022 to 20-Aug-24 | 0.68 ± 0.00 | 0.69 ± 0.00 | 126 | 0.85 | 0.392 |

The NDVI values revealed significant differences between treatments only on one measurement date out of six, where Walnut-AF outperformed Walnut-Control. Across the entire study period, the overall mean NDVI values showed no significant treatment effect. Notably, NDVI values varied substantially across measurement dates, with higher values observed during summer and a sharp decline in October.

Table S9: Leaf water potential (Bar) of walnut trees, 2012-2014

| Date | Trees-Control (Mean ± SE) | Trees-AF (Mean ± SE) | Welch t-test | | |
|---|---|---|---|---|---|
| | | | df | t | p |
| 22-Jun-12 | -11.40 ± 0.24 | -11.50 ± 0.21 | 27 | -0.22 | 0.830 |
| 10-Jul-13 | -13.60 ± 0.27 | -13.50 ± 0.43 | 15 | 0.085 | 0.933 |
| 16-Jul-14 | -12.00 ± 0.21 | -12.70 ± 0.56 | 12 | -1.01 | 0.334 |
| 03-Sep-14 | -13.90 ± 0.71 | -15.40 ± 0.85 | 17 | -1.31 | 0.206 |
| 22-Jun-12 to 3-Sep-14 | -12.60 ± 0.12 | -13.10 ± 0.13 | 84 | -0.99 | 0.321 |

Leaf water potential showed no statistically significant differences between treatments across any measurement dates or in the overall LWP during the study period. Temporal variability in LWP values was relatively low, with both treatments displaying similar trends over the measurement period.

Table S10: SPAD values of walnut trees (2012-2024)

| Date | Trees-Control (Mean ± SE) | Trees-AF (Mean ± SE) | Welch t-test | | |
|---|---|---|---|---|---|
| | | | df | t | p |
| 22-Jun-12 | 30.5 ± 0.6 | 28.3 ± 0.5 | 29 | -1.84 | 0.075 |
| 24-Jul-12 | 38.3 ± 0.6 | 37.1± 0.6 | 28 | -1.40 | 0.172 |
| 21-Sep-12 | 45.2 ± 0.7 | 41.4 ± 0.8 | 29 | -2.99 | **0.005** |
| 10-Jul-13 | 36.1 ± 0.6 | 34.2 ± 1.1 | 24 | -1.42 | 0.168 |
| 16-Jul-14 | 35.1 ± 1.8 | 41.9 ± 0.9 | 19 | 3.02 | **0.007** |
| 03-Sep-14 | 36.1 ± 2.1 | 35.0 ± 1.9 | 16 | -0.38 | 0.713 |
| 18-May-22 | 33.5 ± 1.9 | 26.1 ± 1.0 | 18 | -3.27 | **0.004** |
| 20-Jul-22 | 39.2 ± 1.1 | 39.1 ± 0.7 | 23 | -0.12 | 0.908 |
| 25-Aug-22 | 40.8 ± 0.7 | 41.3 ± 0.5 | 27 | 0.55 | 0.583 |
| 19-Jul-23 | 36.8 ± 1.6 | 38.6 ± 1.1 | 13 | 0.879 | 0.395 |
| 22-Aug-23 | 41.5 ± 1.6 | 42.1 ± 2.0 | 16 | 0.25 | 0.808 |
| 26-Oct-23 | 38.2 ± 3.5 | 36.1 ± 1.5 | 6 | -0.55 | 0.603 |
| 05-Jun-24 | 30.9 ± 1.0 | 29.0 ± 1.3 | 27 | -1.07 | 0.293 |
| 20.Aug.24 | 45.6 ± 1.7 | 43.9 ± 1.8 | 26 | -0.65 | 0.519 |
| 22-Jun-12 to 20.Aug.24 | 37.6 ± 0.4 | 36.4 ± 0.4 | 378 | -1.57 | 0.117 |

Table S11: Light intensity (kLux) in each fortnight (F1, F2) from early May to August 2022

| Dates | MAPs-Control (Mean ± SE) | MAPs-AF (Mean ± SE) | Mann Whitney test | |
|---|---|---|---|---|
| | | | W | p-adjusted |
| 1F-May-22 | 97.0 ± 4.6 | 92.5 ± 4.6 | 18,264 | 0.456 |
| 2F-May-22 | 99.9 ± 4.3 | 79.7 ± 4.5 | 25,006 | **0.000** |
| 1F-June-22 | 104.2 ± 4.2 | 73.2 ± 4.6 | 20,868 | **0.000** |
| 2F-June-22 | 90.0 ± 4.2 | 61.6 ± 4.4 | 22,723 | **0.000** |
| 1F-July-22 | 102.4 ± 4.0 | 62.9 ± 4.4 | 24,832 | **0.000** |
| 2F-July-22 | 95.2 ± 4.0 | 56.2 ± 4.0 | 28,704 | **0.000** |
| 1F-Aug-22 | 103.0 ± 3.7 | 42.7 ± 2.8 | 23,447 | **0.000** |
| 2F-Aug-22 | 86.8 ± 3.8 | 31.9 ± 2.5 | 25,025 | **0.000** |

Table S12: Soil temperature (ºC) in the coldest and warmest spell of 2022-24

| Dates | MAPs-Control (Mean ± SE) | MAPs-AF (Mean ± SE) | Welch t-test | | | Remark |
|---|---|---|---|---|---|---|
| | | | df | t | p | |
| 04-Jan-23 | 6.45 ± 0.37 | 7.15 ± 0.37 | 23 | -37.56 | **0.000** | Coldest Spell |
| 05-Jan-23 | 5.56 ± 0.34 | 6.46 ± 0.37 | 23 | -73.02 | **0.000** | |
| 06-Jan-23 | 4.94 ± 0.35 | 5.93 ± 0.39 | 23 | -76.05 | **0.000** | |
| 4-Jan-23 to 6-Jan-23 | 5.65 ± 0.71 | 6.52 ± 0.62 | 71 | -52.11 | **0.000** | |
| 12-Aug-23 | 26.67 ± 1.44 | 23.82 ± 1.18 | 23 | 36.33 | **0.000** | Hottest Spell |
| 13-Aug-23 | 26.93 ± 1.46 | 24.05 ± 0.96 | 23 | 22.55 | **0.000** | |
| 14-Aug-23 | 26.34 ± 1.46 | 23.56 ± 0.86 | 23 | 21.33 | **0.000** | |
| 15-Aug-23 | 26.35 ± 1.67 | 23.31 ± 0.91 | 23 | 17.10 | **0.000** | |
| 12-Aug-23 to 15-Aug-23 | 26.57 ± 1.51 | 23.68 ± 1.01 | 95 | 43.64 | **0.000** | |

- Total number of authors: 6
- Total number of words (including references): 7378
- Total number of words (excluding references): 6238
- Abstract number of words: 217
- Number of words in Supplementary Information: 2115