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Effect of Irrigation Methods on Black Truffle Production

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Abstract: Spain is one of the main producers of black truffle (*Tuber melanosporum* Vittad.), a fungus of great economic importance. Black truffles are usually cultivated in *Quercus ilex* orchards, as water availability is one of the most important factors influencing truffle production. Optimizing watering systems is essential to reduce the amount of water wasted. Nevertheless, up to now, no study has been carried out comparing the efficiency of different irrigation systems in truffle plantations. The aim of this study was to compare the efficiency of two different irrigation systems, namely a drip irrigation system and a micro-sprinkler system, in a *Quercus ilex* plantation situated in Burgos, Spain. Our data showed that there were no differences between the two irrigation systems in terms of truffle yields, the number of truffles, quality (based on truffle size), or the date of truffle harvesting. However, when other parameters were taken into consideration, such as the economic and environmental impact of installing and running these systems, drip irrigation was deemed the superior irrigation system because it uses less water. This study validates for the first time the use of drip irrigation rather than a micro-sprinkler system (the most commonly used in truffle plantations) because of its greater water use efficiency, which is an increasingly important consideration given future climate change scenarios marked by global water scarcity.

Keywords: truffle farming; truffle cultivation; micro-sprinkler irrigation; drip irrigation system; irrigation system improvement

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1. Introduction

Tuber melanosporum Vittad. is an ectomycorrhizal ascomycete that needs to be associated with a plant, usually a member of the Fagaceae family, to complete its life circle and to produce its fruiting bodies, which are known as black truffles [1–3]. Wild black truffles are mainly found in Italy, France, and Spain. However, owing to the high demand for black truffles and decreasing wild production levels, black truffles are also produced in plantations worldwide [4–6], although the harvesting season is limited to winter in the Northern Hemisphere [7]. Truffle harvesting has historically pursued a commercial purpose [8]: Global truffle production is currently approximately 120,000 kg year^{-1}, generating an annual turnover of about EUR 50 million for truffle growers [9]. Truffle orchards are a highly profitable investment that farmers can use to provide diversification and increase their profits [10]. In addition, truffle plantations have been shown to have beneficial socioeconomic impacts on their surrounding areas [11,12]. Plantations have been increasing in recent decades due to the greater economic benefits of truffle production than any other forest product in many Mediterranean forests [13]. Spain is one of the largest black truffle producers in the world [14] because its soil and climatic conditions are suitable for black truffle cultivation [8], and it is one of the few places in the world to have a vast number of naturally productive sites [15]. In fact, within Spain, the area with the highest production of black truffles is restricted to the northeastern region [16], where the present study was conducted. Although some conditions, such as cool temperatures and a moderate supply of water, are known to be essential for ascocarp formation [17–19], the optimal conditions for cultivation are not well understood, making annual crop yields unpredictable, varying from season to season and from one area to another [14], which may mean a risk in fulfilling an ever-increasing demand.

In recent years, droughts have become frequent due to climate change [20]. The damaging effects of long periods of drought in spring or fall on black truffle yields are widely known by experts and growers [21,22], mainly resulting in an important stress on black truffle crops, risking their fructification [22]. According to the European Environment Agency [23,24], water resources in Europe are expected to decrease as a result of the imbalance between water availability and demand. Other studies have shown that rainfall is the most important climatic factor in truffle yields, which are reduced by these drought events [25,26]. In fact, Spanish truffle production showed the strongest correlation with summer rainfall, as reported in a study by Thomas and Büntgen [27]. Plantation management could help to slow the decline in yields [28], and its practices should be adjusted to reduce soil warming and increase water availability [29]. Already in 1982, Le Tacon et al. [30] stated that irrigation can weaken the link between summer rainfall and truffle production, thus mitigating the influence of unfavorable climatic conditions on production returns. In Spain, watering systems are becoming popular in truffle orchards to complement rainwater to ensure the standard production and quality of truffles [31–33] are maintained throughout the growing season [29]. Water-efficient systems, without compromising crop productivity, will be essential for future climate change scenarios; therefore, the study of the optimization of irrigation systems is crucial so that a minimal amount of water is wasted. Here, we focus for the first time on testing and comparing two different irrigation systems, namely a micro-sprinkler irrigation system and a drip irrigation system, to assess their effect on truffle production. Both are among the most typical micro-irrigation strategies [34,35], as they are able to ensure production while reducing water consumption [36]. Micro-sprinkler irrigation, the most commonly used watering system in truffle farming, is based on the application of small water drops to the surface of the soil, imitating rainwater, to wet the area all around the host tree [17,37]. This type of watering is known to be effective and can also be used to supply plants with diluted water-soluble fertilizer [38]. Also, it reduces water consumption compared with other non-automatic watering systems [39]. This system applies water at a low flow rate and low pressure, using a low volume of water, resulting in low energy costs [40] and, therefore, is a practical and economical solution in plantations [41]. However, it is known that, with micro-sprinkler irrigation, the water distribution may not be homogeneous, as it is dependent on abiotic factors such as the wind, which can leave some areas not wetted [42]. This could represent the main disadvantage that micro-sprinkler irrigation has, as the main indicator of irrigation quality is water application uniformity [43]. Drip irrigation is based on the application of water to one area of the soil, near the stem of the tree [44]. Water is driven through a network of pipes and applied in a localized droplet-like manner using drippers in the pipe [45], providing a controlled and regulated supply of water while minimizing evaporation losses [46], making it 90% to 95% efficient [45]. In addition, Yang et al. [47] observed that drip irrigation showed significantly a more favorable effect on uniformly wetting the entire root zone, which can greatly favor the crop as it ensures that the water reaches the root of the plant [48]. However, this system also has disadvantages such as the high cost of its installation due to equipment cost or the necessity of an automated system [49].

Given the significance of water for fructification in truffle orchards, as well as the need for efficient water resource management due to its impact on the economic profitability of truffle production, it is important to understand which type of watering system would be the most suitable for sustainable truffle production in the future [50]. For this reason, the aim of this study was to analyze for the first time whether a micro-sprinkler irrigation system or a drip irrigation system is optimal for the production of truffles in a *T. melanosporum* plantation based on truffle quantity and quality (i.e., size), net irrigation rate, and energy consumption, and the economic profitability of its installation.

2. Materials and Methods

2.1. Experimental Site

This study was conducted in a truffle orchard comprising nearly 5 ha of mycorrhizal holm oaks (*Quercus ilex* L.; 5 × 5 m spacing, equivalent to 400 plants per ha) that was established in 2013 in Burgos, northern Spain. The orchard is located in an area with a flat terrain (1–2% slope) and a subhumid Mediterranean climate, with cool summers and frosts between December and February. The average annual temperature is 11.4 °C, and the annual precipitation is 460.56 mm. Specifically, during the months in which irrigation was applied in the present study, from April to October 2021, there was a rainfall of 244 mm. In the months of July and August, rain was practically nonexistent (Table 1). The average temperature during these months was 15.48 °C. The orchard is located on a calcareous rock. The agricultural soil is stony, alkaline (pH 8.36), low in organic matter, and has a coarse clay texture (32.56% clay, 48.44% sand, and 19.00% silt). The soil conditions of the plantation are homogeneous in the whole area, with no significant differences between the different irrigation sectors. In order to avoid the effect of any minimal differences in the soil, sectors were distributed to prevent these changes.

Table 1. Monthly mean (tm), monthly mean maximum (T), monthly mean minimum (t), and monthly mean precipitation (P) in the study area during irrigation months (from April to October 2021). Data were obtained from the AEMET Station located in Encinas de Esgueva (Valladolid).

Month	tm (°C)	T (°C)	t (°C)	P (mm)
April	9.38	16.50	3.01	54.00
May	12.73	20.66	4.81	36.54
June	17.14	26.02	8.87	66.99
July	20.00	29.76	9.37	1.62
August	20.89	31.61	9.97	2.23
September	16.71	25.03	9.41	47.50
October	11.49	21.23	2.97	35.12

2.2. Experimental Design

The plantation was divided into nine equal blocks of 0.5 ha each (30 × 190 m), with 220 trees per block. Weeds were removed annually from around the base of the trees, trees were pruned to improve tree structure, and tilling was performed before installing an irrigation system. Only six of the nine blocks were used in this study to make it balanced. Blocks 3, 6, and 9 were watered with a micro-sprinkler irrigation system, while blocks 2, 5, and 8 were watered with a drip irrigation system (Figure 1).

The truffle brûlé around each tree has a diameter of 2.5 m (i.e., the potentially productive area around each tree). Both watering systems were set up so as to be able to water this circular area around each tree. Micro-sprinklers with a 70 L h^{-1} capacity were placed every 5 m at a distance of 0.5 m from tree stems. For the drip irrigation system, each block comprised 6–7 rows of trees. In each row, there were four parallel drip lines 0.5 m from tree stems, two on each side of the stems. Each dripline had integrated self-compensating drippers spaced 0.33 m apart. Each dripper dispensed 1 L h^{-1}, applying 26 L h^{-1} to the truffle brûlés. Therefore, 14.28 L m^{-2} h^{-1} were applied to brûlés using the micro-sprinkler system and 5.30 L m^{-2} h^{-1} using the drip irrigation system. The irrigation period lasted from April to October 2021. The plantation was irrigated for a total of 60 h, applying 14,500 L h^{-1} of net irrigation with the drip irrigation system and 39,000 L h^{-1} of net irrigation to the plots irrigated with micro-sprinkler system, resulting in a total volume of 870 m^3 $year^{-1}$ and 2340 m^3 $year^{-1}$, respectively.

Figure 1. Drip irrigation system in a *T. melanosporum* orchard.

Trees were watered weekly; however, the amount of water varied according to the climate conditions. Every block was watered throughout the day for the same length of time, and at the same time throughout the irrigation season; however, owing to the different flow rates of the two irrigation systems, trees irrigated using the micro-sprinkler system and those irrigated using drip irrigation did not receive the same amount of water.

2.3. Black Truffle Sampling

Truffles were harvested individually in each block over a 114-day period between December 2021 and March 2022 with the aid of trained dogs. Truffles were harvested once a week except when harvesting was not possible because of adverse weather conditions (13 harvest days in total).

The collected truffles were cleaned manually to remove any soil before being counted and weighed. The weight of each truffle and the total truffle mass per block were recorded. The quality of a black truffle is determined by its shape and weight. Ideally, truffles should be regular in shape and weigh more than 20 g, but not be too heavy so that it is easier to sell the truffle without damaging it. However, we assessed truffle quality based on weight alone because weight is easier to measure than shape (Table 2).

Table 2. The number of truffles in each weight class collected in blocks watered using either a micro-sprinkler or a drip irrigation system during the harvesting season.

Weight Class	Total	Micro-Sprinkler	Drip Irrigation
0–20 g	442	210	232
21–40 g	270	147	123
41–60 g	145	72	73
61–80 g	62	31	31
81–100 g	40	20	20
More than 100 g	44	22	22

2.4. Statistical Analysis

For each group, the data of interest comprised the weight in grams of every single truffle collected, the total weight of truffles collected in each block, and the total number of truffles. We performed analyses to assess the effect of the different watering systems on truffle production. The type of watering system was considered as a fixed variable. The effect of the number of truffles collected was considered to be a possible confusion value, so it was included as a fixed variable when the interaction with the watering system was significant and as an independent variable when it was not. To control the variation between dates of collection and between blocks, dates and blocks were considered as random variables so that the conclusion could be extended to the entire orchard and the entire harvesting season.

RStudio was used for data analysis, using the package ImerTest, which implements the adjustment of mixed models from the package Ime4 using the function Imer [51,52]. A variance table type III using the Satterthwaite method was used to test the significance of fixed variables, with a *p*-value of 0.05 considered the threshold for significance. For random variables, a likelihood ratio test with a chi-square distribution was used. Each model was validated as to normality and constant variance of errors.

3. Results

3.1. General Data Analysis

In total, 1003 truffles with a total mass of 34.904 kg were collected from 3 ha, i.e., an average yield of 11.63 kg ha^{-1}. The average weight of an individual truffle was 33.98 g, with a standard deviation of 32.82 g and a standard error of 1.04 g (Figure 2). Truffles produced in blocks that were watered with the micro-sprinkler irrigation system had an average weight of 33.88 g, with a standard error of 1.55 g, while truffles produced in blocks that were watered with the drip irrigation system weighed an average of 34.08 g, with a standard error of 1.37 g.

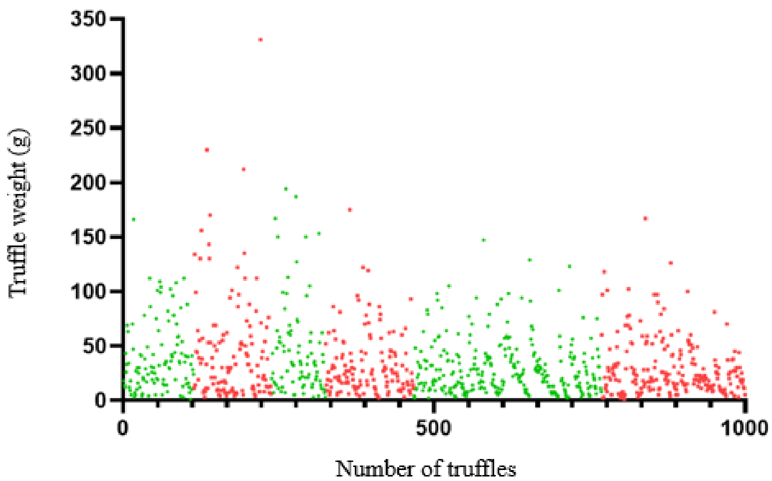

Figure 2. Point cloud showing the weight of the 1003 truffles collected during the study depending on the irrigation system: drip irrigation (in green) or micro-sprinkler irrigation (in red).

3.2. Effects of Irrigation System on Truffle Total Weight

The total mass of truffles collected in blocks watered with the micro-sprinkler irrigation system did not differ significantly from the total mass of truffles collected in blocks watered using drip irrigation ($F_{1,4}$ = 0.000; *p*-value = 0.99). Furthermore, the type of irrigation system and the date of collection did not significantly affect truffle weight (*p*-value = 0.962; Figure 3). Overall, the total weight of truffles collected on each harvest day showed a general downward trend between the first and last day of harvest, with a second peak between days 50 and 70. With the date considered as the number of days since the beginning of the harvesting season, the association between collection date and truffle weight had a *p*-value of <0.01 and an $F_{69,1}$ = 23.1. This same *t*-test for date-squared had a *p*-value = 0.02 and an $F_{69,1}$ = 6.2.

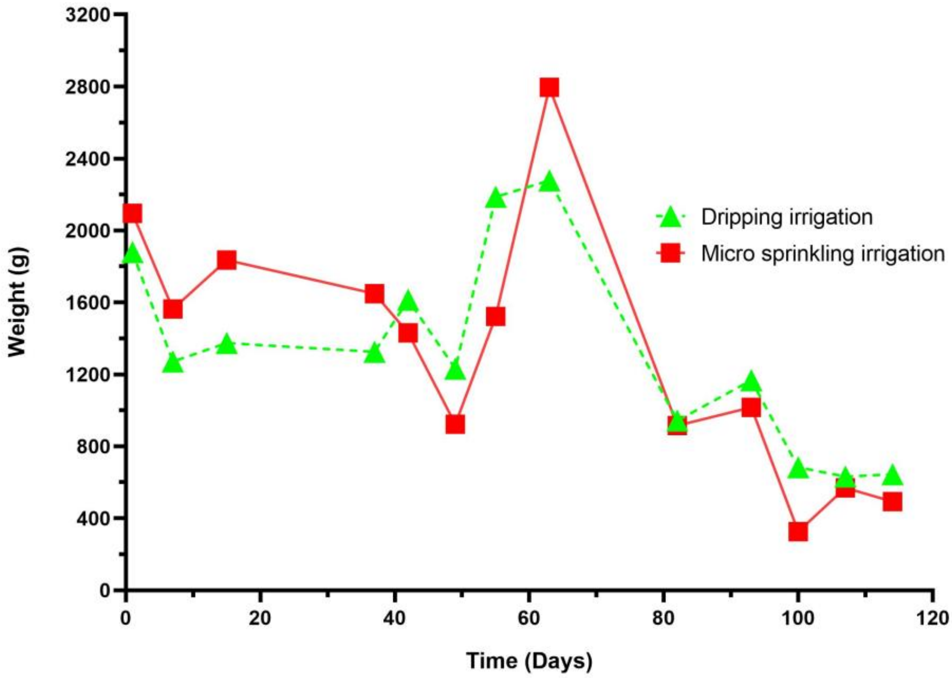

Figure 3. Comparative graph showing the weight of truffles collected in blocks watered via drip irrigation (green triangles) or a micro-sprinkler irrigation system (red squares) over the course of the harvesting season. Truffle weight is the total weight (g) of truffles collected on each harvest day during harvesting season.

3.3. Effects of Irrigation System on Truffle Size

Truffles were classified according to their weight and irrigation system. No significant differences were found between the weight of the truffles and the two different irrigation systems tested (Satterthwaite variance analysis; $F_{1,4}$ = 0.08; *p*-value = 0.79). The number of truffles collected in each weight class was very similar for both irrigation systems (Table 2). Most of the truffles (85%) belonged to the three lowest weight classes. Correlations between the quality (weight class) and the number of truffles collected were also non-significant (p = 0.11; Figure 4). Furthermore, no correlation was found between the date of truffle collection and truffle weight (Figure 4C,D) under either of the irrigation systems (*p*-value in the $\chi_1{}^2$ of 0.96).

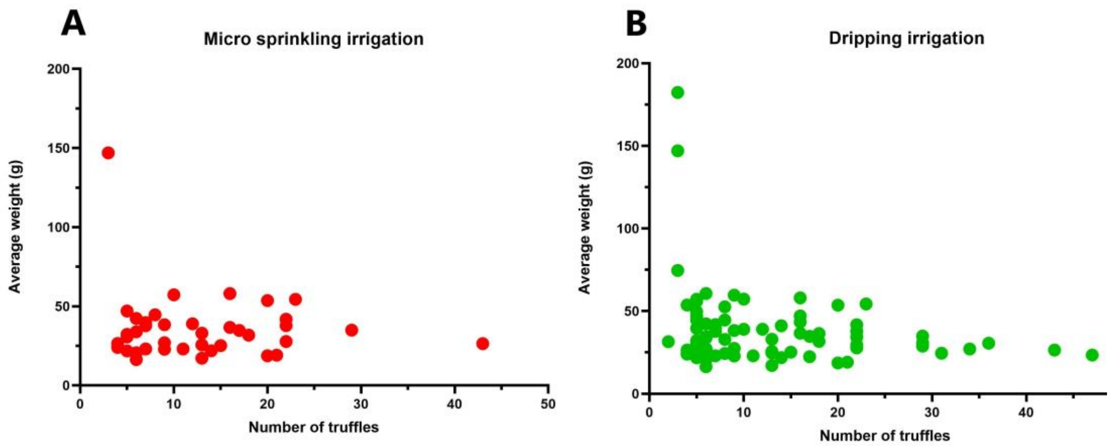

Figure 4. *Cont.*

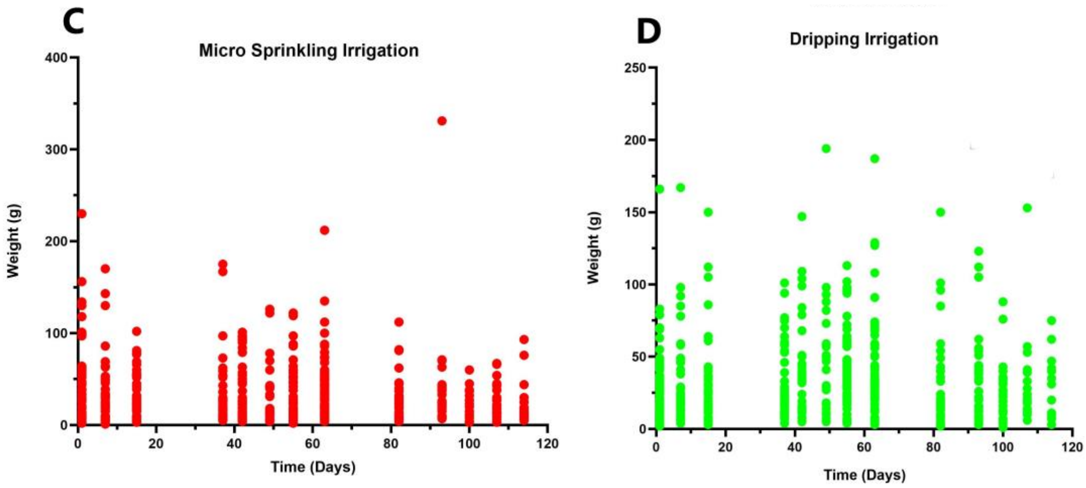

Figure 4. Average truffle weight and the number of truffles collected each harvesting day in blocks watered with either a micro-sprinkler watering system (**A**) or a drip irrigation system (**B**), and weights of all the truffles collected each harvesting day in blocks watered with either a micro-sprinkler watering system (**C**) or a drip irrigation system (**D**).

4. Discussion

Truffle formation and maturation processes are not completely understood; however, it is believed that mycelial development, and therefore truffle production, depends on water availability, with greater mycelial growth occurring when the entire surrounding area is moistened [53]. Despite the importance of irrigation in truffle cultivation, this is the first time that a study has been conducted comparing different irrigation systems in truffle plantations. In our study, both irrigation systems supplied water to the blocks for the same time period; however, even though blocks watered with the micro-sprinkler system received almost three times as much water as the drip-irrigated blocks, this did not significantly affect truffle yields, the number of truffles, truffle size class, or the date of truffle harvesting. Drip irrigation is therefore more efficient than micro-sprinkler irrigation in terms of water use. Drip irrigation provides sufficient water to the wet part of the root, which should enable water to reach the fungal primordia, providing them with nutrients and enabling them to grow [54]. Low doses of irrigation can increase the amount of root tips and the number of tips colonized by *T. melanosporum* without affecting the aboveground growth of the tree [55]. However, increasing irrigation above the necessary volume of water does not necessarily have a positive effect on the amount of truffle mycorrhization, as demonstrated by Bonet et al. [56]. Another study by Olivera et al. [57] observed that increasing soil moisture only increased the ability of competing fungi to form mycorrhizae. Further studies of irrigation with accurate measurements of soil temperature and water content are necessary to fully understand irrigation needs [58]. In this study, truffle yields peaked in the middle of the harvesting period, which falls within normality [7]. Fruiting is influenced by the conditions to which the host tree and the fungus are exposed throughout the year. In a study of desert truffle (*Terfezia claveryi*), Andrino et al. [59] showed that all factors affecting plant and fungal changes and development from summer to spring could be crucial for fruiting and crop yield.

Given the fact that both irrigation systems resulted in similar truffle yields and quality, for economic and environmental reasons, the choice of irrigation system should be based on water use efficiency (WUE). Drip irrigation effectively maintains plants while using a smaller volume of water than micro-sprinkler irrigation because it only wets a specific area near the root rather than the entire surface of the soil surrounding the tree [60]. In fact, Matyakubov et al. [61] observed that, in cotton crops, drip irrigation reduced water waste and increased raw cotton yield by 24%. Furthermore, regarding the use of this irrigation system, Cao et al. [62] studied the water absorption characteristics of cherries and observed that, by using a variable drip irrigation strategy, i.e., adjusting it to the current requirements, water waste can be reduced. When using a micro-sprinkler irrigation system, a considerable volume of sprayed water can be lost due to abiotic factors such as wind, which may redirect the water to undesirable areas, which promotes weed growth [42]. This also means that the actual volume of water that irrigates the plant could vary. Based on an applied irrigation volume of 400 mm, Ortiz-Calle et al. [60] calculated water savings of 40.9% when using a drip irrigation system rather than a sprinkler irrigation system. Therefore, in areas where strong winds are frequent, water losses due to the use of micro-sprinkler irrigation would be even higher, which implies an extra charge for the farmer. Drip irrigation has been shown to save water and enhance crop production through the frequent transfer of small amounts of water around the periphery of plant roots [63]. The location of the drippers makes the system efficient, providing each tree with just the amount of water it needs while subjecting the tree and fungus to water stress [64]. Although the soil water content takes longer to reach an optimal state when applied using drip irrigation rather than with a micro-sprinkler [65], this promotes plantation development by limiting evapotranspiration and deep percolation [66], contributing to the maintenance of soil moisture. Furthermore, plantations with a drip irrigation system can be irrigated at any time of the day, keeping the soil moist even during periods of high temperatures, such as summer, a crucial stage for the development of *T. melanosporum* [67]. Although the installation cost of both systems is very similar, drip irrigation running costs are significantly lower because this system is more water-efficient than micro-sprinkler irrigation. Precise irrigation methods, such as drip irrigation [34], also reduce energy consumption.

Drip pipes can be dismantled more easily than a micro-sprinkler system, allowing the soil to be tilled in both directions. Although dismantling and reconnecting drip pipes entails a labor cost, there is an economic saving by avoiding the proliferation of weeds [45], which can also reduce the efficiency of micro-sprinkler irrigation. In addition, animals can damage plantations in various ways, including irrigation systems [68]. A flush irrigation system, without external elements, such as a drip irrigation system, is less likely to be damaged by animals than other irrigation systems. However, the disadvantage of a drip irrigation system is that drippers may become blocked; therefore, a good filter system is necessary, and the system needs to be checked frequently [49]. Acids may also need to be poured into pipes to descale the pipelines [69]. However, factors affecting irrigation systems may vary depending on the location of the plantation. Therefore, the selection of an irrigation system should take into account factors such as energy consumption, water availability and quality, labor availability, wind, and fauna.

Over the past few decades, the recurrence of drought periods has increased and is expected to increase further in the coming years, particularly in the truffle-growing area in the Mediterranean [70,71]. Relationships between the monthly water balance and truffle production could change if a truffle orchard experiences warmer and drier conditions than those analyzed by Garcia-Barreda et al. [72] or if regional climate trends show these conditions, as is expected to occur [73]. To combat drier conditions in truffle plantations, irrigation [74] and mulching [75] will need to be implemented. Climatic factors, particularly during the summer, can have a great influence on black truffle fruiting [25,76], with the availability of water being the most limiting factor [17,29,77]. Less water availability also affects the physiological state of the host *Q. ilex*, limiting carbon uptake and arresting growth [78]. The positive effects of summer rainfall [77] and mid-level irrigation on *Q. ilex* root tip colonization [79] and *T. melanosporum* sporocarp production [30] have been reported previously. Adequate or well-scheduled irrigation is a key factor in maintaining the productivity of a plantation [80]. Plantation managers need to understand when and how much water should be applied [81] and the direct effect this has on water use efficiency [82]. Irrigation systems need to work efficiently to promote the efficient use of resources [83]. Therefore, the development of a suitable irrigation system for a *T. melanosporum* plantation must be able to meet the water requirements of the black truffle and be economically profitable in order to guarantee a good take-up by farmers.

5. Conclusions

This is the first study conducted to compare the efficiency of different irrigation systems in truffle plantations. The results showed that truffle production was not significantly affected by the type of irrigation system, which implies that advantages in terms of water retention and the efficient use of water will be key when choosing an irrigation system. Based on these criteria, drip irrigation would be a better system than the micro-sprinkler system. A drip irrigation system would also minimize water losses due to biotic and abiotic factors that can threaten the efficiency of irrigation, making drip irrigation a good choice. Economic issues will determine which irrigation system farmers select. This study validates for the first time the use of drip irrigation in truffle cultivation, which can curb the threat to production caused by worldwide water shortages. The use of drip irrigation may help producers around the world save water and money, and it facilitates land management. Thus, this study paves the way for future research on the water requirements of *T. melanosporum* primordia and their relationship with the host.

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