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**Validation of new sustainable intensification
models of hazelnut orchard**

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1 General introduction

European hazelnut (*Corylus avellana* L.) is a species native to Asia Minor, currently widespread over a wide landscape, ranging from the European continent to western Asia, including the Ural Mountains of Russia, the Caucasus, Turkey and northwestern Iran (Molnar, 2011).

Within the *Corylus* genus, European hazelnut stands out as the most commercially interesting species, cultivated in several temperate regions of the world due to its high adaptability to different climatic conditions. Among nut species, it ranks as the fourth most extensively grown crop (FAOSTAT, 2022) following cashews, almonds, and walnuts.

The plant is characterized by its typical growth as shrub, consisting of multiple stems that develop from adventitious buds located at the base of the stump during the growing season (Rovira, 2021).

European hazelnut is a monoecious species, characterized by male flowers arranged in inflorescences named catkins and female flowers (glomerulus) inserted within mixed buds, comprising a lower vegetative portion and upper fertile internodes (Germain, 1994).

Flowering and subsequent anemophilous pollination occur during mid-winter, between December and February, depending on the variety. However, fertilization takes place during late spring-early summer, specifically in late May - early June (Mehlenbacher, 1991 e Germain, 1994).

This species also exhibits a sporophytic type of self-incompatibility, which determines the presence of plants with pollinator function within each commercial hazelnut orchard to ensure a consistent yearly yielding. This kind of incompatibility is controlled at the sporophytic level by a single gene (S gene) with multiple alleles (Germain, 1994).

For proper plant development, the optimal temperature range is between +12°C and +16°C, with maximums not exceeding +36°C, especially in low humidity conditions, as higher temperatures can damage photosynthetic structures and hinder plant growth (Pasqualotto, 2019).

Additionally, the species is quite sensitive to water stress, influenced by its reduced stomatal regulation capacity (Tombesi, 1994). The ideal amount of annual precipitation is between 800 and 1,000 mm, well distributed during the year, and

without prolonged summer drought, when some of the most important plant physiological processes occur (Fideghelli and De Salvador, 2009).

The best soil conditions for profitable hazelnut cultivation involves the establishment of an orchard in fertile, deep soils with good drainage and water-holding capacity that are neutral or slightly acidic (pH 5.8 to 7.8) (Gispert et al., 2005).

To date, Turkey (657,761.5 t) and Italy (121,085 t) dominate the inshell hazelnut production, together supplying about 75% of the world's total production. Other major hazelnut producers include Azerbaijan (53,697.2 t), USA (44,815.6 t), Chile (33,939 t) and China (24,263.3 t). In Europe, countries such as France, Spain, and Poland also play significant roles in hazelnut sector, with local productions of 12,030, 9,084 and 6,079 tons, respectively (FAOSTAT, 2022). In Italy, hazelnut cultivation is mainly spread in four regions, Latium, Piedmont, Campania and Sicily, which account for about 95 % of the total production (Table 1.1).

Table 1.1. *Harvested area, total production, and average yield of European hazelnut in the main Italian hazelnut regions (ISTAT, 2022).*

Region	Harvested area (ha)	Production (tons)	Yield (tons/ha)
Piedmont	27,075	39,788	1.47
Latium	25,095	30,780	1.23
Campania	22,120	34.828	1.57
Sicily	13,993	15,986	1.41

In recent years, hazelnut cultivation has enlarged exponentially both in traditional hazelnut areas and in new environments due to increasing demand from the confectionery industry (Pacchiarelli et al., 2022).

This expansion, followed by a general increase at the technological level of agronomic practices, has also led to growth in the yields of producing countries (Figure 1.1).

In general, this increase of cultivated areas has been observed in flat and fertile areas, moving away from the traditional hilly and mountainous environments. The trend is related to the cultivation of more productive varieties and to the innovation in planting and plant training aimed to increase plant density in the orchard.

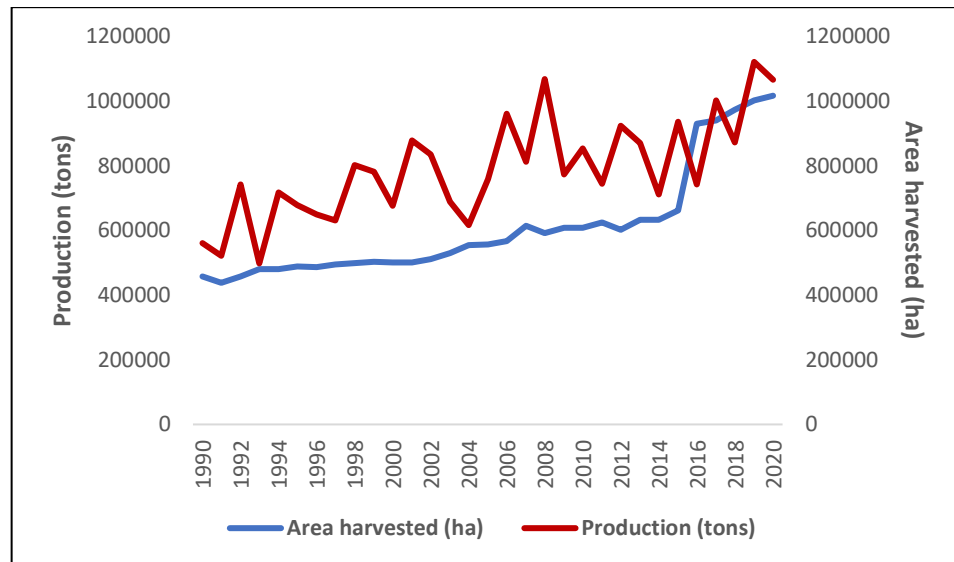


Figure 1.1. Trends in production and total cultivated area in the world over the 30-year period 1990-2020 (FAOSTAT, 2022).

A primary scope of the intensification approach, pursued by researchers, farmers, and technicians is to quickly absorb planting costs by achieving satisfactory yield levels (Ellena et al., 2014, 2018). Similarly, advanced cultivation models offer farmers increased autonomy from manual labor in agricultural practices, encompassing tasks like pruning interventions and sucker removal. Moreover, diminished canopy development, in contrast to conventional methods, not only enhances the efficiency of phytosanitary treatments but also contributes to a more cost-effective and environmentally sustainable approach. This amalgamation of factors leads to diminished expenses and heightened operational efficiency, marked by an overarching commitment to environmental sustainability.

The typical bush shape of hazelnut plant is highly valued by farmers because it allows them to renew plants cyclically by removing diseased, dead, or poorly oriented branches. While this training system is prevalent in Mediterranean environments (Spain, Italy and Turkey), other countries such as United States of America (Oregon), France, and Chile, favor single-trunk (V-shape) forms, facilitating mechanization within hazelnut orchards (Silvestri et al., 2021a).

Typical planting densities for Italian hazelnut orchards, with plants trained as multi-stemmed bushes, involve between 400 and 500 plants ha⁻¹ (Bignami et al., 2005). In recent years, it has been observed a trend towards increased planting density (700-800 plants per hectare), although with minimal use of single-trunk training systems. This

approach has also been facilitated by the introduction and use of mechanical transplanters (Cristofori et al., 2017).

Given the prolonged juvenile or unproductive phase of the hazelnut plant that occurs during the first five years, followed by a three-five years period of low yearly yield during the increasing production phase according to the selected cultivar, dynamic spacing models have been proposed to compensate the unproductive period. This model involves an initial planting layout of 5.0 x 2.5 m (800 plants ha⁻¹) with the removal of half the plants after the tenth to twelfth growing seasons to restore the final layout of 5.0 x 5.0 m, giving a final planting of 400 plants per hectare. However, in the Mediterranean environment, this technique has shown limited interest due to unconfirmed cost-effectiveness (Tous et al., 2004; Bignami et., 2005).

Conversely, this technique is widely used in Oregon (USA), where the introduction of new cultivars with different vigor has allowed the implementation of dynamic tree spacing, using several cultivars differing in bearing and vigor, such as Yamhill and Jefferson, released by the hazelnut breeding program carried out at Oregon State University (Corvallis – OR, USA) in 2008 and 2009, respectively, in alternating rows with the removal of the highest vigor cultivar around the twelfth leaf on ground.

In the Spanish environment, despite a general reduction in areas devoted to hazelnut cultivation, an expansion of new-generation hazelnut orchards has been observed in recent years, thanks to the introduction of some non-suckering rootstocks, most notably as “Dundee”, an interspecific hybrid of *Corylus colurna* x *Corylus avellana* released by the hazelnut breeding program of Oregon State University (USA) in the 1990s (Rovira, 2021).

The design of hazelnut orchards, especially those with high density (4.5 × 3.0 m; 5.0 × 2.5 m) and considerable size (>30 ha), has been greatly simplified by the use of topographic tools (DGNS handheld devices, GIS software), which allow to square the field and determine the planting distance more easily and accurately. In addition, connecting the DGNS system directly to specific mechanical transplanters, it is possible to automate transplant operations and increase the speed of the process (Cristofori et al, 2017).

The evolution of planting layout and training systems of the hazelnut, which has occurred in recent decades, has facilitated the first commercial applications of mechanical pruning performed through rotary-blade bars. The validity of this practice,

to be recommended on large-scale farms, has been confirmed by the positive effects on the quantity and quality of production in the medium term (Silvestri et al., 2021b).

1.1 Aims and scopes of the research and thesis structure

The significant expansion of hazelnut cultivation recorded during the past decade in several countries around the world, concomitant with the revitalization of planting configurations and training systems for hazelnut orchards, coupled with the adoption of non-suckering rootstocks and the implementation of mechanical pruning, is leading the cultivation technique toward new scenarios. This trajectory is increasingly aligned with sustainable intensification, facilitated by the incorporation of precision agriculture techniques and on-farm digitalization.

Based on this evidence, the aims of this research were: I) to assess the carbon sequestration potential offered by the establishment of hazelnut cultivation in grasslands and following the carbon dynamics of hazelnut orchards over several decades; II) to develop a monitoring model using proximal electromagnetic induction sensors to test the effectiveness of sub-irrigation in hazelnut orchards; III) to evaluate the eco-physiological and agronomic responses of Italian cultivar Nocchione subjected to three different training systems as multi-stemmed shrub, symmetrical four-stemmed shrub, and single trunk; IV) to analyze morphological and eco-physiological traits for the evaluation of the effects of the non-suckering clonal rootstock "Dundee" on the grafted cultivar "Tonda di Giffoni". The research activities were organized into four main research tasks, described in chapters as follow:

Chapter I – Carbon sequestration of hazelnut orchards in central Italy

In this study, the potential capacity of hazelnut orchard to sequester carbon dioxide from the atmosphere during its life-cycle has been evaluated, using permanent grasslands as a reference for previous land use. Carbon sequestration capacity was determined by considering the main sources of carbon, respectively, above-ground biomass, below-ground biomass, and carbon stock present in the top-soil until a deep of 30 cm. A new allometric equation was also developed for estimating the dry weight of above-ground biomass, using branch diameter at 60 cm above the soil as the reference parameter.

An article on the subject was published in a peer-reviewed journal (<https://doi.org/10.1016/j.agee.2022.107955>)

Chapter II – Proximal soil sensors for irrigation optimization in sub-irrigated hazelnut orchards

In this trial, a mature hazelnut orchard equipped with a subirrigation system has been subjected to analysis using an electromagnetic induction soil sensor (EMI) with the primary objective of assessing the effectiveness of the instrument in identifying potential problems associated with the underground irrigation water distribution. The overall goal was to comprehensively evaluate the efficiency and longevity of the subirrigation system. To achieve this, two separate evaluations were conducted: one preceding the activation of subirrigation system and another after six hours irrigation treatment. The point data acquired through the instrument were subsequently spatialized using QGIS software to generate maps aimed at discerning variations in apparent electrical conductivity indicative of alterations in the soil's water status attributable to the subirrigation system.

Chapter III – Agronomic and productive evaluation of plants of the “Nocchione” cultivar subjected to three different forms of training

In this trial, an assessment was conducted on the agronomic responses of the plants of Nocchione cultivar, which has been subjected to three different plant shaping (Thesis A: symmetrical four-stemmed bush; Thesis B: sapling form; Thesis C: multi-stemmed bush). The parameters analyzed were the amount of wood removed by annual pruning, measurement of trunk cross-sectional area at 30 cm above the ground, average production per plant, and yield efficiency. Furthermore, nut and kernel traits were undertaken, with a specific focus on evaluating the average kernel/nut ratio and prominent commercial defects related to the plant training system. Within the framework of sustainable intensification, with the adoption of new planting distances and new training systems, the main objective was to discern which of the investigated forms exhibited the best responses to the growing conditions.

Chapter IV – Effect of Dundee rootstock on growth, morphology, and nutrient uptake in Tonda di Giffoni hazelnut

In this trial, the effects of the non-suckering rootstock Dundee on the grafted cultivar Tonda di Giffoni, characterized by a medium to high aptitude for producing suckers, were evaluated. A comparative analysis was conducted with self-rooted plants of the same cultivar and the same rootstock. Specifically, the assessment encompassed a

thorough examination of morphological parameters (stem height and diameter), leaf biometric traits, eco-physiological parameters (stomatal conductance), as well as macro- and micronutrient content on the leaves. Additionally, observations on the phenology of the plant material were recorded. It is noteworthy that this trial was executed on potted plants, with field assessments planned for years to come. The main goal of this study was to advance hazelnut orchard practices in the future by employing plants trained as single trunks. This approach is considered more conducive to intensive, mechanized orchard management, in line with the overall goal of improving environmental and economic sustainability.

An article on the subject was published as a conference paper (<https://doi.org/10.17660/ActaHortic.2023.1379.31>)

2 Chapter I

Carbon sequestration of hazelnut orchard in central Italy

Abstract: The cultivation of European hazelnut (*Corylus avellana* L.) is showing a rapid increase due to the high global demand for hazelnuts from the confectionery industry also affecting Italy, which is the second largest hazelnut producing country after Turkey. Like most tree crops, hazelnut tree planting includes land preparation often carried out with a preliminary ground breaking and subsequent soil tillage during the first growing seasons to avoid weed competition, whereas after three years the soils are usually permanently grass covered. The aim of this work is to develop an estimation model in order to quantify carbon sequestration based on the age of hazelnut orchards, in comparison to that of neighboring permanent grasslands as reference of previous land use. The results demonstrate that soil carbon stock (sCS₃₀) tends to decrease between 23% and 58% during the first 3–5 years after hazelnut cultivation. This carbon depletion is only partially compensated by the carbon sequestration of the hazelnut trees, variable from about 0.6–3.3 Mg C ha⁻¹. Adult hazelnut orchards showed high levels of sCS₃₀, as well as higher carbon stock (CS) from tree biomass, resulting in similar, or even higher carbon sequestration potential of the grassland. In some cases, excessive nitrogen fertilization seems to decrease the carbon sequestration potential of the soil. In conclusion, the land use change from grassland to hazelnut cultivation has a short-term negative impact in terms of soil carbon sequestration. In orchards with optimal fertilization and management, sCS₃₀ is recovered and exponentially increased, highlighting the high carbon sequestration potential offered by this kind of perennial nut crop.

Key words: *Corylus avellana* L., biomass estimation, allometric equation, carbon stock, organic matter.

1. INTRODUCTION

Soil organic carbon (SOC) represents about 70% of total terrestrial carbon (C), accounting for three times the carbon stored in the aboveground biomass (AGB) and twice that in the atmosphere (IPCC, 2014). Due to the ongoing negative effects of climate change, both the largest terrestrial C pools, namely the soil and the AGB, need to be carefully managed to avoid disturbances that lead to substantial loss of C. Carbon in both pools can be increased by establishing perennial tree crop cultivations without any loss of productive areas, also contributing to the delivery of other products (e.g., food, fiber, energy feedstock) and ecosystem services (Glover et al., 2010). In this context, European hazelnut (*Corylus avellana* L.) represents a kind of perennial nut crop that is currently expanding the area of cultivation at the global level and could offer a very good opportunity to increase C at the ecosystem level, thus contributing to mitigate climate change.

European hazelnut is distributed in the temperate zone of the northern hemisphere (Boccacci et al., 2013), but is spreading rapidly also in new areas such as Chile, South Africa, and Australia (Silvestri et al., 2021a). The total cultivated area in the world is about 660,000 ha, and in-shell nut production is concentrated in Turkey (765,000 t), Italy (98,670 t), Azerbaijan (72,104 t), United States (70,310 t), Chile (62,557), Georgia (33,400 t), and Spain (8,000 t) (FAOSTAT, 2022). Its substantial spread has occurred in recent years due to a steady increase in demand from the confectionery industry. Italy is the second largest hazelnut producer in the world, with approximately 95,696 ha of cultivated areas mainly concentrated in Piedmont (39,788 t), Campania (34,828 t), Latium (30,780 t), and Sicily (15,986 t) (ISTAT, 2022).

Hazelnut grows in a variety of soils, but it prefers a neutral or slightly acidic soil (pH between 5.5 and 7.8), well drained, fertile, rich in organic matter, and active limestone not exceeding 7–8% (Gispert et al., 2005). For this reason, volcanic soils such as those of the Vico Lake area, in central Italy, are excellent for its cultivation. Although hazelnut has a great adaptability to climatic conditions, it prefers environments with average annual temperatures between +12 and +16 °C, with a well distributed rainfall throughout the year of about 800–1000 mm (Bignami et al., 2011). The Viterbo province (Latium Region, Central Italy), where the study area is located, showed all these requirements for the hazelnut tree cultivation. The province is the first Italian district of hazelnut production, with a cultivation surface around 23,900 ha and an

increase of about 6,000 ha in the last 10 years (ISTAT, 2022). In this area, most of the land subjected to the new hazelnut cultivation was previously used as pasture or croplands (mainly cereals). It is well known that land-use change causes perturbation of the ecosystem and thereby influences the C fluxes and stocks (Lal, 2005). In particular, the conversion from grassland to cropland, both for herbaceous and woody species, has the effect of releasing extra soil carbon into the atmosphere, mainly due to the higher mineralization of organic matter due to the improvement of soil aeration by tillage (Poeplau and Don, 2013; Tang et al., 2019). A meta-analysis on the effect of land-use changes on carbon stock (Guo and Gifford, 2002) reported a decrease in soil carbon stock after conversion from pasture to any other land use.

Very few studies report the effect of land conversion to hazelnut orchards on soil carbon stock. Hazelnut is a medium-size multi-stemmed shrub, characterized by high suckers' emission, developing from buds located at the base of the trunk or stump, and it can reach a height of 6-7 m (Rovira, 2021). This behavior makes the quantification of biomass more difficult to estimate than in the other trees. On the other hand, orchards with these structural characteristics allow to sequester significant amount of atmospheric C (Smaje, 2015) and, with low soil tillage, prevent the mineralization of soil organic matter (Scandellari et al., 2016).

Two studies carried out in Turkey (Gol, 2009; Oral et al., 2013) reported that the conversion from forest to hazelnut orchard in northern Turkey induced a decrease in soil organic matter between 23% and 36% during the first years of plantation. Similarly, a recent study by Zhang et al. (2021) reported a decrease of SOC after establishment of a hazelnut orchard until the first 5-6 years, whereas from the 8th year, the SOC pool became to be higher than in the non-cultivated land.

A recent paper by Granata et al. (2020) monitored the net photosynthetic rate and the CO₂ sequestration capability of hazelnut trees in central Italy, subjected to routine horticultural management. The results of the work reported that, on average, the hazelnut orchard system, sequestered an annual amount of CO₂ of $58.8 \pm 9.1 \text{ Mg ha}^{-1} \text{ year}^{-1}$, corresponding to an amount of C equal to $16.0 \pm 2.5 \text{ Mg C ha}^{-1}$. Part of this carbon stock, namely in-shell hazelnuts and pruned wood, leaves the orchard system, whereas other plant portions, namely leaves, catkins and small branches, remain in the orchard contributing to its carbon stock, together to the seasonal root system renewal. The aim of the research was to evaluate the carbon sequestration potential offered by the hazelnut cultivation on soils previously cultivated as grassland and follow the C

dynamics of hazelnut orchards for several decades (more than 50 years-old orchards). Carbon sequestration was evaluated considering the main C pools: a) above-ground biomass (AGB), b) below-ground biomass (BGB), and c) soil organic carbon stock to a depth of 0-30 cm (sCS₃₀).

For AGB, a new allometric equation was developed to estimate the dry weight of the branches (BDW). Indeed, the allometric equation to estimate the biomass of cultivated hazelnut trees is lacking in the literature, and only two studies reported allometric equations of hazelnut trees in natural forest ecosystems (Alberti et al., 2008; Škema et al., 2018).

2. MATERIAL AND METHODS

2.1 Study area

The study area covers the rural district around Vico Lake situated in Viterbo province (central Italy), which represents one of the most important hazelnut districts in Italy and in the world. The increase of surface destined for hazelnut cultivation in the Viterbo province has been characterized by two distinct phases of progressive expansion. The first phase was observed in the years 1970s-1980s, with an increase of about 8,000 ha (Gasbarra et al., 2001), while the second phase was registered in the years 2010s-2020s, with an increase of about 6,000 ha (ISTAT, 2022). Conversely, during the period 1990s-2010s, few new hazelnut orchards were planted, therefore, it was not possible to find available orchards between 10- to 25-years-old to complete the chrono-sequence. The field trials involved three different areas in Viterbo province, specifically around the Vico Lake (Municipalities of Nepi, Caprarola and Ronciglione), as reported in Figure 2.1. Each area includes hazelnut orchard sites with different age (Figure 2.2), in detail:

1. Area 1 NEP (Nepi): grassland (NEP1); 3 years-old (NEP2); 5 years-old (NEP3); 35 years-old (NEP4); 50 years-old (NEP5);
2. Area 2 CAP (Caprarola): grassland (CAP1); 4 years-old (CAP2); 45 years-old (CAP3);
3. Area 3 RON (Ronciglione): grassland (RON1); 3 years-old (RON2); 40 years-old (RON3).



Figure 2.1. Location of study sites. Area 1 NEP: (Nepi - VT), 270 m a.s.l., Cambic Endoleptic Phaeozems (IUSS WRB, 2015); Area 2 CAP: (Caprarola - VT), 420–480 m a.s.l., Cambic Endoleptic Phaeozems; Area 3 RON: (Ronciglione - VT), 640 m a.s.l., Luvisc Phaeozems, Haplic Luvisols, and Dystric Andosols.



Figure 2.2. Examples of young and old hazelnut orchards used for this study located in the same site (CAP2 and CAP3).

In each area, the plants were represented by the main hazelnut cultivars "Tonda Gentile Romana" and "Nocchione", with spacing layout varying from 4.5×3.0 m to 8.0×4.0 m (Table 2.1), trained as multi-stemmed bush and managed applying an integrated pest

and disease control. Irrigation supply was ensured during the summer in all selected orchards.

Table 2.1. Description of experimental sites (Cultivar: TGR= Tonda Gentile Romana; N = Nocchione).

Site	Site code	Latitude	Longitude	Date of planting (age, years)	Planting scheme (m x m)	N° plants/ha	N° of main branches/plant	Cultivar
Nepi	NEP1	42°16'48"	12°17'43"	grassland	-	-	-	-
Nepi	NEP2	42°16'30"	12°18'02"	2017 (3)	4,5 x 3	740	5	TGR
Nepi	NEP3	42°16'46"	12°17'58"	2015 (5)	4,5 x 3	740	4	N
Nepi	NEP4	42°16'45"	12°17'50"	1985 (35)	5 x 5	400	8	TGR
Nepi	NEP5	42°16'35"	12°18'03"	1970 (50)	4 x 4	625	7	TGR
Nepi	NEP6	42°16'44"	12°17'38"	1960 (60)	8 x 4	313	8	TGR
Caprarola	CAP1	42°19'15"	12°14'19"	grassland	-	-	-	-
Caprarola	CAP2	42°18'53"	12°14'35"	2016 (4)	5 x 5	400	5	N
Caprarola	CAP3	42°18'55"	12°14'37"	1985 (35)	5 x 5	400	10	TGR
Ronciglione	RON1	42°17'52"	12°09'00"	grassland	-	-	-	-
Ronciglione	RON2	42°17'48"	12°09'31"	2017 (3)	5 x 3	667	4	TGR
Ronciglione	RON3	42°17'46"	12°09'34"	1980 (40)	5 x 4,5	444	6	TGR

According to Köppen classification, the climate of the study area corresponds to CSa: hot and dry summer Mediterranean climate, with the warmest month's average temperature above +22°C. Long term meteorological data was downloaded from a station of Latium region, placed between Ronciglione (Area 1) and Caprarola areas (Area 2) at an altitude of 520 m a.s.l. Data from the last 10 years (2010-2020) reported a mean annual air temperature (MAT) of +15.5°C and an annual precipitation (AP) of 946 mm. The warmest and driest months were June, July, and August, whereas the highest monthly precipitation was recorded in November (Figure 2.3).

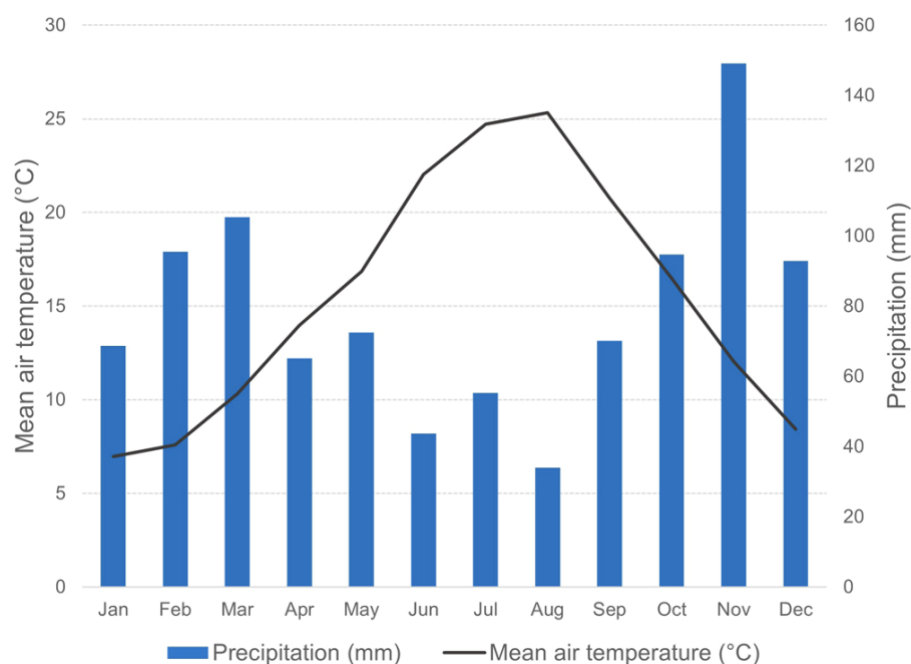


Figure 2.3. Mean monthly precipitation and air temperature of the study area. Data source: ISPRA, Istituto Superiore per la Protezione e la Ricerca Ambientale, <http://www.scia.isprambiente.it> (from 2010 to 2020).

Another meteorological station, active only from 2016 to 2019, was available near Area 1 (Municipality of Nepi). The data comparison of the two stations between 2016 and 2019 reported as lightly colder and drier climate near NEP. During the period 2016 - 2019, site 1 showed MAT of +14.9 °C and AP of 757 mm, whereas CAP and RON showed MAT of +15.7 °C and AP of 926 mm.

The Vico volcanic region was generated by volcanic activity of the Pleistocene and the depression of Vico Lake, which testify to the collapse of the caldera. The volcanic activity of Vico was estimated between 0.8 and 0.09 M-years and produced thick pyroclastic deposits with alkalipotassic composition, varying from latitic to trachylatitic, alternated to trachytic lavas and ignimbrite formations (Baiocchi et al., 2006; Colombo et al., 2007). The study area is located within the soil region “Volcanic hilly areas of central and southern Italy”, in particular to the two soil systems “volcanic plateau” and “volcanic caldera” of the Bolsena, Vico and Bracciano lakes (Napoli et al., 2019). The soil distribution of the area follows a climo-toposequence from the most elevated areas around Vico lake caldera (700-900 m a.s.l.), where Andosols are very frequent, from the plateau dissected by streams (200-300 m a.s.l.) southward Vico Lake, where Phaeozems and Luvisols are more common than Andisols (Colombo et al., 2007; Napoli et al., 2019). The experimental hazelnut orchards were subdivided

into three areas in the municipality of Nepi (NEP), Caprarola (CAP), and Ronciglione (RON). Area 1 is the most southern and lowest in altitude, situated in a wide portion of volcanic pyroclastic plateau around 270 m a.s.l. Area 2 is situated between 420 and 480 m a.s.l., along moderately steep slopes formed by streams dissecting the volcanic pyroclastic plateau. The most common soils of both the areas are Cambic Endoleptic Phaeozems (IUSS WRB, 2015), according to Napoli et al. (2019). Area 3 is situated in a plain along the border of Vico valley, at an altitude of about 640 m a.s.l.

According to the regional map (Napoli et al., 2019), the soil sub-system of the area spans between Luvic Phaeozems, Haplic Luvisols, and Dystric Andosols (IUSS WRB, 2015), the latter generally situated in the forest. The soils cultivated with hazelnut trees are generally Phaeozems or Luvisols, mainly characterized by loam or clay-loam texture, good structure and drainage, scarce or no stoniness, no calcium carbonate, neutral or sub-acid pH, and variable organic carbon from moderately low (around 1 g 100 g⁻¹) to high (around 3 g 100 g⁻¹). Such kind of soils, as well as the climate, showed high suitability for the hazelnut cultivation, indeed this area is the first Italian district of hazelnut production for extension and yield.

2.2 Assessment of Soil Carbon Stock (sCS₃₀)

In 2020, each site was delimited with a 1-ha plot, representative of the entire orchard. In each plot, soil samples from three random points were collected using mini-pits dug to about 40 cm depth. Soil samples were collected at two different depths, 0-15 cm and 15-30 cm. Using the same mini-pits, samples for the bulk density (n = 3 per plot) were also collected using cylinders of known volume (100 cm³) at the same depth as used for collecting soil samples. All samples were oven dried (+60 °C) to constant mass, except for those for bulk density, which were oven dried at +105 °C until constant mass. The mineral soil samples were sieved at 2 mm, and all the analyses were performed on the fine soil fraction (<2 mm). All samples were characterized for particle size distribution using the pipette method to verify the comparability between the different stages of the chrono-sequence. Total C and nitrogen (N) were measured in all soil samples (n = 3 per layer), in finely ground aliquots by dry combustion (Thermo-Finnigan Flash EA112 CHN, Okehampton, UK). As a result of the absence of carbonates, total C corresponded to organic C content. The SOC and N stocks were calculated for each soil layer according to Boone et al. (1999), considering the C or N concentration, the bulk density, the depth of the layer, and the content of rock

fragments. The sCS_{30} was calculated for each layer according to the following equation:

$$sCS_{30} (Mg\ ha^{-1}) = SOC_{30} \times BD \times D \times 10$$

Where SOC_{30} is the organic carbon content ($kg\ kg^{-1}$) of a layer (e.g. 0-15 cm; 15-30 cm), BD is the bulk density ($kg\ m^{-3}$), D is the reference depth (m) and 10 is the multiplicative coefficient to transform $kg\ m^{-2}$ into $Mg\ ha^{-1}$. The sCS_{30} was obtained as the sum of the stock of the two layers.

2.3 Estimation of hazelnut tree dry biomass

To our knowledge, studies on the estimation of hazelnut AGB in agricultural environments lack, whereas two papers reported allometric equations of wild hazelnut trees in forest ecosystems (Alberti et al., 2008; Škëma et al., 2018). In this natural forest context, hazelnut branches tend to grow in height to reach the sunlight, in competition with the other more vigorous forest species. For this reason, hazelnut trees in forest tend to show long and thin branches already during the first years. In cultivated hazelnut tree orchards, the branches are periodically pruned, and they do not compete for sunlight with other tree species. For these reasons, the shape of cultivated hazelnut trees tends to be similar to a large round bush, especially when multi stemmed trained, from 1 to 2 m in young orchards to 3-4 m or more, according to the cultivar vigor and plant age, in the older ones.

Škëma et al. (2018) investigated the biomass allocation of hazelnut shrub in Lithuanian forest, and its relationship with morphometric traits, such as the diameter of the stems at different heights aboveground, the height of the stems, and the complex index D^2H . Alberti et al. (2008) estimated the total biomass above ground of hazelnut shrub considering the density of hazelnut wood and the volume of the plant, by the following allometric equation:

$$V = 0,5 \pi/4 (Dm)^2 Hm n$$

where V is the shrub volume of a hazelnut plant, Dm is the average diameter of branches measured (1.3 m from the surface), Hm is the average length of the branches measured, and n is the number of branches of the hazelnut plant. The use of species-

specific allometric equations is preferred because trees of different species can differ in architecture and in wood density (Alberti et al., 2005). Allometric equations remain strictly empirical relationships with local validity, thus species- and site-specific (Zianiset al., 2005). During this work, the model developed by Alberti et al. (2008) in the Italian Alps was tested for the cultivated hazelnut trees. To validate the model in the context of the work, 10 branches of different hazelnut trees were collected, and the morphometric traits were measured. All the predictions obtained by the model proposed by Alberti et al. (2008) underestimated the measured biomass. For this reason, we considered the model unsuitable for our scopes.

A new predictive equation was calibrated using 282 branches from 44 different hazelnut trees. The experiment was divided into three key steps (two field trials and one in the laboratory). Briefly, branches were collected by cutting them at the base of the tree stump and then weighed (fresh weight, BFW), as shown in Figure 2.4. Furthermore, length (h) and diameters at 0.3 (D0.3), 0.6 (D0.6), and 1.3 m (D1.3) from the soil surface of each branch were measured. Ten fractions of branches representative of the entire dataset (different tree ages and fields) were selected to determine the ratio between the fresh and dry weights of the branches (BFW/BDW). These branches were weighed (BFW) and then placed in an oven at $+ 104 \pm 1$ °C, until constant weight (branch dry weight, BDW). The BFW/BDW ratio was used to recalculate the BDW of the entire dataset of 282 branches.



Figure 2.4. Field measurements to calibrate the predictive equation for hazelnut tree biomass.

Statistical relationships between variables of the calibration set database were evaluated by Pearson's parametric correlation. The predictive equation to estimate BDW was calculated using a forward stepwise multiple linear regression, setting F-to-enter = 3. The normal distribution of the input data and the residuals was tested by the Shapiro-Wilks's test. Pearson's correlations between variables and residuals have also been tested to check the eventual bias of the equation. The quality of the model was evaluated by R^2 adj, F-value, p-values, and mean standard error of calibration (MSEC). An external validation was performed measuring the morphometric parameters of 15 additional hazelnut branches from the same experimental areas. The mean standard error of prediction (MSEP) was calculated to determine the reliability of the equation. All the statistical analysis were performed using "Statistica 7.0" (Statsoft Inc., Tulsa, OK, U.S.A.).

2.4. Assessment of hazelnut trees carbon stock

The amount of CO₂ sequestered from the atmosphere can be evaluated by analyzing the carbon stock in several hazelnut orchards, with different age, planting density, and number of branches per plant. The process of eliminating the water component and obtaining the dry weight of the above-ground biomass (AGB) is necessary to calculate the amount of organic carbon (OC). The percentage of OC in hazelnut dry biomass is estimated at 44.65% of the total dry biomass (Picchi et al., 2018). Knowing the amount of AGB per hectare, obtained by the number of plants and the number of branches per plant, the total amount of organic carbon in above-ground biomass was calculated using the ratio described above. The quantification of the below-ground biomass (BGB), in order to estimate the CS sequestered by the roots, was calculated using the Root/Shoot ratio (R/S) of 0.26. This coefficient expresses the mean of the R/S values reported in the literature for several types of trees (Cairns et al., 1997; Brown, 2002; Alberti et al., 2005).

The sum of the carbon of the total living biomass, both above and below ground, and the soil organic carbon (sCS_{30}) provides the total amount of carbon stock (CS tot, in Mg ha⁻¹) of hazelnut orchards at the ecosystem level. The biomass of hazelnut yield and pruning has not been considered because it is removed from the ecosystem, and most of the sub-products (husks and pruning materials) are used to produce wood chips and pellets for energy (Silvestri et al., 2021b).

3. RESULTS

3.1 Assessment of soil carbon stock

All soils analyzed are characterized by the volcanic origin of the parent material, mainly ashes and tephras. The textural classes of the soil samples span between Sandy-Clay-Loam, Loam, and Clay-Loam, with a clay content varying between 18% and 35% (Table 2.2). The coarse fragments, estimated in the field, were characterized by small and medium gravel and varied from 0% to 5% in volume. All the studied soils are free from calcium carbonate. The bulk density is generally lower than 1.3 g cm^{-3} , with the only exception of the NEP3 and NEP4 samples, which showed a slightly higher BD of 1.37 and 1.44 g cm^{-3} , respectively. Soil organic carbon at the two reference depths, namely SOC_{15} and SOC_{30} , showed a very high correlation (Pearson's $r = 0.95$); therefore, the data elaboration and the results are focused on 30 cm as the reference depth (SOC_{30} and sCS_{30}). The SOC content showed moderate to high values for agricultural soils in most of the sites, except for soil within hazelnut orchards younger than five years. In such sites, the SOC_{30} content was lower than 10 g kg^{-1} , apart from RON2 in the Ronciglione area, where SOC_{30} varies between 12.4 and 14.1 g kg^{-1} , as shown in Table 2.2. The total nitrogen of the topsoil (TN_{30}) usually provided values between 1.0 and 1.8 g kg^{-1} , although few replicates showed higher values and equal to 2- 2.5 g kg^{-1} . All RON3 replicates (Ronciglione area, 40 years-old hazelnut orchard) showed the highest values, between 2.5 and 4.4 g kg^{-1} . The same site also showed the lowest C/N ratio, 6 on average (Table 2.2), which indicated an unbalanced ratio between organic carbon and total nitrogen, and then a probable rapid mineralization of the organic matter.

Table 2.2. Soil features of the experimental sites. The values show the mean \pm standard deviation of the three sampling points. (1): bulk density; (2): soil organic carbon at the depth of 0–30 cm; (3): ratio between organic carbon and total nitrogen; (4): soil carbon stock 0–30 cm, letters defined homogeneous groups ($p < 0.05$) for LSD test after ANOVA.

Site	Site code	Date of planting (age, years)	Sand (%)	Clay (%)	Textural class	BD ⁽¹⁾ (g cm ⁻³)	SOC ₃₀ ⁽²⁾ (g kg ⁻¹)	C/N ⁽³⁾	sCS ₃₀ ⁽⁴⁾ (Mg ha ⁻¹)
Nepi	NEP1	Grassland	31	26	Loam	1.14	1.55 \pm 0.29	11.7 \pm 3.9	47.0 \pm 11.6 (ab)
Nepi	NEP2	2017 (3)	38	35	Clay Loam	1.13	0.92 \pm 0.16	13.1 \pm 2.8	31.1 \pm 5.3 (a)
Nepi	NEP3	2015 (5)	44	23	Loam	1.37	0.86 \pm 0.07	7.7 \pm 0.8	35.3 \pm 3.9 (a)
Nepi	NEP4	1985 (35)	31	26	Loam	1.44	1.40 \pm 0.26	8.5 \pm 0.8	60.1 \pm 9.8 (ab)
Nepi	NEP5	1970 (50)	45	30	Clay Loam	1.23	2.13 \pm 0.50	12.3 \pm 0.7	78.5 \pm 18.5 (bc)
Nepi	NEP6	1960 (60)	31	26	Loam	1.14	3.05 \pm 1.02	28.9 \pm 10.5	103.6 \pm 32.2 (c)
Caprarola	CAP1	Grassland	49	17	Loam	1.29	2.69 \pm 0.65	13.3 \pm 0.5	72.0 \pm 23.3 (b)
Caprarola	CAP2	2016 (4)	51	24	Sandy Clay Loam	1.18	0.96 \pm 0.18	10.8 \pm 0.9	34.4 \pm 10.3 (a)
Caprarola	CAP3	1985 (35)	50	18	Loam	1.38	4.18 \pm 0.51	12.2 \pm 1.2	124.5 \pm 26.6 (c)
Ronciglione	RON1	Grassland	36	33	Clay Loam	1.34	3.87 \pm 1.09	25.7 \pm 7.3	106.7 \pm 18.2 (b)
Ronciglione	RON2	2017 (3)	39	30	Clay Loam	1.1	1.35 \pm 0.09	9.9 \pm 3.0	43.4 \pm 2.0 (a)
Ronciglione	RON3	1980 (40)	38	28	Clay Loam	1.04	1.77 \pm 0.24	6.0 \pm 1.5	55.0 \pm 3.3 (a)

The LSD test after ANOVA of the sCS₃₀ values showed significant differences in the hazelnut orchards with different ages. Young orchards (3-5 years) showed sCS₃₀ between 22.9 and 45.8 Mg ha⁻¹, with data belonging to the same statistical group (a) and statistically different from the CS of the older orchards, with the only exception of RON3. Although RON3 was a 40 years-old orchard, SOC₃₀, and then sCS₃₀ showed values very slightly higher than the young orchards (3–5years) and not statistically different. Compared to the 3 years-old orchards besides, RON3 showed an increase of sCS₃₀ of only 12 ton ha⁻¹, on average. In contrast, the other old Nepi orchards (NEP 4, 5, 6) and Caprarola (CAP3) showed a mean increase in sCS₃₀ from the young orchards of about 58 ton ha⁻¹, resulting significantly different from the 3- and 5 years-old orchards.

3.2 Estimation of hazelnut trees dry biomass and carbon stock

The calibration data set was obtained by measuring 49 branches from 15 different trees, and different diameter traits were detected for each branch (D0.3, D0.6, D1.3, and h). The D0.3 varied between 2.5 and 9.1 cm (mean value \pm standard deviation; 5.31 \pm 1.80), D0.6 between 1.5 and 8.4 cm (mean value \pm standard deviation; 4.68 \pm

1.61), D1.3 between 1.2 and 6 cm (mean value \pm standard deviation; 3.45 ± 1.14), and h between 1.8 and 4.1 m (mean value \pm standard deviation; 2.88 ± 0.53). The moisture content of the branches, determined to obtain the biomass dry weight (BDW), ranged from 40.67% to 50.59% of the fresh weight. The average value of the BFW/BDW ratio, used to recalculate the BDW of all branches, was 0.46. The BDW showed mean values between 0.16 and 6.67 kg (mean value \pm standard deviation; 2.46 ± 1.38).

The Shapiro-Wilks test confirmed that the data distribution of each parameter was normal (Figure 2.5); therefore, the data were suitable for parametric statistical methods, such as Pearson's correlation and multiple linear regression analysis. The r coefficients showed very high correlation between branch diameters, as well as between branch diameters and BDW (Table 2.3).

Table 2.3. Pearson coefficients (*r*, in bold, $p < 0.05$). D0.3, D0.6, D1.3: diameters of the hazelnut branches at 30, 60 cm and 1.3 m from the soil surface; h: height of the hazelnut branches (m), BDW: Biomass dry weight (kg).

	D 0.3	D 0.6	D 1.3	h	BDW
D 0.3	-				
D 0.6	0.98	-			
D 1.3	0.81	0.84	-		
h	0.05	0.08	0.32	-	
BDW	0.88	0.90	0.81	0.20	-

In contrast, the heights of the branches (h) showed no correlation with all the other variables. The h varied between 1.8 and 4.1 m, but this variation was strongly driven by farmers through pruning methods and plant training systems. Therefore, it is possible to find similar heights of branches in 4-5 year-old orchards, as well as of 40 year-old orchards, showing trees with large diameter and high biomass.

The measured calibration data were used for a forward-stepwise multiple linear regression, with which the equation was obtained to estimate the biomass of the trees above-ground biomass, highlighting that the trait D0.6 was the most related to the BDW, with an adjusted R^2 of 0.78. The equation to estimate BDW is reported below and showed a standard error of estimation of 0.65 (Figure 2.5). The residual distribution showed a normal distribution and no significant relationships with D0.6 and BDW were observed. Furthermore, an external validation was carried out measuring 15 different branches. The mean value for D0.6 was 4.31 ± 2.07 cm and for

BDW was 2.21 ± 1.78 . The mean standard error of prediction (MSEP) was 0.62 kg, which was very similar to the calibration error (MSEC = 0.65 kg).

$$BDW (kg) = 0.784 D0.6 - 1.199$$

$R^2_{adj} = 0.78$; $F(1,44) = 164.77$; $p < 0.00001$; $MSEC = 0.65$ kg. This new equation allowed us to estimate the BDW (kg branch^{-1}) for each plot in the different study area (Table 2.4). Total tree biomass (AGB+BGB) is related to the age of the orchard and to the number of plants per hectare, as expected. The highest values of biomass per hectare have been found in NEP5 (50 years-old, 25.06 ± 6.04 Mg ha^{-1}) and CAP3 (45 years-old, 21.01 ± 4.65 Mg ha^{-1}), whereas values around 15 Mg ha^{-1} have been found in NEP4 (35 years-old), NEP6 (60 year-old), RON3 (45 year-old). Such biomasses correspond to CS from about 6 to about 10 Mg ha^{-1} (Table 2.4). In young orchards, the average values of biomass/ha varied between 1.3 and 7.5 Mg ha^{-1} , for a corresponding CS varying between about 0.6 and 3.4 Mg ha^{-1} . Therefore, the difference in CS in tree biomass between old and young hazelnut orchards varied, on average, from about 5 to about 10 Mg ha^{-1} .

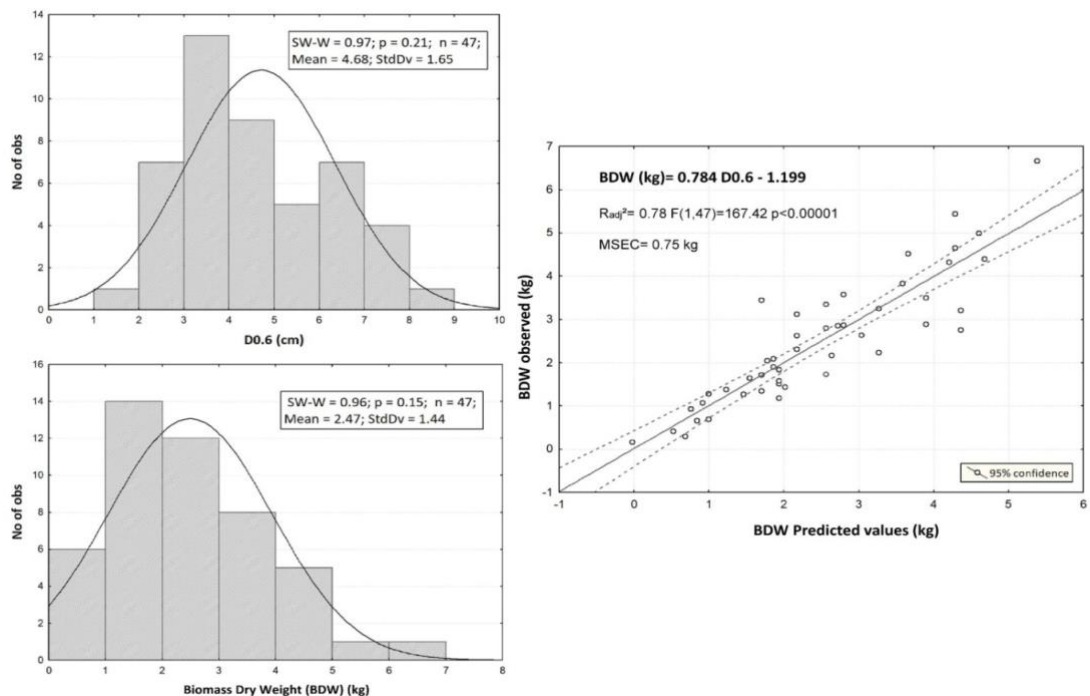


Figure 2.5. Distribution of the output and input variables used to calibrate the regression for prediction of BDW. The histograms show the curve of normality and the results of the Shapiro-Wilks test of normality. On the right, the BDW predicted vs. observed values and the results of the regression.

4. DISCUSSION

Analysis of hazelnut orchards with different ages, as well as the comparison with nearby reference areas used as permanent grassland, allowed us to model the impact and the evolution of carbon sequestration in this agricultural system. The establishment of a new hazelnut orchard causes a first phase (e.g. 3-5 years) characterized by a decline in the SOC stock (Figure 2.6), as confirmed in all the sites studied, which showed a decrease of -23% in Nepi (from 47 to 34 - 38.7 Mg ha⁻¹), of -52% in Caprarola (from 72 to 35 Mg ha⁻¹), and -58% in Ronciglione (from 107 to 45.5 Mg ha⁻¹).

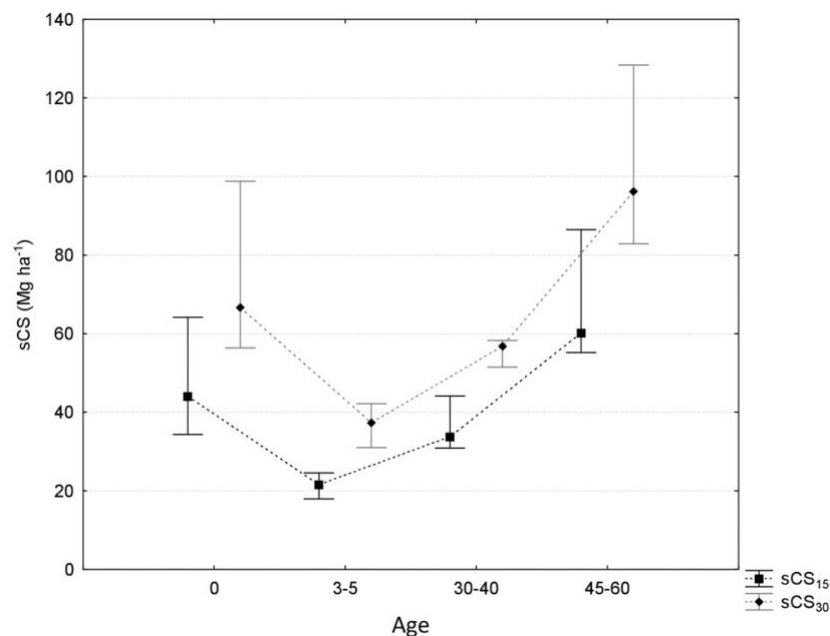


Figure 2.6. Evolution of sCS_{15} and sCS_{30} from grassland conversion to the different age of the hazelnut orchards at the three experimental areas. The groups were statistically different for the F test (ANOVA, $p < 0.001$), with $F(3,32) = 19.4$ for sCS_{15} and $F(3,32) = 17.5$ for sCS_{30} . The points of sCS_{15} and sCS_{30} are slightly shifted to make the graph more readable, but they represent the same four age classes: 0, 3–5, 30–40, and 45–60.

It is important to note that the grassland reference plot in RON showed a very high sCS_{30} (106.7 ± 18.2 Mg ha⁻¹), probably distorting the mean of the results. The high variability of the loss of sCS_{30} among the study areas (Table 2.4) was probably due to different land preparation and management practices. The number of tillage operations to control weeds and the pedoclimatic conditions during tillage, as well as the amount

of nitrogen fertilization, can strongly influence the mineralization of the organic matter. According to many authors, the magnitude of SOC dynamics during land-use change also depends on several soil characteristics, including texture (Tang et al., 2019) and microbial community composition (Lauber et al., 2008).

Table 2.4. Data of hazelnut tree biomass, as well as carbon stock (CS), of the studied hazelnut orchards. (1): branch diameter at a height of 0.6 m, used for the equation; (2): aboveground biomass (trunk, branches); (3): belowground biomass (roots).

Site	Site code	Year of planting (age, years)	N° Plants/ha	N° of main branches/plant	D 0.6 ⁽¹⁾ (cm)	AGB ⁽²⁾ (kg)	BGB ⁽³⁾ (kg)	Tot. Biomass/ha (Mg ha ⁻¹)	CS/ha (Mg ha ⁻¹)
Nepi	NEP2	2017 (3)	740	5	3.15 ± 0.79	1.42 ± 0.41	0.37 ± 0.11	6.63 ± 1.89	2.96 ± 0.85
Nepi	NEP3	2015 (5)	740	4	4.12 ± 0.55	2.03 ± 0.43	0.53 ± 0.11	7.56 ± 1.62	3.38 ± 0.72
Nepi	NEP4	1985 (35)	400	8	5.87 ± 1.6	3.41 ± 1.26	0.89 ± 0.33	13.73 ± 5.07	6.13 ± 2.26
Nepi	NEP5	1970 (50)	625	7	7.33 ± 1.4	4.55 ± 1.1	1.18 ± 0.28	25.06 ± 6.04	11.19 ± 2.69
Nepi	NEP6	1960 (60)	313	8	6.92 ± 3.21	4.22 ± 2.52	1.1 ± 0.65	13.32 ± 7.94	5.95 ± 3.54
Caprarola	CAP2	2016 (4)	400	5	2.11 ± 0.39	0.52 ± 0.23	0.13 ± 0.06	1.31 ± 0.58	0.58 ± 0.26
Caprarola	CAP3	1985 (35)	400	10	6.85 ± 1.18	4.17 ± 0.92	1.08 ± 0.24	21.01 ± 4.65	9.38 ± 2.07
Ronciglione	RON2	2017 (3)	667	4	2.62 ± 0.43	0.86 ± 0.34	0.22 ± 0.09	2.88 ± 1.14	1.29 ± 0.51
Ronciglione	RON3	1980 (40)	444	6	7.48 ± 2.1	4.66 ± 1.65	1.21 ± 0.43	15.65 ± 5.53	6.99 ± 2.47

Considering a minimum change in sCS₃₀ of -14 Mg ha⁻¹ in NEP and a maximum of -64 Mg ha⁻¹ in RON (Table 2.2), the mean sCS₃₀ loss due to the conversion of grassland to hazelnut orchards was approximately -37 Mg ha⁻¹ (-44%) in the short term, that is, 3-5 years. These losses were slightly higher than the data of land use change from forest to hazelnut orchards in northern Turkey, reported by Oral et al. (2013), indicating a loss of 23-36% during the first four years after planting. Lower changes in sCS₃₀ due to the conversion from grassland to cropland have also been reported by Poeplau and Don (2013), who calculated a mean loss of -19 ± 7 Mg ha⁻¹ in Europe. However, the results of this study were in line with several other studies on land conversion from grasslands to croplands. A meta-analysis by Guo and Gifford (2002) showed sCS₃₀ loss of -59% with a land use change from pasture to cropland. Evrendilek et al. (2004) reported as CS₃₀ reduction of -43% after 12 years of grassland conversion.

This loss of soil carbon was only partially compensated by the CS stored in the AGB of the hazelnut tree, which, in the first 3-5 years, accounted for 0.58-3.38 Mg C ha⁻¹, as shown in Table 2.4. Then, the total carbon loss varied between -11 Mg ha⁻¹ in NEP

and -62 Mg C ha^{-1} in RON. Annual pruning biomass was not considered for this study, because such biomass leaves the hazelnut orchard system, and it can be burned, composted, or used for energy. Previous studies (Monarca et al., 2013; Di Giacinto et al., 2014) reported an average value of pruned biomass for young hazelnut orchards (Viterbo province) of $0.8 \text{ Mg ha}^{-1} \text{ year}^{-1}$, which corresponds approximately to $0.35 \text{ Mg C ha}^{-1} \text{ year}^{-1}$. After this first phase, a positive trend can be observed at all the study areas, where over a few decades, an increase in plant biomass and a reduction in soil tillage correspond to a continuous increase in carbon stock within hazelnut orchards (Figures 2.6 and 2.7). At the NEP sites, the amount of $s\text{CS}_{30}$ (Mg C ha^{-1}) increases from 34.06 (3rd year) to 109.55 Mg C ha^{-1} (60th year, +221.64%); in CAP sites $s\text{CS}_{30}$ (Mg C ha^{-1}) increases from 34.98 (4th year) to 133.88 Mg C ha^{-1} (35th year, +282.73%), while the RON sites showed the lowest value of the increase in $s\text{CS}_{30}$, from 44.69 (3th year) to 61.99 Mg C ha^{-1} (40th year, +38.71%) (Table 2.2).

The general trend of $s\text{CS}_{30}$ showed good correlations with the age of the hazelnut orchards and higher values than natural grassland for the older orchards (> 35 years) (Figure 2.6). This assumption has not been confirmed at the sites of the farm in Ronciglione (Table 2.4), where the difference in $s\text{CS}_{30}$ between 3- and 40 years-old orchards was only about 12 Mg C ha^{-1} , and the $s\text{CS}_{30}$ remained lower than grasslands in the 40 years-old orchards. On the one hand, the grassland site in Ronciglione (RON1) showed a very high $s\text{CS}_{30}$ (more than 100 Mg C ha^{-1}), which could set the reference level of $s\text{CS}_{30}$ higher than the usual in the area.

However, the $s\text{CS}_{30}$ of mature hazelnut orchards at the Ronciglione farm (RON3) showed lower values than the others of the same age in Caprarola and Nepi. In addition, RON3 showed the lowest C/N ratio, on average 6.0, which testified an unbalanced C/N ratio with probable rapid mineralization of soil organic matter. Although C/N ratio does not constitute a direct measure of SOM mineralization (Ostrowska and Porębska, 2015), this index is often used for qualitative measurements. According to the literature, soil C/N ratios lower than 10 can be associated with high rates of organic matter mineralization and a surplus of available nitrogen (Riffaldi et al., 1996; Bengtsson et al., 2003). Although the C/N ratio could depend on the organic matter fractions in the soil (Piñeiro et al., 2006), some authors pointed out that input of nitrogen from external sources, such as fertilizers, could strongly influence the C/N ratio of the soil (Sakin et al., 2010).

According to farmer interviews, different types and amounts of fertilizers have been used over the years in the farms selected for this study work. In NEP and CAP farms, an annual fertilization of about 60 kg ha⁻¹ year⁻¹ of nitrogen has been used in the younger orchards, and about 100 kg ha⁻¹ year⁻¹ in the older ones (> 30 years). Both growers used mineral and organo-mineral fertilizers with high percentages of nitrogen (>20%). In both farms, the C/N ratio of the hazelnut soils was often higher than 10, with minimum average values of 7.7 in NEP 3 (5 years-old, Table 2.2).

In the RON farm, 150 kg ha⁻¹ year⁻¹ of nitrogen is normally used in young orchards (<10 years), whereas adult orchards are characterized by about 60 kg ha⁻¹ year⁻¹ of nitrogen by granular organo-mineral fertilizer and by high and frequent foliar fertilization during the whole growing season. More than 300 kg ha⁻¹ year⁻¹ of nitrogen is applied during 8 treatments of foliar fertilization. In this case, the C/N ratio of the soils in the adult hazelnut orchard (40 years) was only 6.0 ± 1.5 (Table 2.2), testifying an inverse correlation between the amount of nitrogen fertilization and soil C/N ratio. Although more detailed monitoring is needed to demonstrate the relationships between foliar fertilization, the soil nitrogen content, and the organic carbon mineralization, it is highly probable that such high nitrogen fertilization could influence the C/N ratio of the soil and could increase the organic matter mineralization. Further studies should be done to understand: i) the amount of foliar nitrogen fertilization falling on the soil surface; ii) the effects of such fertilization on soil organic matter mineralization. These questions are fundamental in defining the best practices for sustainable hazelnut tree cultivation in terms of fertilization and preservation of soil organic matter.

Regarding the other two farms, namely NEP and CAP, the strong direct relationship between sCS₃₀ and the age of the orchard is clear, as reported in Table 2.3. The data highlighted a loss of organic carbon during the first years of the orchard, probably due to agronomic operations for planting, namely deep tillage, leveling, and topsoil refinement, as well as continuous soil harrowing to control weeds during the first years. In general, after 3 years of planting, the soil is left permanently grass covered, because the competition between hazelnut trees and grass become slow. The grass is usually mowed and left on the ground 2-3 times during summer to facilitate the hazelnut harvesting operations in September. No-till practices in orchards that have more than 3-4 years probably tend to decrease soil organic matter mineralization, increasing the potential for soil carbon sequestration. The mature hazelnut trees have a large canopy during the summer that tends to cover most of the interrow with

shadow, diminishing the soil temperature. It is shown that a reduction in soil temperature tends to decrease microbial biomass and activity, as well as organic matter mineralization (Curtin et al., 2012).

Additionally, hazelnut trees provide a considerable quantity of leaves, catkins and small fragments of branches and sprouts that remain on the soil surface, as forest litter, and contribute to soil carbon pools. Bignami et al. (2005) estimated an amount of leaves in hazelnut orchards that varied between about 0.5 and 3 kg per plant, which, for 500 plants per hectare on average, contributed approximately to 0.1-0.6 Mg C ha⁻¹ year⁻¹. The contribution of tree biomass to the total carbon stock of the hazelnut orchard system (CStot) was lower than the carbon sequestration capacity of the soil. If the variation of sCS₃₀ from young to mature orchards varied between 12 and 52 Mg ha⁻¹, the variation of the carbon sequestered by hazelnut tree biomass varied between about 5 and 10 Mg ha⁻¹. This limited contribution of the tree biomass to the carbon sequestration is mainly due to the annual pruning activity, which removes a large biomass from the system. According to previous studies, adult hazelnut orchards in Viterbo province produced an annual pruning biomass between 1-4 Mg ha⁻¹ year⁻¹ (Monarca et al., 2013; Di Giacinto et al., 2014; Silvestri et al., 2021b), corresponding approximately to 0.45 - 1.8 Mg C ha⁻¹ year⁻¹. The valorization of such pruning biomass, namely composting or energy production, could strongly decrease the carbon footprint of the system.

Considering all study areas, a general model of the CStot increasing with the age of the hazelnut orchard was proposed in Figure 2.7.

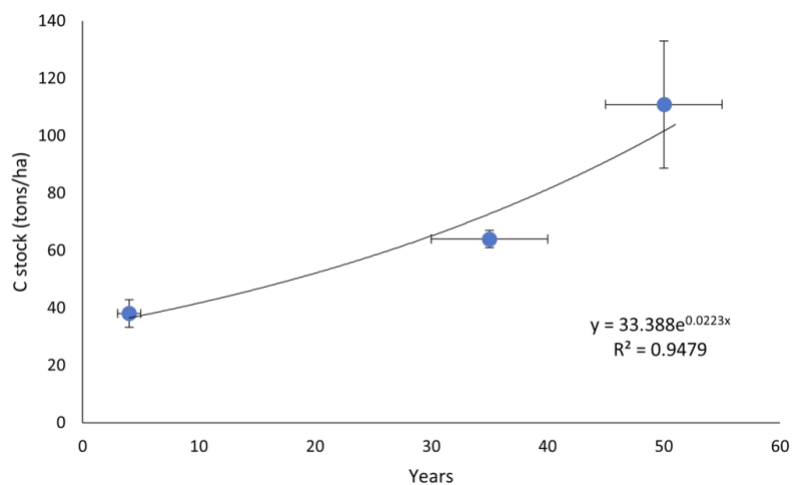


Figure 2.7. Model of total carbon stock ($CStot = sCS_{30} + CS$ tree biomass) related to the ages of the hazelnut orchards.

The effects of climate and soil features on SOM dynamics can be considered homogeneous in the three areas. Mean annual temperature and annual precipitation are very similar in the three areas, and the soil physical-chemical features that influence carbon dynamics, such as texture, pH, and calcium carbonate can be considered homogeneous. The results of the model reported mean annual C sequestration of hazelnut orchard system of 1.3 Mg ha⁻¹ year⁻¹ and 3.1 Mg ha⁻¹ year⁻¹, for orchards from 5 to 35 years and from 35 to 50 years, respectively. These values of carbon sequestration are consistent with those reported for long-term (> 25 years) agroforestry management in central Europe (Aertsens et al., 2013; Cardinael et al., 2017), as well as with the fruit and olive orchards cultivated with organic amendments and intercrops in Mediterranean climate (Vicente-Vicente et al., 2016).

The model of CStot increases with the orchard age showed an exponential curve, but it is not possible to know the trend after 60 years, because older orchards lack in this area.

Further studies are needed to improve this model, including orchards around 10 and 20 years-old and considering other areas. In addition, monitoring the evolution of sCS₃₀ due to the conversion of already cultivated land (e.g. cropland or vineyards) is also needed. It is expected that a conversion from grassland to hazelnut orchard should have low or negligible modifications of sCS₃₀ also in the short term, and then a positive effect on carbon sequestration could be already recordable after the first years of the cultivation.

5. CONCLUSIONS

Land use change from permanent grasslands to hazelnut orchards showed a rapid decrease in sCS₃₀, clearly detectable in the young orchards planted from 2015 to 2017 (3-5 years-old). This decrease was very variable and strongly site dependent, since it varied on average from -14 Mg C ha⁻¹ in NEP, -32 Mg C ha⁻¹ in CAP, and -64 Mg C ha⁻¹ in RON. This loss of soil organic carbon agrees with most of the literature reporting carbon stock changes due to the grassland conversion into croplands. However, adult hazelnut orchards (>35 years) usually showed sCS₃₀ higher than grassland, demonstrating the ability of such cultivation to sequester carbon in the medium-long term, contributing to mitigate a portion of anthropogenic CO₂ emission into the atmosphere. Carbon sequestered by hazelnut tree biomass only partially

affected the changes in C_{Stot} of the system, since the variation with the orchard age was very lower than sCS_{30} . Carbon sequestered by annual pruning biomass, as well as hazelnuts, was not considered for this study because it leaves the orchard system.

The relevance of the present study is to provide a first information on the evolution of carbon sequestration in expanding tree cultivation in the Mediterranean and temperate climate, such as hazelnut orchards. This work should be integrated by additional studies to optimize the modeling of carbon sequestration throughout the life of a hazelnut orchard, to determine the most efficient management to preserve soil organic carbon, and to detail the carbon footprint of the entire crop system.

6. References

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3 Chapter II

Proximal soil sensors to monitor irrigation supply in sub-irrigated hazelnut orchards

Abstract: Water availability and the soil traits play pivotal roles in influencing both the quality and quantity of crop yields.

European hazelnut is classified as a moderately sensitive species to water deficiency, and the impact of water stress is observable in the diminished leaf functionality and assimilative capacity of the crown. The agronomic practice of water distribution can significantly affect various aspects of hazelnut tree eco-physiology. When the water supply is not regular, several issues may appear, such as orchards with low water availability during the growing season or characterized by waterlogging. These concerns could become particularly pronounced in subirrigation systems, where monitoring the effectiveness of the water distribution becomes a complex challenge, especially in the case of dated sub-irrigation systems. This study has been conducted in a private hazelnut orchard located near the Vico lake, in the municipality of Caprarola (Viterbo, Italy). The aim of this work was to develop a model for monitoring the distribution of water supply in sub-irrigation, using proximal electromagnetic induction sensors (EMI), to verify the long-term efficiency of the sub-irrigation system. Tests were carried out under two different soil water conditions: before and after the application of irrigation treatments.

The output of this trial will contribute to validate the efficiency of both short-term and long-term distribution of water supply in sub-irrigation when compared to other irrigation systems, such as the widely used localized drip administration in Italian hazelnut districts. The findings will be instrumental in evaluating soil characteristics and the relationships between soil and water.

Keywords: *Corylus avellana* L., precision farming, proximal soil sensors, apparent electrical conductivity, sub-irrigation system.

1. INTRODUCTION

The European hazelnut (*Corylus avellana* L.) is one of the most important nut crops in the world. Italy is the second largest producer worldwide (121,085 t) after Turkey (657,761 t) (FAOSTAT, 2022).

An ever-increasing demand for hazelnuts by the confectionery industry has led to an expansion of cultivation even in environments that were not traditionally interested to hazelnut cultivation (Bocacci et al., 2013). This spread into environments with orographic features that facilitated mechanization of hazelnut orchards, often took place at the cost of sacrificing soil and climatic conditions optimal for hazelnut cultivation.

Furthermore, European hazelnut is quite sensitive to water stress, mainly due to its low capacity in stomatal regulation (Tombesi, 1994). Such species needs about 800-1,000 mm of well-distributed water throughout the year, especially during the spring-summer period, where some of the most important plant physiological processes occur as flower induction and differentiation, fruit development, vegetative growth (Fideghelli and De Salvador, 2009). Observations indicate that water stress leads to a reduction in photosynthetic activity, early leaf drop, decreased yield percentage, and heightened susceptibility to diseases (Girona et al., 1994; Tombesi, 1994; Bignami and Natali, 1996; Bignami et al, 2000; Dias et al., 2005). Additionally, there is an increase in empty nuts and a simultaneous decline in percent kernel (Bignami et al., 2011).

Therefore, hazelnut production cannot disregard artificial water supply to ensure adequate productions to meet growing market demands, as widely confirmed by the scientific literature (Bignami et al., 2011). Hence, to mitigate these adverse phenomena, ensuring a constant irrigation supply during the driest summer months becomes imperative. In this context, during the last decades, in specialized hazelnut orchards it has been introduced the agronomic practice of localized irrigation, such as drip irrigation and sub-irrigation applications (Cristofori et al., 2014). The subirrigation system is shown to be effective in improving hazelnut productivity and nut quality. However, a notable drawback of this practice is related to the difficulty in detecting leaks or failures in the system, such as breaks, system senescence, or disruption in water delivery caused by obstruction in the dripline.

In addition, the persistent impacts of climate change expose the cultivation to face increasing water shortage, prolonged drought events and intense heat waves. Given

this context, there is significant importance in adopting strategies to manage water resources sustainably.

Especially in the case of sub-irrigation system, monitoring the localized distribution of irrigation water is very complicated if compared to other kinds of irrigation systems (Martinez and Reza, 2014). To determine the punctual distribution of water in the orchard, minimizing water wastage particularly from potential system breaks, it is essential to have a detailed understanding of the physical-hydrological characteristics of the soil and their spatial variability. This knowledge makes it possible to implement management based on precision agriculture, ensuring irrigation interventions only when necessary.

Innovative solutions such as proximal sensing, especially electromagnetic induction sensors, can be used to analyze in detail, the spatial variability of the soil. The use of this kind of tool allows to get detailed mapping of the apparent electrical conductivity (ECa) of the soil. This parameter exhibits a strong correlation with various chemical and physical soil characteristics, including texture, salinity, stoniness (Dolittle and Brevik, 2014), soil depth (Priori et al., 2013), soil water retention (Ortuani et al., 2016) and soil moisture (Martini et al., 2017).

The aim of the trial was to monitor the right distribution of water supply in the orchard, when sub-irrigated, testing the effectiveness of the electromagnetic induction sensor (EMI) for detecting correct or incorrect water distribution along the various rows of the orchard.

2. MATERIALS AND METHODS

The trial was carried out on a mature hazelnut orchard of about two hectares during July 2022, located nearby the Vico Lake area (Caprarola, Viterbo province, Italy). The hazelnut orchard used for the trial, grown using cv. Nocchione, is equipped with a sub-irrigation system established in the orchard about 20 years ago. In this trial, soil samples subjected to laboratory analysis and an electromagnetic induction sensor survey for measuring apparent electrical conductivity were carried out, respectively.

2.1 Proximal soil sensing

Electromagnetic induction sensors (EMI) allow obtaining indirect information on the various physical properties of the investigated soil, such as texture, water content,

salinity, soil depth, stoniness, and porosity. These kinds of sensors can input current into the soil by producing an electromagnetic field, which in turn will create an induced electric current in the soil and, subsequently, a secondary electromagnetic field. The secondary electromagnetic field, whose intensity is related to the characteristics of the soil, is detected, and processed by the sensor without it being in contact with the soil. Further, these sensors operate in proximity to the ground surface, since do not require direct contact with the soil surface.

For the field trial, the EMI sensor from the manufacturer GF Instruments (Czech Republic), model GF Mini Explorer, was used. The sensor operates at 30 kHz, with a vertical dipole orientation. The data were acquired at walking speed, between 3 and 4 km h⁻¹. Additionally, it is designed to investigate three different depths simultaneously, 0-50 cm, 50-100 cm, and 100-180 cm, respectively.

During the test, the continuous sensing mode was used. This mode allows the instrument to detect point readings of the apparent electrical conductivity of the ground every second. In addition, each reading is associated with its coordinates, determined by the instrument's GPS receiver.

During the field test, two different conditions were analyzed; a) survey under dry soil conditions (absence of rain for more than one month and irrigation system turned off and not yet activated during the current growing season); b) survey carried out under wet soil conditions after six hours of sub-irrigation system operating (first seasonal water supply). For both investigations, the detection of ECa values was carried out by following the same path covered by the pipes of the sub-irrigation system, with the aim of intercepting anomalies in ECa values that could be attributed to the potential issues of irrigation system efficiency. After obtaining the ECa values, the data were transferred to a computer for the initial processing phase. This involved the compilation of a preliminary georeferenced map, incorporating the conductivity values derived from the collected data.

Before spatialization process, the data were cleaned of any potential interference errors (exceptionally high or negative values), disturbances related to the presence of metallic material (e.g., irrigation system fittings at the beginning of a row), outliers related to unknown causes (single point with high values in the middle of points with low values, or vice versa).

In conclusion, the point data were spatialized using the Ordinary Kriging function of QGIS software to obtain a continuous map of the entire hazelnut orchard investigated.

In addition, further information of ECa could be obtained through the difference of the two spatializations.

2.2 Soil sampling

Soil samples were collected following a preliminary assessment of ECa values using a proximal soil sensor (EMI). This initial investigation was conducted under conditions of low water availability according to the seasonal weather conditions. Following the sensor survey, the point data obtained were analyzed and spatialized with QGIS software, enabling the release of a continuous map of the plot based on the previously surveyed ECa values.

Subsequently, four distinct points within the hazelnut orchard were selected according to the spatialized map, which had varying and extreme ECa values corresponding to different textural characteristics (Figure 3.1).



Figure 3.1. Samples of soil collected in the experimental hazelnut orchard located in Caprarola municipality (Viterbo province, Italy).

For each individual spot identified, three different soil samples were taken at three different depths by hand auger. The three depth ranges were 0-30 cm, 30-50 cm, and

50-70 cm, respectively. Once the samples were taken, they were transferred to the laboratories of the University of Tuscia and subjected to an analytical study of the soil texture.

2.3 Data processing

Regarding soil moisture, spatialization of collected data in pre- and post-irrigation was achieved using QGIS software, giving the possibility to highlight the differences between the two conditions, expressed in terms of electrical conductivity (mS/m).

These differences, represented graphically by a map of ECa values, makes it possible to check whether the sub-irrigation system had a homogeneous distribution of water, helping to identify potential issues like system breaks, highlighting areas characterized by a greater potential difference and water content, or the presence of drier areas, indicating possible problems like pipeline obstructions or specific textural characteristics, such as limited areas with higher sand content.

The tool allows to investigate the ECa values at three different depths, however, attention focused only for the intermediate depth, 50-100 cm, since the drip pipes of the sub-irrigation system are buried at a depth of 40 cm.

2.4 Texture analysis

Texture analyses of samples taken within the hazelnut orchard, on the four orchard sites previously described, were carried out at the laboratories of the University of Tuscia. The analytical tool employed for texture classification of the collected samples is called PARIO, a sensor equipped with a system based on the ability to detect the sedimentation of suspended particles within a solution. Its operation is based on Stokes' law, which allows to calculate the resistance of a fluid on a spherical body moving within it.

Before proceeding with the soil texture analysis, the samples taken, due to the high degree of moisture, possessed a massive shape that prevented proceeding directly with the analysis. Consequently, it was necessary to crush the samples into smaller portions and left them to dry in an oven at +30°C for 24 hours. Afterwards, they were reduced into further small portions with the laboratory mortar (Figure 3.2).

Following the reduction into smaller parts, the samples were sieved with a 2-millimeter sieve to separate the fine soil, later used for analysis with PARIO, and the coarser part

represented by skeleton. Then, from the sieved sample, 30 g was taken for analysis with the PARIO instrument (Figure 3.2).

The first step toward starting the instrument is to prepare a 1-liter solution consisting of three several elements: distilled water, an aliquot of the dry and sieved sample, and sodium hexametaphosphate (NaPO_3).

The preparation procedure involves adding the soil sample to sodium hexametaphosphate, whose function is to promote the separation of aggregates and their dispersion in solution, especially clays. Subsequently, distilled water is added until 500 milliliters is reached. The soil, water and hexametaphosphate solution was placed in agitation, using a magnetic stirrer, for about 2 hours.

After the agitation, the solution was filtered with a 63-micron sieve to separate the sands fraction. This fraction was then placed in a metal capsule and dried in an oven at $+105^\circ\text{C}$.

The remaining part of the sample, consisting of silt and clay, was used for measurement with the PARIO instrument, after further addition of distilled water to reach the measurement cylinder quantity of one liter. Before starting the instrument reading, it is necessary to perform its calibration (Figure 3.2), using a cylinder with a volume of one liter, filled with distilled water. The instrument is connected to a computer, and through its software, parameters are introduced with which PARIO is able to measure the clay and silt content. The parameters introduced are the time of the measurement, in this case eight hours, the weight of the dispersant (2 g of NaPO_3), the weight of the analyzed sample (30 g), and then the weight of the sandy fraction screened and dried in the oven.



Figure 3.2. Preparation of the sample for analysis in the laboratory mortar (on left), and PARIO Instrument Calibration (on right).

After the instrument calibration, which takes a few seconds in the cylinder containing only distilled water, and manual agitation of the sedimentation cylinder for 60 seconds, the sensor can be moved from the calibration cylinder to the cylinder containing the soil sample.

After the eight-hour measurement period, on the computer screen it displays the graph illustrating the distribution of particles over time, the assigned texture class, and a graphical representation using the texture triangle. This instrument thus allows to directly measure the clay and silt content.

3. RESULTS

3.1 Apparent electrical conductivity and sub-irrigation system

In this study, two different tests were carried out using the proximal sensor (EMI), one in the pre-irrigation condition, on a soil on which it was supposed to rain for more than one month, while the second was replicated following the activation of the sub-irrigation system and left on for six hours.

The collected images refer to the two different conditions: pre-irrigation and post-irrigation and to the three different depths of detection, 0-50 cm, 50-100 cm and 100-150 cm, respectively.

3.2. Pre- and post-irrigation mapping

For the sub-irrigation system under investigation, the following maps with point ECa values were obtained (Figure 3.3).

Specifically, the blue points generally indicate areas of the hazelnut orchard characterized by higher clay content, denoted by higher ECa values than the average. Conversely, points marked in red indicate areas where the sand content is more prominent, resulting in lower ECa values than the average.

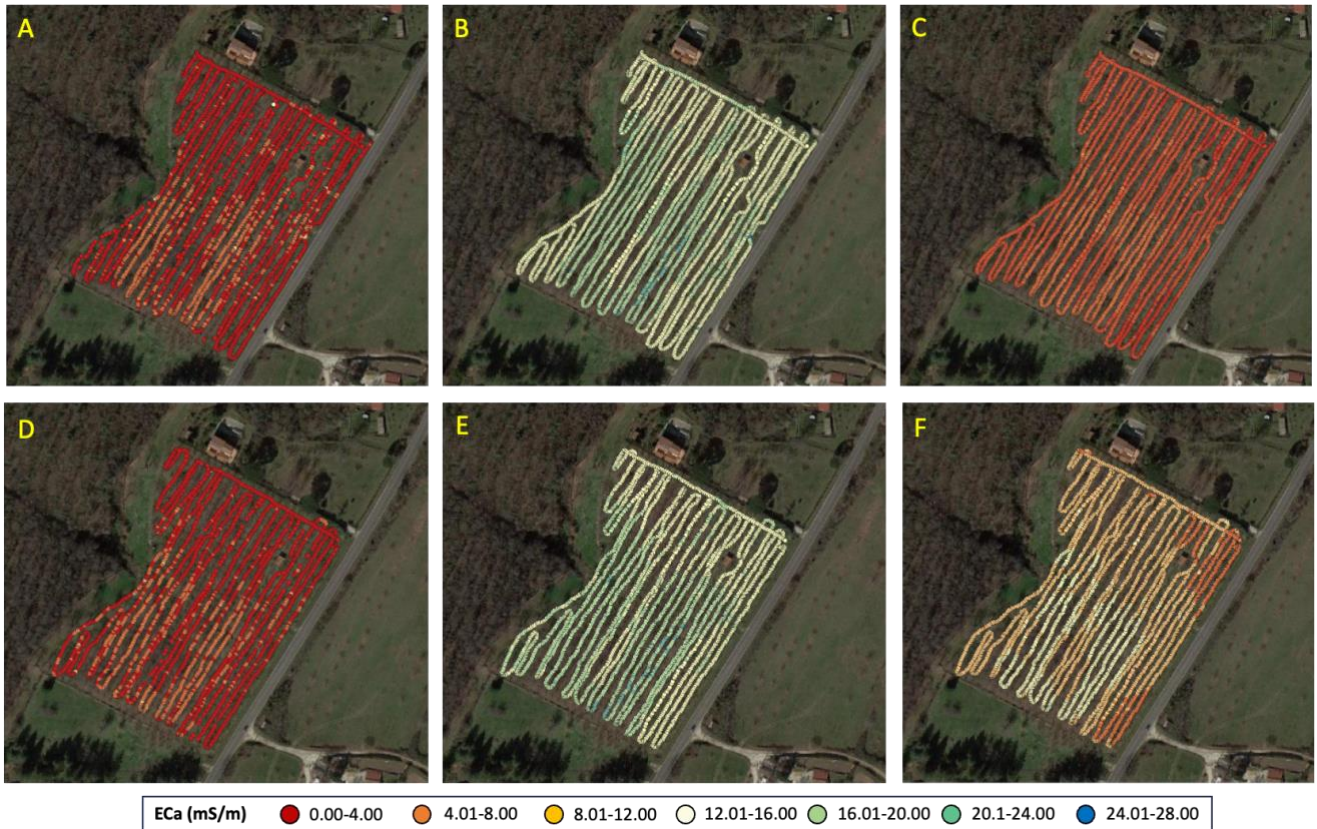


Figure 3.3. Surveys of ECa values in the pre-irrigation treatment, 0-50 cm (A), 50-100 cm (B), 100-180 cm (C). Surveys of ECa values in the post-irrigation treatment, 0-50 cm (D), 50-100 cm (E), 100-180 cm (F).

From the analysis of the maps obtained (Figure 3.3), representing the pre- and post-irrigation conditions, variations in ECa values are evident, particularly within the 50-100 cm depth interval, in which the pipes of the sub-irrigation system are located, and in the third survey layer (100-180 cm).

In addition, the difference in ECa values between post- and pre-irrigation conditions can be calculated.

This difference was further distributed across the entire plot through the spatialization of data, maintaining a representation showing how this difference varies in space (Figure 3.4). This also makes it possible to assess whether there are visually significant differences in conductivity variation that might suggest abnormalities in the subirrigation system.

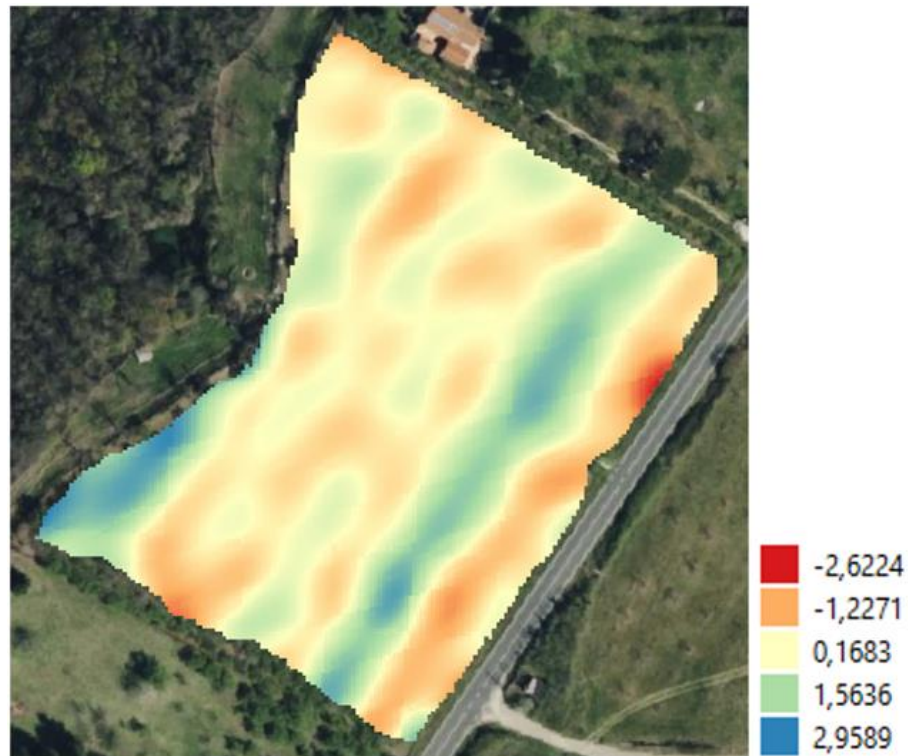


Figure 3.4. Spatialization of the difference between the electrical conductivity values found at the second depth interval.

From the Figure 3.4, it can be observed the presence of areas marked by linear variations, with a difference of about 4 mS/m. Given the relatively small magnitude of this difference, also minor variations in textural composition or slight differences in the flow rate of the sub-irrigation system can contribute to obtain a complex representation that may be hard to interpret accurately.

Despite the non-uniform distribution of water, the hypothesis of any breaks in the system can be considered invalid since direct inspection of the field did not reveal any areas or points with significantly higher moisture content than the entire plot.

In addition, the generated maps do not indicate substantial differences in electrical conductivity enough to assume a leak or break in the system.

3.3 Texture analysis with PARIO

After the eight hours provided by the instrument, the time required for the measurement of clay and silt sedimentation, the following texture triangles (Figures 3.5, 3.6, 3.7, and 3.8) are obtained for the four samples analyzed and belonging to the intermediate depth collection level (30-50 cm).

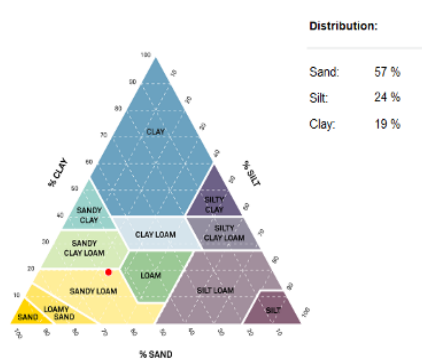


Figure 3.5. Texture analysis of soil sample 1.

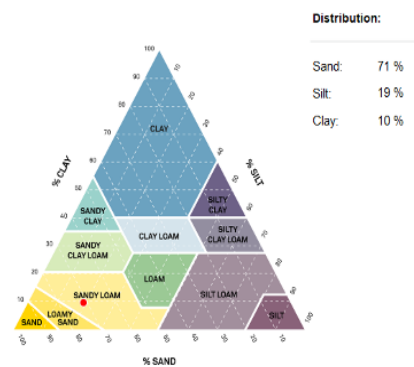


Figure 3.6. Texture analysis of soil sample 2.

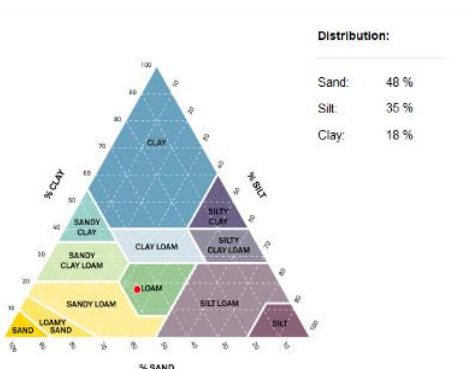


Figure 3.7. Texture analysis of soil sample 3.

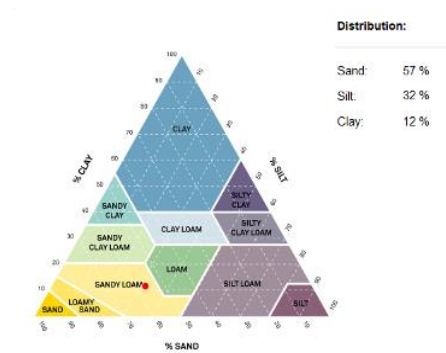


Figure 3.8. Texture analysis of soil sample 4.

Textural analyses performed with PARIO instrument are characterized by direct measurement of clay and silt. Sand content, on the other hand, is obtained by difference. The analysis revealed some variability in the textural characteristics of the hazelnut orchard across the four surveyed spots. Notably, spot number 2, situated in the northernmost part of the orchard, exhibited an exceptionally high sand content when compared to the other surveyed spots. Specifically, the recorded value was of 71% (Sandy-Loam soil). Furthermore, to prove that the sand content estimate is correct and thus the whole analysis performed, it was possible to weigh the amount of sand in the sample and calculate its percentage (Table 3.1).

Table 3.1. Measured weight and percentage of sand in the four sampling soils of the sub-irrigated hazelnut orchard.

Sample	Sample weight (g)	Sand weight (g)	Sand %
1	27.581	16.287	59.1
2	27.594	20.484	74.2
3	27.876	13.289	47.7
4	27.883	16.068	57.6

The calculated percentage of sand content allows us to say that the results obtained with the PARIO instrument are correct, since the direct and estimated results are very similar to each other.

Knowing the texture-related values of the four points analyzed and having carried out the spatialization of the difference in electrical conductivity (Figure 3.9), it can be shown how point 2, a point with a sandier texture, is in a zone with a lower conductivity. While the other three points with lower sand content and higher clay content were in a zone with higher conductivity values.

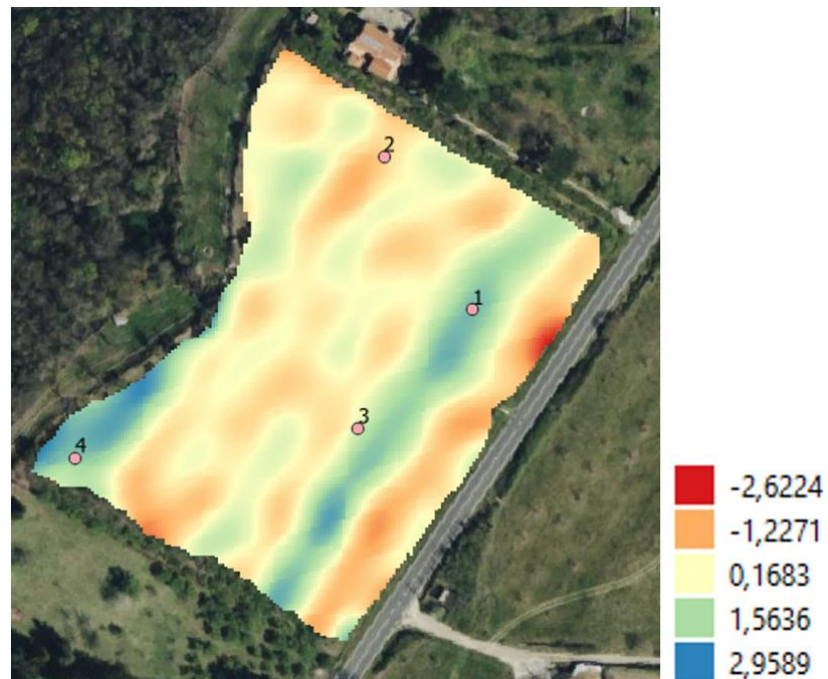


Figure 3.9. Map of the difference in electrical conductivity revealed in the hazelnut orchard and points where soil samples were taken.

Furthermore, the correlation between ECa values of the four-soil samplings and their texture analysis was conducted, as reported in Figure 3.10 and 3.11. Pearson's correlation test highlighted a strong negative correlation with sand content, indicating that an increase in sand content is associated with a decrease in ECa values (Figure 3.10). On the contrary, it can be observed how an increase in clay content positively affects ECa, leading an increase in these values (Figure 3.11).

Despite these interesting outcomes obtained, for a sturdier assessment of the correlation between ECa and the texture analysis of the soil samplings, generally, it is preferable using a larger dataset.

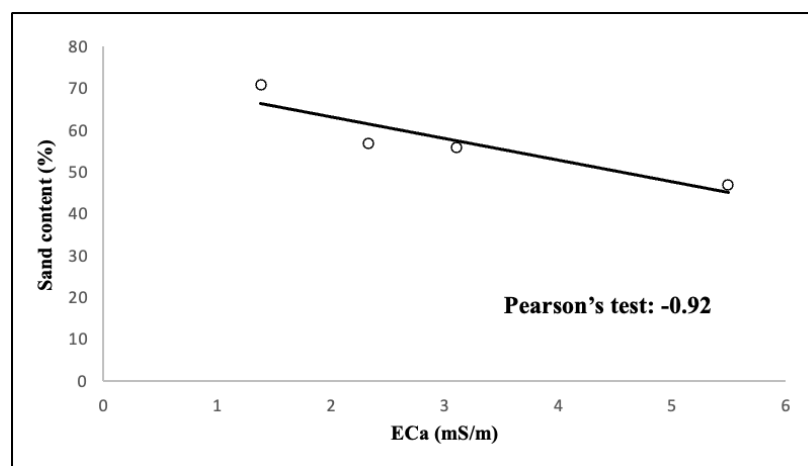


Figure 3.10. Comparison between ECa values (mS/m) and texture analysis of four samplings soil identified on the hazelnut orchards. The picture represents the correlation with the sand content (%). Pearson's test was applied to assess the relation between them.

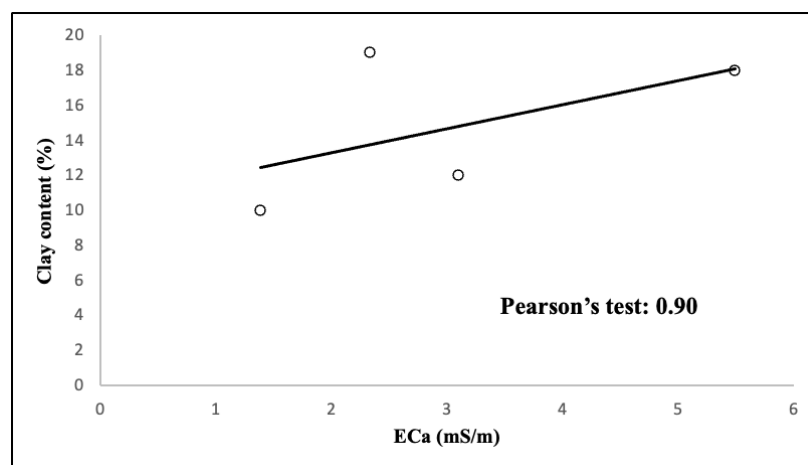


Figure 3.11. Comparison between ECa values (mS/m) and texture analysis of four samplings soil identified on the hazelnut orchards. The picture represents the correlation with the clay content (%). Pearson's test was applied to assess the relation between them.

4. DISCUSSION

The sub-irrigation system highlights high water use efficiency due to minimized water evaporation, achieved by direct application of it within the soil (Ayars et al., 1999), and does not hinder the displacement of farm vehicles within the orchard for cultivation operations. However, it presents some drawbacks. Among these, a particularly noteworthy challenge is the difficulty in assessing the functionality and identifying potential breaks or occlusions in the buried drip pipes (Martinez and Reca, 2014). In this context, the objective of this study was to evaluate the efficiency of a proximal sensor in determining the functionality of a 20 years-old sub-irrigation system.

From the spatialization of ECa values measured under dry soil conditions (first investigation with the proximal sensor), and subsequent identification of four specific points, whose samples were subjected to textural analysis, it was possible to observe the presence of wide differences within the same hazelnut orchard. Generally, the blue-colored points denoted higher ECa values suggested areas with elevated clay content, while red-colored points indicated regions with higher sand content and lower ECa values. The northernmost point of the orchard corresponding to number two exhibited the highest values in sand content (over than 70%). This observation was confirmed by the recorded lowest ECa values in this specific spot, directly related to the high sand content.

Comprehensive analysis of the textural characteristics of the soil in the hazelnut orchard holds pivotal significance for the subsequent evaluation of the sub-irrigation system's efficiency. This importance arises from the fact that a soil with high sand content, indicative of reduced water retention and storage capacity, may potentially impact the readings obtained from the proximal sensor. Specifically, elevated sand content could result in lower apparent electrical conductivity (ECa) values, raising concerns about potential system breakdowns or diminished sub-irrigation efficiency. A second survey was conducted using the proximal sensor under soil conditions subjected to six hours of irrigation. The instrument was able to detect apparent electrical conductivity (ECa) values at three different depths, with particular emphasis placed on the intermediate depth, ranging between 50 and 100 cm. The high interest in this intermediate depth derives from the fact that it is the portion mainly served by the sub-irrigation system.

From the spatialization of the point data obtained through the two investigations under different soil water conditions (pre- and post-irrigation), a third map was generated, which showed differences in ECa values. The linear variations recorded, with a maximum deviation of 4 mS/m, and the absence of areas characterized by significantly higher moisture levels, as confirmed through direct field observation, allowed us to discard the hypothesis of breaks within the sub-irrigation system.

5. CONCLUSION

The use of proximal sensors such as the EMI sensor can be employed in detailed mapping and soil monitoring, in order to optimize the management and irrigation of hazelnut orchards. The findings obtained with this study contribute to the evolving field of precision agriculture, offering valuable insights for optimizing irrigation strategies and enhancing the sustainability of hazelnut orchards.

The interaction between soil texture characteristics and water content highlights the importance of considering the former factor to better understand the complexity of the impact of the sub-irrigation system on the association between water content and electrical conductivity.

Although the data obtained from the investigation showed lines of change in ECa values after irrigation treatment, we have no data to explain the causes with certainty. In conclusion, the use of proximal soil sensor (EMI) to monitor irrigation water distribution and the possibility to detect abnormalities along the sub-irrigation system seems to require further investigation, which will be carried out in the next years to develop a detailed model, to be used on a large scale.

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4 Chapter III

Influence of the plant training system on yield and nut traits of the European hazelnut cultivar Nocchione

Abstract: The European hazelnut (*Corylus avellana* L.) exhibits a distinctive shrubby growth pattern related to the recurrent development of suckers emerging either at the base of the trunk or of the stump, according to the plant growing system.

The traditional multi-stemmed shape continues to be used in traditional hazelnut districts such as those in Italy and Turkey. The suckers generated from adventitious buds enable a gradual renewal of the plant, by selecting lignified and well oriented suckers in the stump. Despite these advantages, suckers are long juvenility organs that compete with the plant for water and nutrient uptake, increase the risk of developing plant diseases, and hinder proper mechanization of cultural practices. Therefore, suckering is deemed a necessary agronomic operation during each growing season.

During the last decade, an enlargement in hazelnut cultivation has been recorded, both in traditional environments and in new hazelnut introducing areas, with a trend toward cultivation intensification through the adoption of high-density planting layouts and new training systems, such as the single trunk sapling. In this context, the activities of this study were undertaken, with the aim of studying the agronomic behavior of different training system applied to hazelnut plants of the cultivar Nocchione during their progressive increase in plant maturity. The three different training system, under investigation were the following: regular four-stemmed shrub (Thesis A), single trunk sapling form (Thesis B) and traditional multi-stemmed shrub (Thesis C).

Key words: *Corylus avellana* L.; sustainable intensification; training systems; pruning wood; yield efficiency.

1. INTRODUCTION

The European hazelnut (*Corylus avellana* L.) is a temperate nut crop which tends to produce several suckers annually from the base of the stump, developing as a multi-stemmed shrub. This natural shape is valued and still used as training system by growers in many traditional hazelnut districts, such as Italy, Spain and Turkey (Silvestri et al., 2021a), since allow them to gradually renew the plants selecting new lignified suckers (Silvestri et al., 2021b). However, several drawbacks characterize this training system, such as reducing the effectiveness in pest and disease control increasing environmental condition that promote pathogen developed (Gonçalves et al., 2019; Tomasone et al., 2009), increased competition for water and nutrients (Tous et al., 1994a), extension of juvenile phase, hinder mechanized cultivation operations (Pacchiarelli et al., 2022), and in some cases even reduction in nut yield (Mehlenbacher and Smith, 1992).

Anyway, in other countries, such as Oregon (USA), France and Chile, growers prefer to shape the hazelnut plants as a single-trunk and open vase, in order to increase the penetration of sunlight within the rows and into the inner portion of the plant canopy (Me et al., 2001), allowing an easier suckering, increasing planting density and facilitating nuts harvesting (Germain and Sarraquigne, 1997). However, the adoption of single-trunk training system implies an increased aptitude for sucker production (Pacchiarelli et al., 2022). A possible solution to this issue could come from the use of non-suckering rootstocks, such as seedlings of *C. colurna*, commonly used in Serbia, or interspecific hybrids of *C. colurna* x *C. avellana*, among all Dundee and Newberg, released by the Oregon State University breeding program and widely used in Spanish environment (Rovira, 2021).

The type of training system is relevant not only for facilitating mechanization of agronomic practices, but also for light penetration into the plant canopy. The physiological behavior of trees is greatly influenced by the amount and location of light interception, resulting in yield and fruit quality optimization (Germain and Sarraquigne, 1997). Further studies have confirmed the influence of different training systems applied on the same cultivars on parameters such as fruit and kernel weight (Farinelli et al., 2005).

Nowadays, the cultivated area got over 700,000 hectares worldwide, being established in typical countries and in new introducing areas, such as Chile, South Africa and

Australia. In this context, a new generation of specialized hazelnut orchards has been established generally in flatter and more fertile areas, to make easier the mechanization of agricultural practices (Pacchiarelli et al., 2022).

During the last decade, especially in new hazelnut areas, an increase in intensive hazelnut orchards approach has been observed (Fideghelli and De Salvador, 2009), with the adoption of new forms of training system (single trunk shape) and new planting patterns characterized by higher density (4.5 m x 3.0 m), often carried out through the use of transplanting machines (Cristofori et al., 2009), as opposed to traditional hazelnut orchards, characterized by plants generally trained as multi-stemmed bush or vase shape, with a density of 400-500 plants ha⁻¹, and made by hand planting from one to four rooted suckers per hole (Cristofori et al., 2017).

The innovative model of tree planting using DGNSS handhelds that interface with specially designed Geographic Information System (GIS) software, enables high-precision operations with savings in time and costs (Cristofori et al., 2023).

European hazelnut is a temperate nut species with a long juvenile and unproductive period lasting about 4-6 years after planting, according to the chosen cultivars. For this reason, some approaches of dynamic spacing have been applied on it (Tous et al., 1994b). The goal of this cultural practice is increasing the yield during the first twelve years, through the plantation of double number of the plants (800 plants ha⁻¹, spaced 5.0 m x 2.5 m), and then removing half of the plants, reaching the final tree density (400 plants ha⁻¹, spaced 5.0 m x 5.0 m). The high cost of this system has determined in the past a narrow use of this agricultural practice (Bignami et al., 2005). However, recently a new dynamic spacing approach using two different cultivars with different vigor in alternate rows was developed in Oregon (USA). The management of this system involves removing the most vigorous plants when canopy branches begin to touch each other.

To increase light penetration inside the canopy, avoiding physiological disorder that could stimulate the low yield and a decrease in kernel quality (Roversi and Mazzone, 2005), and alternate bearing (Azarenko et al., 2005), annual pruning practices on mature plants are needed. Generally, pruning operations are still carried out by hand, requiring high labor and time, of about 20 labor hours per hectare (Franco and Pancino, 2009). For these reasons some growers tend to neglect these operations, with negative implications related to physiological imbalances, as described previously.

In this context, mainly in extensive hazelnut orchards, mechanical pruning approaches are getting more frequent (Ellena et al., 2014). Further confirmation, related to the economic viability of this operation was highlighted by (Silvestri et al., 2021b), who also described the type of rotation to be carried out between hedging and topping operations, so that excessive loss of production is avoided.

In this scenario, the purpose of the following study was to conduct an agronomic evaluation of three different training systems applied on plants of the cultivar Nocchione, during their increasing productivity stage, to identify which of them presented a greater suitability for use in a high-density hazelnut orchard. The training systems under investigations were the following: regular four-stemmed shrub (Thesis A), single trunk sapling form (Thesis B) and traditional multi-stemmed shrub (Thesis C).

2. MATERIALS AND METHODS

2.1. Localization of the study site

The trials of this study were carried out on a private farm in the municipality of Caprarola, in Viterbo province, about 270 m a.s.l. (Coordinates: 42°16'42.56" N; 12°17'49.59" E).

The plot under investigation covers an area of about 9.1 ha, and it is cultivated using Nocchione as main cultivar. The hazelnut orchard was established in 2016, using a planting layout of 4.5 m x 3.0 m, with a density of 740 plants ha⁻¹. The orchard is equipped with a subirrigation system with two drip wings per row, 30 cm in depth and 80 cm from the row. The investigated area presents a loamy-clay soil with acidic pH, low organic matter content, low C/N ratio and rapid mineralization processes, medium cation exchange capacity, low assimilable phosphorus and high potassium concentration and unbalanced Mg/K ratio due to an excess of K content.

The field trials were carried out for five consecutive growing seasons, from 2019 to 2023, although the measurements taken in 2019 and 2020 were conducted before the PhD period. However, for a greater completeness of the study, these data were included in the statistical analysis.

2.2. Description of the cultivar used in the trial

The Nocchione cultivar is widespread in Latium region and in Nebrodi area of Sicily region. The plant is characterized by high vigor, with a semi-erect growing, high suckering attitude, intermediate female flowering, early male flowering, and high productivity. The husk is just little longer than the nut. Further, the nut is large (3.0 - 3.2 g), spheroidal in shape with a thick shell. The kernel/nut ratio is medium-low (38-40%). The kernel shows a spheroidal shape with high blanching ability (80-85%).

2.3. Pruning models used in the trial

The field trial was established when the young plants were at their third leaf in the field. Until that point, the plants had never been pruned, with the only exception for the annual removal of suckers. In total 30 plants were selected, 10 representing each shaping treatment in comparison. Thesis A was represented by plants pruned as regular four-stemmed bush (Figure 4.1); Thesis B was represented by plants trained as single trunk sapling form (Figure 4.2), while Thesis C was represented by plants trained as traditional multi-stemmed shrub (Figure 4.3).



Figure 4.1. Thesis A, representation of regular four-stemmed shrub. Images acquired in February 2020, July 2022 and April 2023, respectively.



Figure 4.2. Thesis B, representation of plant trained as single trunk sapling form. Images acquired in February 2020, July 2022 and April 2023, respectively.



Figure 4.3. Thesis C, representation of plant trained as traditional multi-stemmed shrub. Images acquired in February 2020, July 2022 and April 2023, respectively.

During the first year of the survey (February 2019), at the end of the winter rest and slightly before budbreak, the first manual pruning interventions were carried out, in order to determine the training system selected for each treatment. Over the following years, from 2020 to 2023, the pruning practices, with the aim to maintain the training system, were carried out in every February (Table 4.1). The agronomic evaluation to assess the influence of different training systems was carried out for five consecutive growing seasons from 2019 to 2023.

Table 4.1. Pruning operations carried out on selected plants in the compared training treatments from 2019 to 2023.

Intervention	Period	Pruning
1	February 2019	Plant training
2	February 2020	Maintenance
3	February 2021	Maintenance
4	February 2022	Maintenance
5	February 2023	Maintenance

2.4. Assessment of agronomic parameters

For each plant representing the different shaping and pruning thesis, the following agronomic parameters were evaluated over the period of investigation:

- Pruning wood weight (kg plant⁻¹);
- Plant production (kg plant⁻¹);
- Measurements of the diameter of the main branches at 30 cm above the ground to calculate the trunk cross sectional area (TCSA, cm²) at the end of each growing season. In the case of multi-stemmed shrub (Thesis A and C), the total TCSA was expressed as the sum of the sections of all main branches per plant;
- Yield efficiency (kg cm⁻²) expressed as ratio of yield/TCSA.

2.5. Assessment of nut and kernel traits and incidence of main commercial defects

The hazelnuts harvested during each growing seasons were subjected to evaluation of nut and kernel traits. A sample of 150 nuts was analyzed for each thesis. Hazelnuts were assessed for nut, kernel and shell weight, major and minor equatorial diameter of the nut and kernel, and nut and kernel height using a digital caliper (± 0.03 mm). The roundness index for both nut and kernel were determined according to the formula [(larger diameter + smaller diameter)/2]/height. The kernel/nut ratio was also determined.

2.6 Evaluation of the main commercial defect incidence

Hazelnut samples subjected to carpological characterization were also evaluated for the incidence of major commercial defects, such as blank, poor filled, shriveled, mould, black tip, double kernel and nut split. Defect incidences were expressed as

percentage values according to the protocol issued in the hazelnut breeding program in progress at the Oregon State University (Mehlenbacher et al., 1993).

2.7 Statistical analysis

Statistical analysis was applied to all investigated parameters except for the incidence of main commercial defects, whose values are expressed as percentages. The program used to assess the significance of differences was Jamovi 2.4.12 software. All the data were subjected to two-way analysis of variance (ANOVA) considering the effects of year, treatment, and their interactions. Additionally, nut and kernel traits were subjected to one-way ANOVA to analyse the influence of the treatment for each individual year.

Differences were accepted as statistically significant when $p < 0.01$. Fisher test was applied on dataset previously analyzed by Anova to identify significance among the samples.

3. RESULTS

3.1. Amount of pruned wood

Figure 4.4 represents the amount of pruned wood expressed as mean values \pm standard deviation of kilos wood per plant for each thesis during the five years of investigation. In 2019, the first pruning operations, carried out to establish the different training systems produced about 5.0 kg plant⁻¹ in thesis B, in comparison to 2.2 kg plant⁻¹ observed in thesis A. Further, the amount of pruned wood in thesis C (0.7 kg plant⁻¹) was approximately 7 times less than what obtained in treatment A.

Over the following years of survey, it can be observed as the different pruning treatments tend to produce a similar amount of pruned wood among the treatments, mainly during the seasons 2022 and 2023, whit the treatment B characterized by a slightly lower amount of wood produced by pruning interventions in comparison to the other thesis. Considering the interaction between years and treatments, no significant differences were observed during the last two growing seasons.

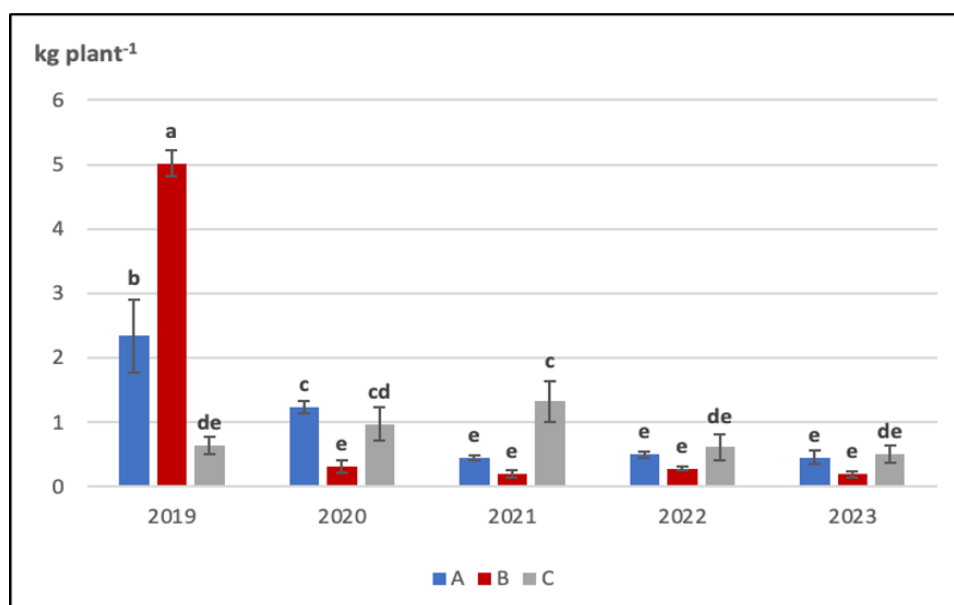


Figure 4.4. Amount of wood produced by pruning interventions during the five years of the trial (Thesis A: regular four-stemmed shrub; Thesis B: single trunk sapling form; Thesis C: multi-stemmed shrub). Data are represented as mean values \pm standard deviation. Different lowercase letters on the columns indicate significant differences of the interaction between thesis \times year (Fisher's test, $p < 0.01$).

3.2. Plant production

Nut production, recorded during the five survey years was significantly affected by the pruning treatments and by the year of evaluation, as reported in Figure 4.5.

In the growing season 2019, the first year of the trial, treatments did not affect yield, which showed no significant differences between treatments. From the 2020, the productions tend to increase year by year for all the pruning treatments. In general, thesis B (single-trunk sapling) seems being more penalized by the pruning interventions, always showing the lower values of production. During the growing season 2021, a late frost occurred on the night of April 8-9, with a minimum temperature of - 8 °C, affecting the shoot growth and fruit set and compromising the entire yearly production. During the growing season 2022 and 2023, the yield of thesis A and C tend to align their values, which are approximately twice as high as that observed for thesis B. Further, thesis A seems being able to recover faster the production losses determined by the initial more severe pruning, in comparison to thesis B. During the last year of the trial, when the plants almost got a fully maturity production, treatments A and C showed the higher yields, respectively 4.77 ± 0.26 and 4.83 ± 0.15 kg plant⁻¹.

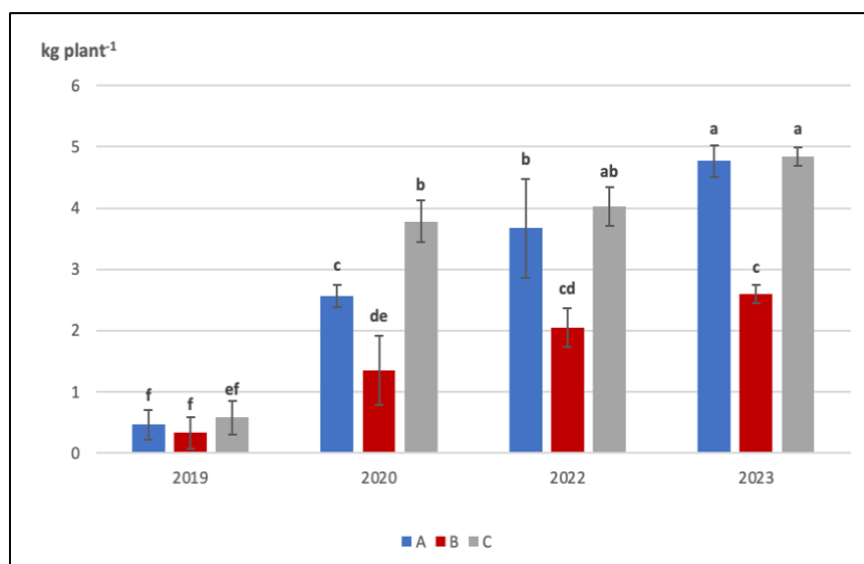


Figure 4.5. Annual nut production (kg plant⁻¹) of the plants subjected to three different training systems during the survey (Thesis A: regular four-stemmed shrub; Thesis B: single trunk sapling form; Thesis C: multi-stemmed shrub). Data are represented as mean values \pm standard deviation. Different lowercase letters on the columns indicate significant differences of the interaction between thesis x year (Fisher's test, $p < 0.01$).

3.3. Plant vigor (TCSA)

As expected, during all the survey years, thesis B showed the lowest value of TCSA (Figure 4.6), when compared with the other treatments, since the applied treatment involves only one single trunk as a training system. Among the multi-stemmed shrub training systems, thesis C (control) showed the highest values, being represented by a larger number of branches per plant surveyed, while for thesis A (regular four-stemmed bush), intermediate values of TCSA were observed between treatment B and treatment C.

Among the treatments, thesis A was characterized by the highest vegetative growth, increasing from $40.33 \pm 2.63 \text{ cm}^2$ in 2019 to 106.03 ± 2.36 in 2023, with a percentage increase of +162.91%, unlike thesis B (+139.39%) and thesis C (+112.33%). In addition, thesis A noticed greater homogeneity among the branch sizes during all investigation years, with the lowest standard deviation values.

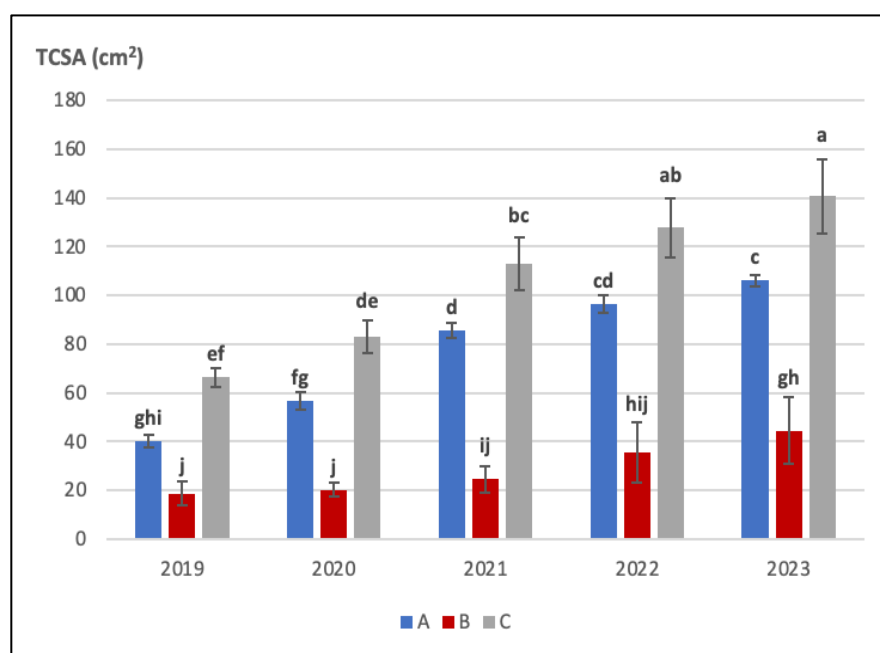


Figure 4.6. Sum of trunk cross sectional area ($\Sigma TCSA$) (cm^2), measured at 30 cm above the ground, on the plants subjected to different training systems (Thesis A: regular four-stemmed shrub; Thesis B: single trunk sapling form; Thesis C: multi-stemmed shrub). Data are represented as mean values \pm standard deviation. Different lowercase letters on the columns indicate significant differences of the interaction between thesis \times year (Fisher's test, $p < 0.01$).

3.4. Yield efficiency

Yield efficiency (YE) recorded during the five year-survey is shown in Figure 4.7. The lack of yield values (kg plant^{-1}), for the 2021 growing season, determined the inability to calculate YE values for the same year.

In general, the plants trained as a single-trunk sapling (Thesis B), although characterized by the lower production, highlighted the highest values of YE, if compared with the other treatments, getting $0.0600 \pm 0.0104 \text{ kg cm}^{-2}$ in 2022, and $0.0623 \pm 0.0197 \text{ kg cm}^{-2}$ in 2023. On the other hand, thesis C (multi-stemmed shrub) showed the lowest values of YE, although had shown the greater values for the nut production. Considering the interaction between years and treatments, no significant differences were observed during the analyzed growing seasons.

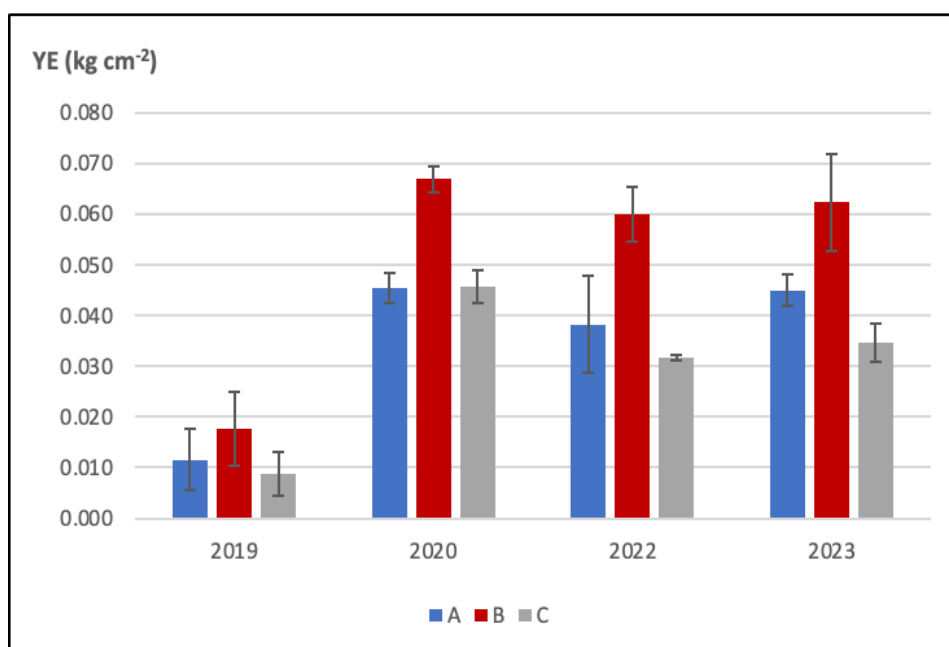


Figure 4.7. Yield efficiency (kg cm^{-2}) of the plants subjected to three different training systems during the survey (Thesis A: regular four-stemmed shrub; Thesis B: single trunk sapling form; Thesis C: multi-stemmed shrub). Data are represented as mean values \pm standard deviation. No significant differences were observed among the treatments from the interaction of years \times thesis.

3.5. Nut and kernel traits

Nut and kernel traits have been affected by the treatments applied, the year, and their interaction, as shown in Table 4.2. Specifically, Table 4.3 displays the differences between treatments for each survey year. Regarding nut and kernel weight, significant differences were observed when the interaction between thesis x year has been evaluated, showing how the effect exerted by the treatment varies among the years. In particular, a significant difference in kernel weight between years, with the highest values recorded in 2019 (averaging 3.13 ± 0.59 g) was recorded. In comparison, the weights for subsequent survey years 2020, 2022, and 2023 were 2.39 ± 0.82 g, 2.40 ± 0.53 g, and 2.53 ± 0.14 g, respectively. Distinct variations in nut weight were also noticed among the different treatments, with treatments A and B exhibiting the highest values (2.83 ± 0.59 and 2.79 ± 0.57 , respectively). Like nut weight, kernel weight displayed a comparable trend across the years, with thesis A and B consistently showing higher values. Overall, the highest values for kernel weight were observed in 2019 and 2023 (1.13 ± 0.22 and 1.05 ± 0.17 , respectively).

The regular four-stemmed shrub and single-trunk sapling form, in addition to showing the highest values for nut and kernel weight, also recorded the best values for shell weight as average values among all survey years (1.75 ± 0.37 and 1.75 ± 0.39 g). This parameter represents an interesting trait since this product may be used for agronomic or energy purpose.

The kernel/nut weight showed some variability among survey years. Among them, the highest values were observed in 2023, with a ratio over 40% ($41.29\% \pm 4.57$), significantly higher than the previous years. Moreover, from the analysis of the different treatments, the best values were recorded for treatments A and B ($38.64\% \pm 5.78$ and $38.88\% \pm 5.11$, respectively).

Regarding the evaluation of treatment effects on nut and kernel traits for each individual year, a considerable variability was recorded, as displayed in Table 4.3. For the first year of survey trial (2019), the greatest results related to nut and kernel weight were achieved by treatment C, 3.31 ± 0.61 g and 2.18 ± 0.42 g, respectively. On the contrary, in the subsequent years (2020 and 2022), treatment A and B showed the best values for the same parameters. During the last growing season of evaluation, thesis B gained the highest weights for nuts and kernels, 2.61 ± 0.20 g and 1.57 ± 0.17 g, respectively. In conclusion, no significant differences among the treatments were detected for the parameter nut/kernel weight (% kernel) for the seasons 2019 and 2022.

Conversely, the best results for the same parameters were achieved by thesis B in 2020 with a value of $41.43 \pm 4.60\%$ and by thesis A in 2022 with a value of $38.73 \pm 3.10\%$.

Table 4.2. Nut and kernel traits of cultivar *Nocchione* subjected to different training systems (Thesis A: regular four-stemmed shrub; Thesis B: single trunk sapling form; Thesis C: multi-stemmed shrub). Data are represented as mean \pm standard deviation. Different lowercase letters on the columns indicate significant differences among pruning treatments (Fisher's test, $p < 0.01$).

Year	Thesis	Nut weight (g)	Kernel weight (g)	Shell weight (g)	% Kernel
2019	A	3.04 ± 0.47 b	1.08 ± 0.24 a	2.00 ± 0.29 b	34.89 ± 6.38 b
	B	2.96 ± 0.61 b	1.08 ± 0.18 a	2.06 ± 0.42 ab	34.80 ± 4.29 b
	C	3.31 ± 0.61 a	1.18 ± 0.23 a	2.18 ± 0.42 a	35.15 ± 5.75 b
2020	A	2.84 ± 0.74 b	1.17 ± 0.28 a	1.74 ± 0.42 c	39.53 ± 5.63 a
	B	2.84 ± 0.64 b	1.19 ± 0.28 a	1.66 ± 0.37 cd	41.43 ± 4.60 a
	C	1.64 ± 0.38 d	0.56 ± 0.19 c	1.09 ± 0.23 e	33.78 ± 6.68 b
2022	A	2.80 ± 0.41 bc	1.08 ± 0.18 a	1.71 ± 0.26 cd	38.73 ± 3.10 ab
	B	2.51 ± 0.39 c	0.91 ± 0.15 b	1.60 ± 0.26 cd	36.30 ± 2.43 b
	C	1.89 ± 0.33 d	0.68 ± 0.16 c	1.21 ± 0.20 e	35.80 ± 4.00 b
2023	A	2.53 ± 0.21 c	1.06 ± 0.15 ab	1.47 ± 0.14 d	41.70 ± 4.38 a
	B	2.61 ± 0.20 c	1.05 ± 0.14 ab	1.57 ± 0.17 d	40.13 ± 4.59 a
	C	2.46 ± 0.21 c	1.03 ± 0.14 ab	1.43 ± 0.17 d	42.05 ± 4.59 a
Average	A	2.83 ± 0.59 a	1.11 ± 0.24 a	1.75 ± 0.37 a	38.69 ± 5.78 a
	B	2.79 ± 0.57 a	1.10 ± 0.24 a	1.75 ± 0.39 a	38.88 ± 5.11 a
	C	2.33 ± 0.85 b	0.85 ± 0.33 b	1.50 ± 0.56 b	35.81 ± 6.42 b
2019	Average	3.13 ± 0.59 a	1.13 ± 0.22 a	2.09 ± 0.39 a	34.97 ± 5.55 d
2020		2.39 ± 0.82 b	0.95 ± 0.39 b	1.46 ± 0.44 b	38.31 ± 6.54 c
2022		2.40 ± 0.53 b	0.89 ± 0.23 bc	1.51 ± 0.32 b	36.94 ± 3.46 bc
2023		2.53 ± 0.14 b	1.05 ± 0.17 ab	1.49 ± 0.17 b	41.29 ± 4.57 a
Source of variation					
Year		**	**	**	**
Thesis		**	**	**	**
Year x Thesis		**	**	**	**

Table 4.3. Nut and kernel traits of cultivar *Nocchione* subjected to different training systems (Thesis A: regular four-stemmed shrub; Thesis B: single trunk sapling form; Thesis C: multi-stemmed shrub). Data are represented as mean \pm standard deviation. Different lowercase letters on the columns indicate significant differences among pruning treatments for each year of investigation (Fisher's test, $p < 0.01$).

Year	Thesis	Nut weight (g)	Kernel weight (g)	Shell weight (g)	% Kernel
2019	A	3.04 \pm 0.47 b	1.08 \pm 0.24 b	2.00 \pm 0.29 b	34.89 \pm 6.38 a
	B	2.96 \pm 0.61 b	1.08 \pm 0.18 b	2.06 \pm 0.42 ab	34.80 \pm 4.29 a
	C	3.31 \pm 0.61 a	1.18 \pm 0.23 a	2.18 \pm 0.42 a	35.15 \pm 5.75 a
2020	A	2.84 \pm 0.74 a	1.17 \pm 0.28 a	1.74 \pm 0.42 a	39.53 \pm 5.63 b
	B	2.84 \pm 0.64 a	1.19 \pm 0.28 a	1.66 \pm 0.37 a	41.43 \pm 4.60 a
	C	1.64 \pm 0.38 b	0.56 \pm 0.19 b	1.09 \pm 0.23 b	33.78 \pm 6.68 c
2022	A	2.80 \pm 0.41 a	1.08 \pm 0.18 a	1.71 \pm 0.26 a	38.73 \pm 3.10 a
	B	2.51 \pm 0.39 b	0.91 \pm 0.15 b	1.60 \pm 0.26 a	36.30 \pm 2.43 b
	C	1.89 \pm 0.33 c	0.68 \pm 0.16 c	1.21 \pm 0.20 b	35.80 \pm 4.00 b
2023	A	2.53 \pm 0.21 b	1.06 \pm 0.15 a	1.47 \pm 0.14 b	41.70 \pm 4.38 a
	B	2.61 \pm 0.20 a	1.05 \pm 0.14 a	1.57 \pm 0.17 a	40.13 \pm 4.59 a
	C	2.46 \pm 0.21 b	1.03 \pm 0.14 a	1.43 \pm 0.17 b	42.05 \pm 4.59 a

3.6. Incidence of main commercial defects

Table 4.4 summarizes the main commercial defects detected in the sampled nuts during the nut and kernel characterization, expressed as percentage values.

A notable prevalence of commercial defects incidence was observed in the year 2020, while the lowest incidence was recorded in 2022. Specifically, during 2020, treatments B and C exhibited the lowest percentages of good nuts, 68.67% and 28.00%, respectively. These low values of good nuts were mainly related to the high incidence of shriveled nuts observed in that growing season.

Thesis A consistently demonstrated greater nut quality across all investigation years, achieving its peak performance in 2022, where 96% of the entire dataset included defect-free nuts. Moreover, this treatment exhibited remarkable consistency in delivering high-quality nuts throughout the evaluation years.

The predominant commercial defects recorded included "shriveled", "poor filled" and "blanks" (empty nuts).

Table 4.4. Incidence of main defects revealed on the cultivar “Nocchione” subjected to different training systems (Thesis A: regular four-stemmed shrub; Thesis B: single trunk sapling form; Thesis C: multi-stemmed shrub). Data are represented as percentage mean values.

Year	Thesis	Shriveled (%)	Blank (%)	Mold (%)	Black tip (%)	Poor filled (%)	Split (%)	Double kernel (%)	Good (%)
2019	A	-	7.32	1.22	-	6.10	-	-	85.37
	B	-	16.84	-	1.05	1.05	-	-	81.05
	C	0.83	4.17	0.83	1.67	1.67	-	-	90.83
2020	A	5.33	6.00	-	-	8.67	-	-	80.00
	B	24.67	1.33	0.67	-	4.67	-	-	68.67
	C	43.33	2.00	1.33	-	25.33	-	-	28.00
2022	A	4.00	-	-	-	-	-	-	96.00
	B	2.00	-	-	-	2.00	-	2.00	94.00
	C	6.00	-	-	-	6.00	-	-	88.00
2023	A	1.67	-	-	-	8.33	6.67	1.67	81.67
	B	3.33	-	-	-	8.33	6.67	1.67	80.00
	C	1.67	-	-	-	10.00	3.33	-	85.00

4. DISCUSSION

The trial carried out in this five-years study aimed evaluating different training systems applied on the cultivar Nocchione, mainly focusing on single trunk sapling form, that is increasingly appreciated and adopted in the establishment of new hazelnut orchards, since these shape facilitates mechanical harvesting, allow more sunlight penetration (Germaine and Sarraquigne, 1997), and reducing shading effects that can cause a decrease in yield efficiency (Kempler et al., 1994; Hampson et al., 1996).

Assessing the amount of pruning wood can provide useful information, related to the labor required for its disposal and removal from the soil, and related to the plant biomass produced, that can be used as an energetic source (Silvestri et al., 2021b).

In this context, the study highlighted how the adoption of different training system influenced the amount of pruned wood, mainly during the first years. Thesis B (single trunk sapling) exhibited significantly higher values if compared to what observed for thesis A and C, due to the establishment of the single-trunk shape.

Over the years, pruning interventions tended to produce similar amounts of wood between treatments. This was particularly observed during the last two seasons of investigation (growing seasons 2022 and 2023).

The first-year production (kg of in-shell nuts per tree) was not affected by the pruning treatments, despite several pruning operations that involved the removal of large and different amounts of wood from the plants, depending on the treatments applied. This was probably due to plants were still characterized by a juvenile stage (unproductive phase), which has led to a uniformly reduced production across all plants, resulting in a lack of significant variation in yields. However, this condition changed extremely from the second year, in which the production between the thesis highlighted wide differences, mainly in the case of treatment B (single-trunk sapling). Specifically, thesis B showed lower production values, indicating a production potential compromised by pruning interventions when compared to the other thesis (A and C). After the 2021 season, in which yields were totally compromised by a late frost recorded between April 8th and 9th, treatments A and C showed good resilience from the adverse weather conditions that had not only compromised their productive activity but also their vegetative activity. Under this scenario, during the 2022 and 2023 seasons, thesis A and C (multi-stemmed shrub) aligned their production values, demonstrating yields almost double to what recorded on thesis B.

The present findings exhibit only partial congruence with the outcomes reported by Ellena et al. (2018). The observed divergence in productivity between the two training systems appears to be determined mainly by intrinsic genotype characteristics. Specifically, in the Chilean environment, the cultivar Tonda di Giffoni showed higher productivity in association with multi-stemmed shrub form; in contrast, the Barcelona cultivar showed completely opposite behavior, with single-trunk plants recording higher productivity (Ellena et al., 2018).

Regarding the vegetative growth, as expected, a significant influence on the various pruning treatments applied, was observed. The parameter employed to investigate the vegetative behavior was the trunk cross sectional area (TCSA), measured at a standardized height of 30 cm above the ground. Thesis B, a single-trunk system, highlighted the lowest TCSA values, indicating a diminished vegetative growth compared to the multi-stemmed shrub counterpart. Thesis C, representing the traditional multi-stemmed shrub with a number of branches higher than four, consistently displayed the greater vegetative growth throughout the investigated

period. On the contrary, thesis A exhibited a moderate degree of vegetative growth in comparison to the other two treatments. In summary, it is evident how the vegetative growth is closely associated to the number of branches, as clarified by the trend observed in this study.

Relatively to yield efficiency, treatment B, which was characterized by the lower productions during the investigation years, it showed the greater values for that parameter, indicating a more efficient use of resources for nut production. On the contrary, treatment C, with higher production values, recorded lower yield efficiency values, suggesting potential inefficiencies in resource allocation. This ratio allows assessing how efficiently a tree is converting resources into productive output, considering the trees structural development (Ebel et al., 1995; González-Altozano and Castel, 2000; Rahemi and Tavallali, 2007).

Nut and kernel traits were significantly influenced by pruning treatments, years, and their interaction. The highest kernel weight was observed in 2019, with subsequent years showing a decline. During the year 2022, treatment C, appeared to be greatly influenced by the type of training system adopted, showing the lowest values for nut and kernel weight. Notably, the regular four-stemmed shrub and single-trunk sapling forms consistently displayed higher values for nut and kernel weight, as well as shell weight, demonstrating their potential for agronomic and energy purposes. In addition, thesis A, during the survey years, also showed greater constancy in the several parameters analyzed, thus showing greater potential for the introduction of this training system in the specific soil and climatic conditions of the study area, compared to the other thesis analyzed.

In summary, regarding the prevalence of primary commercial defects and the proportion of good nuts, treatment A consistently demonstrated superior outcomes (achieving 85% of defect-free nuts), in contrast with the other thesis, where the defect incidence was generally higher.

This suggests that the observed success of treatment A in mitigating commercial defects and promoting the production of good-quality nuts is closely linked to the management practices that facilitate enhanced air circulation and adequate light exposure within the canopy. The maintenance of such conditions emerges as a critical factor in optimizing nut quality and minimizing commercial defects in nut production (Kempler et al., 1994; Hampson et al., 1996; Cristofori et al., 2015).

5. CONCLUSION

The purpose of this work was to compare the suitability of three different training systems applied on plants of the Nocchione cultivar, mainly grown in Viterbo province, that represents one of the most important hazelnut districts in Italy and in the world. In a context where it can observe an increase in the cultivated area with higher planting density, accompanied by the adoption of different training system than those typically used (multi-stemmed shrub), special attention has been paid to the single trunk sapling form. This shape also allows easier management of mechanized cultural operations, resulting perfectly in its application in hazelnut orchards characterized by higher planting densities (Germaine and Sarraquigne, 1997).

The reduced vigor of the thesis B (single-trunk sapling form) appears to be more suitable for an introduction in a high-density planting context, where the low tree vigor allows reducing distances along the row, between adjacent plants (Massenti et al., 2022). However, this treatment, highlighted almost half the production compared to plants trained as multi-stemmed shrub (regular and traditional), thus underscoring an inadequacy of the training shape at least during the first productive years.

In conclusion, given the consistency of nut traits and production parameters maintained over the survey years and generally higher values, regular four-stemmed shrub (thesis A) appeared to be the most promising system among those under investigation. In addition, this type of canopy shape (having a very open inner plant portion) increases the efficiency of phytosanitary treatments, with more environmentally friendly management.

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5 Chapter IV

Effect of Dundee rootstock on growth, morphology, and nutrient uptake of the hazelnut cultivar Tonda di Giffoni

Abstract: European hazelnut (*Corylus avellana* L.) is one of the most important tree nut species cultivated around the Mediterranean basin. It is a shrub which annually produces suckers, competing for water and nutrients and hindering mechanized management of mature hazelnut orchards. For this reason, suckers have to be annually removed, and the related operations are expensive and time-consuming. A possible solution to this issue is the use of non-suckering rootstocks such as the interspecific hybrid Dundee (*C. colurna* × *C. avellana*). The aim of this work was to evaluate morphological, physiological and reproductive traits, and the mineral leaf content of the grafted plants, to assess the effect of the clonal rootstock Dundee on the scions of the cultivar Tonda di Giffoni compared to own-rooted plants of both rootstock and cultivar. Each type of plant consisted of 40 replicates placed in 28 cm × 28 cm round perforated pots (15 liters volume), and each one placed 70 cm apart to avoid shading. Considering the leaf biometric traits, leaf size was greater on the grafted plants. Of the macro- and micronutrients in the leaves, grafted plants had higher concentrations of K, Ca, Mg, Mn and B, highlighting a possible positive effect in nutrient uptake. Dundee used as a rootstock induces a longer bud dormancy in Tonda di Giffoni compared to the same self-rooted cultivar, making the plant less susceptible to late frosts. The stomatal conductance values of grafted plants were much lower than those noted for the own-rooted Tonda di Giffoni, underlining the strong influence of the rootstock on the scion. In addition, grafted plants also showed more female flowers than the own-rooted Tonda di Giffoni plants. These preliminary results obtained with potted plants are promising, although long-term observation under field conditions is necessary to better understand the agronomic behavior and yield performance of the grafted plants.

Key words: *Corylus avellana* L.; *Corylus colurna* L.; interspecific hybrid; clonal rootstock; eco-physiological traits.

1. INTRODUCTION

The European hazelnut (*Corylus avellana* L.) is one of the most important tree nut species and is native to Europe and western Asia (Bocacci et al., 2013). However, hazelnut cultivation is rapidly expanding both in traditional environments and in new areas such as Chile (Molnar, 2011; Silvestri et al., 2021). To date, Italy, with approximately 90,000 ha (ISTAT, 2022) of hazelnut growing area, is the second largest producing country in the world after Turkey (FAOSTAT, 2022).

The natural growth of the European hazelnut plant is a multi-stemmed shrub, characterized by high emission of suckers which develop from buds located at the base of the woody stems. Despite the importance and usefulness of suckers for plant recovery (from old, dead, or diseased branches), their presence can compete with the plant for water and nutrients and can hinder mechanized management (mowing, nut harvest, etc.) of hazelnut orchards. For these reasons, the annual removal of suckers is a necessary operation. Sucker removal can be carried out manually in the summer (typical of small farms), using a brush cutter two times per growing season, or by applying authorized herbicides which normally require two-three treatments per growing season. This last sucker control strategy is characterized by environmental impact (Lagerstedt, 1975; Tous et al., 1994; Radicati et al., 1997; Cerović et al., 2009; Fideghelli and De Salvador, 2009; Rovira, 2021; Silvestri et al., 2021). Thus, in general sucker control is expensive and time-consuming, accounting for up to one fifth of the annual costs of managing the hazelnut orchard.

A possible solution to overcome these weaknesses is to use non-suckering rootstocks such as Dundee and Newberg, two *C. colurna* × *C. avellana* hybrids released in Oregon around 1990 and widely recognized as viable clonal rootstocks (Lagerstedt, 1993a; Silvestri et al., 2021). Over the period 2000-2015, field evaluation was carried out at the IRTA-Mas Bové Research Center (Tarragona, Spain), in which the agronomic and eco-physiological behavior of cultivar Negret-N9 grafted onto non-suckering rootstocks Dundee and Newberg was thoroughly assessed in comparison with self-rooted trees of the same cultivar (Rovira et al., 2022). The trial showed improvements in the agronomic traits of the Negret N-9 scion cultivar compared to self-rooted plants that included increases in plant yield and vigor, a significantly reduction in sucker emission, and an increased tolerance to iron chlorosis (Rovira et al., 2014; Tous et al., 2009; Rovira et al., 2022).

Despite these encouraging results, Dundee and Newberg rootstocks gained little interest in Oregon because they are highly susceptible to Eastern Filbert Blight, a fungal disease caused by *Anisogramma anomala* (Peck) E. Müller (Mehlenbacher, 1991; Molnar, 2011). Conversely, in the Mediterranean countries there is interest in their field use, especially for Dundee rootstock.

This study aims to evaluate the morphological and eco-physiological traits of the plants to analyze the effects of the clonal rootstock Dundee on scion of the cultivar Tonda di Giffoni, considered a medium-high suckering cultivar, in comparison to own-rooted plants of the same rootstock and cultivar. This first trial was conducted on potted plants, to be followed by further experiments in the field, in order to guide the plantation of new hazelnut orchards with plants trained to a single trunk, more suited to be used in high-density and in high mechanization level orchard management.

2. MATERIAL AND METHODS

2.1 Plant materials

The trial was conducted during the growing seasons 2021 and 2022, and the plant material used consisted of potted plants of: A) own-rooted Dundee; B) Tonda di Giffoni scions grafted on Dundee rootstocks; C) own-rooted Tonda di Giffoni (TG). Each type of plant material was represented by 40 plants, for a total of 120 plant samples. The bare-root plant material was placed in 28 cm × 28 cm perforated round pots with a capacity of 15 liters, filled with Brill® Typical Tonerde 3 soil (peat and clay 1:2, electrical conductivity 0.25 dS m⁻¹, density 160 kg m⁻², pH 5.8-6.5, porosity 90% v/v). All plants were fertilized once per year with 500 g m⁻³ NPK + micronutrients. During the late spring and summer period, all the plants were subject to watering treatment, using a drip irrigation system. Two drippers were provided for each pot.

2.2 Morphological traits

The stem diameters of the plants were determined during the two growing seasons at a standardized 30 cm above the ground and right above the grafting point using a digital caliper (IP67 Mega Try ± 0.01 mm). Simultaneously, an assessment of plant height was conducted over the same period, utilizing the same set of plants.

For leaf biometric assessments, six leaves per plant of Tonda di Giffoni, Dundee and grafted Tonda di Giffoni/Dundee, were taken in early June by sampling expanded leaves at the second, third and fourth node from the apex. The parameters measured were leaf blade length (cm), leaf blade width (cm), and leaf area (cm²). To determine leaf area, leaf blade length and width, Image J software was used (Collins, 2007).

2.3 Leaf analysis

For the macro- and micronutrients analysis, four leaves per plant were taken, during the season 2021. Leaf samples were shredded in the laboratory and dried at +37°C for 72 h. A 250-mg sample of dried material was placed in tetrafluoromethoxy tubes and microwaved. In each tube, 2 mL of 30% H₂O₂, 0.5 mL of 37% HCl and 7.5 mL of a HNO₃ solution were added to the sample. The samples were raised from an initial temperature of +25 to +180°C in 37 min. Subsequently, the temperature was kept constant (+180°C) for 15 min. After cooling, the samples were diluted to a final volume of 25 mL in Milli-Q water. Acid digestion of the samples was performed using a commercial high-pressure laboratory microwave oven (Mars plus CEM, Italy) operating at a power of 1,800 W equipped for 10 tubes of tetrafluoromethoxy. The readings of the different elements were taken at the following wavelengths: zinc (Zn = 213.857 nm); iron (Fe = 259.939 nm); phosphorus (P = 213.620 nm); boron (B = 249.677 nm); manganese (Mn = 257.610 nm); calcium (Ca = 317.933 nm); potassium (K = 766.490 nm); magnesium (Mg = 279.077 nm); copper (Cu = 324.752 nm). An inductively coupled plasma optical emission spectrometer (ICP OES) with an axial configuration (Optima 8000DV, Perkin Elmer) and ultrasonic nebulizers (U5000AT + CETAC) was used. For each element, the frequency with the lowest interference, high analytical signal and background ratio was chosen.

2.4 Plant phenology observations

Evaluation of phenological traits was carried out weekly during the two growing seasons, from budbreak until leaf senescence, using appropriate phenological codes (Taghavi et al., 2022) and with the support of field-captured images.

2.5 Leaf stomatal conductance

The measurements of stomatal conductance (gs) were carried out using a portable leaf porometer (SC-1, METER Group, Inc., Pullman, WA, USA). For each group of plant

materials (Tonda di Giffoni, Dundee and grafted trees), three plants uniform in vegetative growth were selected and stomatal conductance was measured on two mature, fully expanded, and healthy leaves per plant. All stomatal conductance measurements and data collection were carried out between 12:00 noon and 2:00 pm on June 3 and 29 and September 20, respectively.

2.6 Reproductive parameters

During the last year of investigation, an assessment of reproductive parameters was conducted on the plant material. This involved an examination of each tree, with a two-step approach: initial detection and subsequent counting of both male and female flowers. This evaluation was carried out during the entire flowering period, with observations conducted at regular intervals of seven days. Although the evaluation involved the entire plant material (own-rooted Dundee, Tonda di Giffoni/Dundee, and own-rooted Tonda di Giffoni), our primary focus was directed towards discerning differences between grafted plants (Tonda di Giffoni/Dundee) and own-rooted plants of the cultivar Tonda di Giffoni. Furthermore, the survey focused on careful observation of female flowers, as the primary objective was to assess the productive capacity of the plant material under investigation, and evaluating how the non-suckering rootstock affected this behavior.

2.7 Statistical analysis

All the data acquired during the trial were subjected to analysis of variance (ANOVA) using the statistical processing software DSAASTAT (Onofri, 2007). Fisher's test ($p < 0.05$) was carried out to identify significant differences among the treatment means.

3. RESULTS

3.1 Morphological traits

The average values of stem diameter are shown in Figure 5.1. During the season 2021, the stem diameter of Dundee was slightly larger than the other plant material. In addition, the grafted plants showed lower values in comparison to those recorded for the own-rooted Tonda di Giffoni plants (12.86 ± 1.27 mm and 13.33 ± 1.23 mm, respectively), although these differences were not significant.

From the analysis of the subsequent season (2022), a strong influence attributed to the Dundee rootstock becomes evident. In particular, the grafted plants exhibited the most significant percentage increase in the diameter parameter between the two seasons, with an increase of +15.86%, and specifically the average diameter increased from 12.86 ± 1.27 mm to 14.90 ± 1.48 mm. Furthermore, during the season 2022, a remarkable change can be observed, as grafted plants exhibited a larger diameter compared to own-rooted Tonda di Giffoni plants. This condition contrasts with the previous growing season (year 2021), where grafted plants showed the lowest diameter.

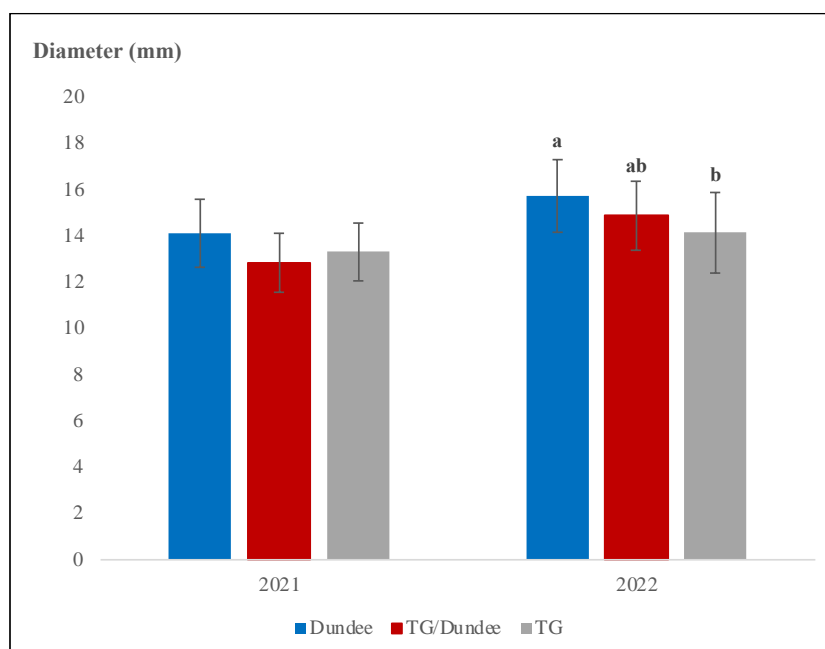


Figure 5.1. Stem diameters of the plants at the end of the seasonal growth over the two years of investigation. Bars represent mean values \pm standard deviation. Different letters on the columns define significant differences for the same growing season (Fisher's test, $p < 0.05$).

Similar behavior was recorded during the evaluation of plant height. Also in this case, the evaluation of this parameter involved two growing seasons, 2021 and 2022, as reported in Figure 5.2. In the first growing season, own-rooted Dundee plants exhibited markedly higher values (146.17 ± 18.65 cm) compared to both grafted plants and own-rooted Tonda di Giffoni counterparts (115.82 ± 10.92 and 124.61 ± 15.12 cm, respectively). Notably, grafted plants demonstrated the least vigor among the investigated plant materials during the first survey season.

Comparing the two investigated growing seasons, the most substantial increase in height was observed for the grafted plants (+51.30 %), rising from 115.82 ± 10.92 cm in 2021 to 174.94 ± 16.913 cm in 2022. In addition, during the second growing season the values associated with grafted plants were significantly higher than those recorded for own-rooted Tonda di Giffoni, underlying the strong impact exerted by the Dundee rootstock (Figure 5.3).

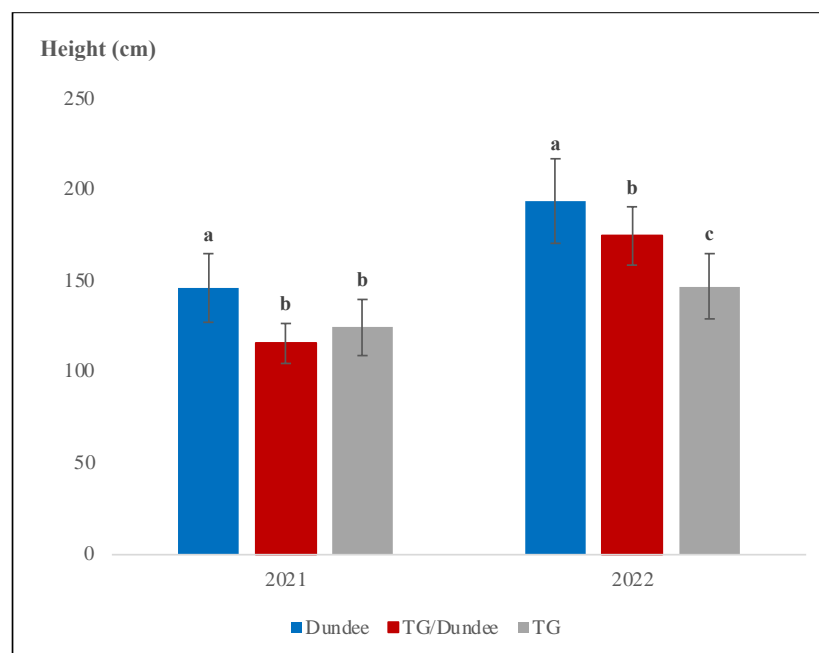


Figure 5.2. Stem diameters of the plants at the end of the seasonal growth over the two years of investigation. Bars represent mean values \pm standard deviation. Different letters on the columns define significant differences for the same growing season (Fisher's test, $p < 0.05$).



Figure 5.3. Comparison of grafted plants (Dundee rootstock) and own-rooted plants of Tonda di Giffoni during the growing season 2022.

The biometric traits of the mature leaves are shown in Table 5.1. Statistical analysis confirms the differences among treatments, with grafted plants having the highest values for leaf blade length (11.86 ± 0.58 cm) and width (11.15 ± 0.91 cm), in contrast to self-rooted Dundee and self-rooted Tonda di Giffoni, which showed the lowest mean values.

This leaf trait highlighted significant differences among the plant materials, with higher values in grafted plants, but no difference between own rooted Tonda di Giffoni and Dundee, that had similar leaf sizes. Leaf area was 40.1% and 54.3% lower in self-rooted Tonda di Giffoni and Dundee, respectively, when compared to those of grafted plants. The results show a promoting effect of the Dundee rootstock on the grafted plants, which are characterized by a greater leaf area.

Table 5.1. Biometric traits of the mature leaves (length, width, and leaf area). Data are presented as mean values \pm standard deviation. Letters define homogeneous groups ($p < 0.05$) for Fisher's test after ANOVA.

Plant material	Length (cm)	Width (cm)	Leaf Area (cm ²)
Dundee	8.07 \pm 0.68 (c)	7.21 \pm 0.53 (c)	45.76 \pm 6.15 (b)
TG/Dundee	11.86 \pm 0.58 (a)	11.15 \pm 0.91 (a)	59.98 \pm 8.68 (b)
TG	9.31 \pm 0.88 (b)	8.33 \pm 0.94 (b)	101.90 \pm 12.96 (a)

3.2 Foliar analysis of macro- and micronutrients

Macro- and micronutrient concentrations (P, K, B, Ca, Mn, Fe, Mg, Cu, Zn) determined on mature leaves showed wide heterogeneity as evidenced by high standard deviations. For this reason, attention was focused only on the nutrients with significant differences between the three plant material groups, for $p < 0.001$, which are calcium, manganese, and magnesium, respectively (Table 5.2).

A significant effect of the Dundee rootstock on Tonda di Giffoni plants was observed, highlighting higher leaf content of the selected nutrients (Ca, Mn, Mg) than those for the own-rooted Dundee and Tonda di Giffoni.

Table 5.2. Macro- and micro- leaf nutrients statistically different ($p < 0.001$) between treatments. Values are presented as mean \pm standard deviation. Letters define homogeneous groups ($p < 0.05$) for Fisher's test after ANOVA.

Nutrient	Dundee	TG/Dundee	TG
P (% d.w.)	0.34 \pm 0.02	0.37 \pm 0.04	0.35 \pm 0.02
K (% d.w.)	1.60 \pm 0.04	1.79 \pm 0.07	1.74 \pm 0.04
B (% d.w.)	0.55 \pm 0.05	0.53 \pm 0.01	0.67 \pm 0.08
Ca (% d.w.)	1.19 \pm 0.15 (ab)	1.42 \pm 0.13 (a)	0.95 \pm 0.02 (b)
Mn (ppm d.w.)	142.93 \pm 8.1 (b)	255.72 \pm 8.37 (a)	229.90 \pm 31.86 (a)
Fe (% d.w.)	0.08 \pm 0.01	0.09 \pm 0.02	0.09 \pm 0.01
Mg (% d.w.)	0.41 \pm 0.03 (b)	0.51 \pm 0.01 (a)	0.36 \pm 0.04 (b)
Cu (% d.w.)	0.31 \pm 0.05	0.34 \pm 0.07	0.31 \pm 0.01
Zn (ppm d.w.)	29.21 \pm 3.72	34.59 \pm 2.33	75.02 \pm 39.47

3.3 Plant phenology observations

Clear differences in phenological stages were recorded in mid-April on the three types of plant material (Figure 5.4). On those dates, Dundee (A) had closed buds that had not yet begun to swell (dormant/quiescent stage), while the self-rooted Tonda di Giffoni (B) showed young leaves with unfolded blades not fully developed. The grafted plants showed an intermediate phenology with almost all buds opened and their first leaves emerging in mid-April.



Figure 5.4. Plant material kept in the nursery and observed in mid-April 2021. In order from the left: Dundee (A), Tonda di Giffoni (B) and grafted trees (C).

3.4 Leaf stomatal conductance

Figure 5.5 shows the mean values of stomatal conductance during the first growing season. The self-rooted Tonda di Giffoni on all survey dates had higher values than the grafted plants and self-rooted Dundee, with maximum values recorded on June 29 and September 20 (436.13 and 422.2 mmol m⁻² s⁻¹, respectively). In addition, no significant differences were observed between grafted plants and Dundee on any date. These results show the strong influence of the rootstock on the Tonda di Giffoni scion, which has reduced stomatal activity compared to the self-rooted plants.

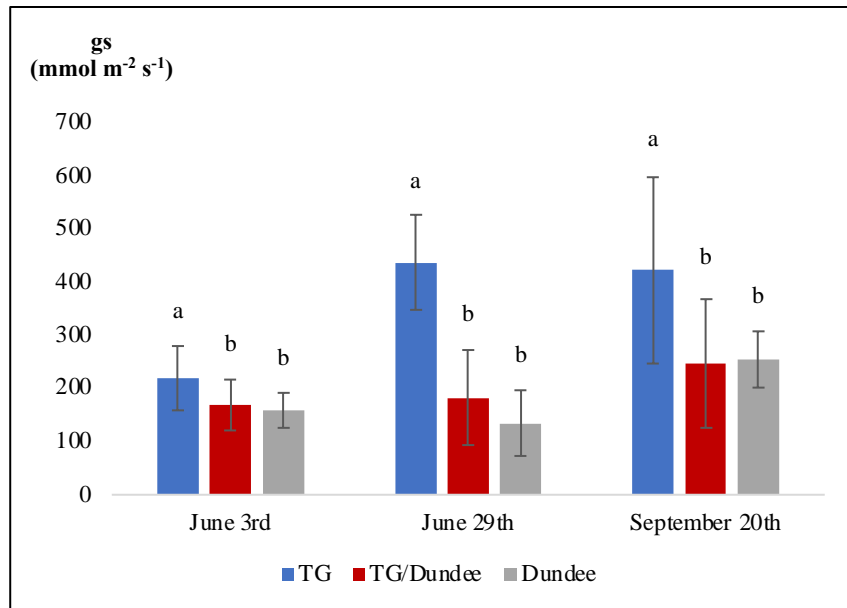


Figure 5.5. Leaf stomatal conductance of Dundee, Tonda di Giffoni and grafted trees on three different dates. Data are represented as mean values \pm standard deviation. Different letters on the columns define significant differences for the same survey date (Fisher's test, $p < 0.05$).

3.5 Reproductive parameters

Significant differences were observed in reproductive parameters, particularly when comparing grafted plants to those of the own-rooted Tonda di Giffoni. In particular, all the grafted plants exhibited the presence of both male and female flowers, in contrast to what observed on the own-rooted TG, where not all the trees displayed both types of flowers. In addition, the influence exerted by the Dundee rootstock was also found on the average number of female flowers recorded on the different plant material, as reported in Figure 5.6. In general, grafted plants showed an average number of female flowers (21.00 ± 5.07), almost three times higher than that observed in own-rooted TG plants (7.27 ± 3.74).

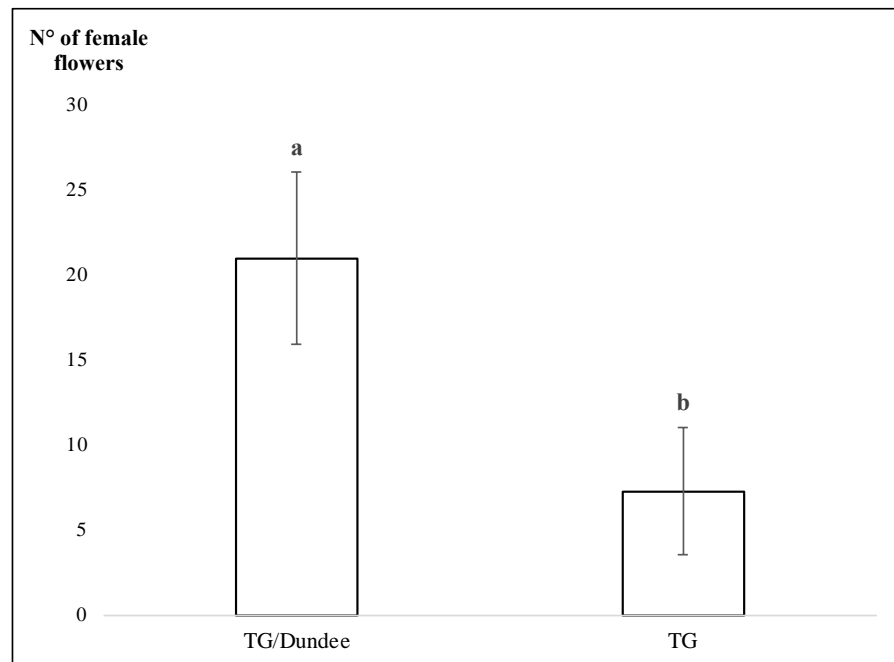


Figure 5.6. Number of female flowers observed on grafted plants (TG/Dundee) and own-rooted plants of Tonda di Giffoni. Letters define homogeneous groups ($p < 0.05$) for Fisher's test after ANOVA.

4. DISCUSSION

The rising costs associated to sucker management, particularly when manual removal is carried out (until 1/5 of the annual orchard management costs), coupled with the drawbacks linked to the persistence of suckers produced each year (Pacchiarelli et al., 2022), and a contemporary shift in hazelnut cultivation towards intensified planting layout, with the adoption of single-trunk training systems, have sparked a heightened interest in the utilization of non-suckering rootstocks in recent years. This increased attention underlines the need for more efficient and economical strategies in orchard management.

Although the plant materials under investigation were confined to pots, the outcomes obtained from this preliminary study revealed some interesting characteristics about grafted plants, especially when compared to own-rooted Tonda di Giffoni counterpart. Relatively to the vigor of the plants, the combination of Tonda di Giffoni scion/Dundee rootstock displayed a higher vegetative growth, evidenced by greater values of diameter and height of the plants, if compared to the own-rooted Tonda di Giffoni. In

addition, the rootstock appears to promote more uniform diameter development of the grafted plants, which showed greater uniformity than observed on plants of other plant material.

Despite these characteristics could determine a faster coverage of the canopy volume assigned to the plants, generally, in an intensified hazelnut cultivation, with a reduction of the distance between adjacent plants, it prefers trees characterized by a medium-low vigor, with the aim to avoid issues associated to shading effects that can cause a decrease in yield efficiency (Kempler et al., 1994; Hampson et al., 1996). However, this behavior of grafted plants, which showed high vigor, was confirmed by other study, both in young and mature trees (Lagerstedt, 1993b; Tous et al., 1997; Tous et al., 2009; Rovira et al., 2014; Rovira et al., 2022).

Concerning leaf morphology, through the analysis of some biometric traits such as leaf length, width, and area, our investigation revealed, also in this case, a clear influence exerted by the Dundee rootstock on the TG scion. Leaves from grafted plants consistently exhibited significantly larger dimensions compared to those observed on the other plant material (own-rooted Dundee and TG), which may be more efficient in light capture (Lusk et al., 2019). As expected, the measurement of leaf area (without petiole), reflected the differences found in leaf length and width, as leaf area is a closely related parameter (Cristofori et al., 2007).

During the examination of foliar macro- and micronutrients, the presence of pronounced heterogeneity, highlighted by high standard deviation values, implied focusing the attention exclusively on those nutrients that showed significant differences among the plant material for $p < 0.001$. In this context, grafted plants displayed markedly elevated levels of calcium, magnesium, and manganese, as illustrated in Table 5.2, if compared to the rest of plant material investigated (own-rooted Dundee and TG). The heightened concentrations of these micronutrients at the foliar level have been linked to increased yield and enhanced fruit quality, as reported by Cristofori et al. (2018).

The strong influence exerted by the Dundee rootstock was also observed during the phenology evaluation. Grafted plants were characterized by an intermediate phenology between own-rooted plants of Tonda di Giffoni (earlier) and own-rooted plants of Dundee (later). In general, the end of dormant period (rest phase), which align with the starting bud break, occurred in grafted plants about ten to fifteen days later than the own-rooted TG. This aspect makes grafted plants particularly interesting for their

introduction in environments characterized by late frosts, as the extended period of dormant phase of the buds, could help them to avoid or minimizing the risk of damage in relation to both vegetative and productive activities.

From a physiological point of view, an examination of stomatal conductance suggests that the known limitations related to a reduced capacity in stomatal regulation (Bignami et al., 2009) do not seem to be improved using rootstock, since for all survey dates, grafted plants consistently exhibited diminished values of stomatal conductance in comparison to those recorded for the own-rooted TG. Anyway, the reduced stomatal capacity in the leaves of grafted plants appears to contradict the report of Rovira et al. (2022), who found that the Negret N-9/Dundee combination positively affected the stomatal capacity of the grafted plant, which had higher values than self-rooted Negret N-9.

In conclusion, the consistent presence of a greater number of female flowers in grafted plants, suggests that the Dundee rootstock seems induce a shorter juvenile phase (unproductive period) in this plant material, when compared to the own-rooted TG. These findings were supported by those released by Ellena et al., (2014), which they noticed a shortened juvenile phase on plants of the cultivar Tonda di Giffoni grafted on the rootstock BA5, obtained by clonal selection of the cultivar Chilean Barcelona. This observation, if confirmed by field assessments in the next years, could represent a significant potential for growers and investors. A diminished unproductive phase would facilitate an earlier transition to plant production, consequently accelerating the economic returns on the initial investment within a shorter timeframe.

5. CONCLUSION

The search for non-suckering rootstocks for commercial hazelnut orchards is still ongoing. Several rootstock selection programs are in place to solve problems related to nut cultivation, such as high vigor, low shelling yield, sucker emission, and high susceptibility to alkaline soils (Vahdati et al., 2021).

In this preliminary study, despite grafted plants exhibited high vigor, as evidenced by increased vegetative growth and larger leaf dimensions, these traits may not immediately position them as ideal candidates for intensified hazelnut planting layout. Nevertheless, the study underlined several interesting traits of the grafted plants, promoting this investigation to be continued in the next few years after the plants have

been placed in the field, with the aim of finding out whether what was observed in this first phase is replicable in the field as well.

Leaf biometric parameters showed a high influence of the rootstock on grafted plants, highlighting for all leaf traits significant differences in comparison to those recorded for the other plant material, also showing the greater size of the leaf blades. Analysis of macro- and micronutrients showed that grafted plants had higher leaf concentrations of Ca, Mg, and Mn, highlighting a possible positive effect of the rootstock. Further, the grafted plants, compared to those of self-rooted Tonda di Giffoni, showed longer bud dormancy, making them potentially more suitable in environments characterized by high incidence of late frosts. Notably, the Dundee rootstock influences the reproductive traits, inducing a shorter juvenile phase.

The medium to long-term observation of this plant material in the field will be crucial to fully understanding the effects of Dundee rootstock on Tonda di Giffoni scions. Furthermore, it is desirable that these experiments will be extended to the other cultivars to determine the influence of rootstocks on their agronomic behavior, yield, and nut traits.

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6 CONCLUSIONS AND FUTURE PERSPECTIVES

During the last decades, hazelnut cultivation expanded rapidly, not only in traditional environments but also in new regions such as Chile, South Africa, and Australia (Molnar, 2011; Boccacci et al., 2013). This positive trend has been driven mainly in response to growing demand for hazelnuts from the confectionery industry.

Given this increasing production, hazelnut cultivation needs significant challenges related to enhancing farm profitability, improving product quality, and ensuring the environmental sustainability of the entire production process.

These considerations could align with the paradigm of sustainable intensification, to pursue the goal to increase production level, while minimizing the environmental impact associated to the processes involved. However, the term ‘intensification’ is very often viewed with a negative meaning, especially those outside the sector. The first references to the binomial sustainable development date back to the 1980s, defining it as development that is economically viable, ecologically sound, and socially acceptable (Latruffe et al., 2016).

Crop intensification occurs not only with a high plant density per unit area, but also when plants and related genotypes with high production potential are used, or when strategies are employed to optimize the production process by mitigating/reducing factors that limit production (plant protection products, fertilizers, water supply, etc.). When intensification follow the principles of sustainability, it involves the integration of knowledge and technology into the production process. This approach combines intensive and productive hazelnut cultivation with high environmental standards in agricultural practices (Buckwell et al., 2014). The result is a greater efficiency in resource use, reflecting a balanced and ecological approach to crop intensification.

In this research, we present the studies carried out in order to assess, understand and design sustainable intensification of the hazelnut orchard. The research activities were conducted in the hazelnut district of the province of Viterbo, recognized as one of the most important in Italy and in the world.

In Chapter I, a comprehensive study was conducted to assess the dynamics of carbon sequestration during the life cycle of hazelnut orchard, using permanent grasslands as a reference for previous land use. Carbon sequestration capacity was determined by considering the main carbon sources, above-ground biomass, below-ground biomass, and carbon stock in the topsoil, up to a depth of 30 cm. The findings indicated that the

establishment of a new hazelnut orchard initially led to carbon losses, mainly related to soil tillage, but after this initial phase, around 30-35 years, it became evident how the carbon content overcame that observed in the stable grassland. This observation underlines the high capacity of this species in sequestering CO₂ from the atmosphere, mitigating in part its negative effects.

In Chapter II, the possibility of using a proximal electromagnetic induction sensor to assess the functionality of a sub-irrigation system, and intercept potential anomalies in the buried drip pipes, was evaluated. This tool allows the detection of soil apparent electrical conductivity (ECa), a parameter closely related to some of the main physical characteristics of the soil and to its water content. During the study, two different interventions were carried out, one under dry soil conditions, and one following an irrigation intervention of six hours. Through direct field observation, and from the findings obtained by the sensor, the investigation concluded that there were no apparent breaks or anomalies in the sub-irrigation system. However, due to relatively modest differences in ECa, there is no certainty whether these variations could be attributed to a change in water content or alterations in textural characteristics. Thus, further investigation to improve the designed model will be necessary.

In Chapter III, an assessment on the agronomic responses of the plants of Nocchione cultivar, which has been subjected to three different plant shaping (Thesis A: symmetrical four-stemmed bush; Thesis B: sapling form; Thesis C: multi-stemmed bush) was conducted. Within the framework of sustainable intensification, with the adoption of new planting distances and new training systems, the main objective was to discern which of the investigated forms exhibited the best responses to the growing conditions. The results showed that the regular four-stemmed bush (thesis A) appeared to be the most promising system among those investigated, showing greater constancy in hazelnut biometric traits and production parameters, maintained over the years of investigation, with generally higher values.

In conclusion, in Chapter IV, a preliminary trial on potted plants was conducted, in which the influence of the non-suckering rootstock Dundee, (interspecific hybrid of *C. colurna* x *C. avellana*), on the cultivar Tonda di Giffoni was evaluated. A comparative analysis was conducted with self-rooted plants of the same cultivar and rootstock. Specifically, the evaluation included a thorough examination of morphological parameters (stem height and diameter), leaf biometric traits, eco-physiological parameters (stomatal conductance), and macro- and micronutrient content in the

leaves. In addition, observations on the phenology of plant material and reproductive parameters (detection and counting of female and male flowers) were recorded. In general, grafted plants, showed higher vigor, delayed vegetative recovery, and higher production of female flowers than Tonda di Giffoni plants. These preliminary results are promising, although long-term observations under field conditions are necessary to better understand the agronomic behavior and yield performance of the grafted plants.

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LIST OF FINALISED PUBLICATIONS

Articles in peer-reviewed journals

- Pacchiarelli, A., Priori, S., Chiti, T., Silvestri, C., Cristofori, V. (2022). Carbon sequestration of hazelnut orchards in central Italy. *Agriculture, Ecosystems and Environments*, 333, 107955. <https://doi.org/10.1016/j.agee.2022.107955>
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Articles in peer-reviewed book series

- Pacchiarelli, A., Silvestri, C., Brunori, E. and Cristofori, V. (2023). Effect of ‘Dundee’ rootstock on growth, morphology, and nutrient uptake in ‘Tonda di Giffoni’ hazelnut. *Acta Horticulturae (ISHS)*, 1379: 221-228. <https://doi.org/10.17660/ActaHortic.2023.1379.31>
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