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# CLIMATE CHANGE AND VITICULTURE IN NORTHERN ITALY: PERSPECTIVES OF ADAPTATION AND MITIGATION

Thesis written with the financial contribution of DOC Prosecco Consortium

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*Chapter IV:*

## General abstract

Climate change is leading to warmer temperatures and more erratic and intense precipitation patterns with future increases in frequency and intensity of extreme events, such as heat waves, heavy precipitations, hailstorm and drought spells. This will especially affect viticulture, because wine quality and style are highly dependent on local microclimate. In particular, climate change will impact on acidity to sugar ratio, the key quality parameter for grapes in sparkling wines production. This will have strong repercussions on the wine sector of north-eastern Italy, the production area of the renowned Prosecco sparkling wine. On the other hand, the relationship is not unidirectional as agriculture influence climate changes with green houses gasses (GHG) emissions during field management. For this reason, combined with the carbon removal by harvest, agricultural lands are considered a net source of carbon released in the atmosphere. Yet, tree crops have been shown to be good C sinks in the short and medium term. Moreover, environmentally sustainable practices under integrated or organic managements (such as reduced tillage, organic fertilization, use of green manures, reduced or no use of chemicals and improvements of natural pest management) are often proposed as a good strategy for lowering the impact of field management. But very low information is available in literature about the effectiveness of vineyard uptake on offset field emissions under environmentally sustainable management. The aims of this study followed these two research lines, to reach new information about i) actual water requirement of a representative vineyard in the DOC Prosecco, variability of heat and drought stress risk in the extensive and variable Prosecco DOC related to grape quality and productivity in the different areas with a special focus on malic acid; ii) carbon footprint reliability and nitrous oxide emissions factors (EF) for organic fertilization, with a specific long term monitoring that allowed for calculate specific emissions factors, and climate impact of sustainable viticulture, with a multiannual comprehensive GHG budget of the vineyard. The average water consumption during growing season for a representative vineyard in the DOC Prosecco was about 450 mm with significant different values in irrigated and not-irrigated years but very stable inside these periods (respectively 523±29 mm and 417±15 mm). The zoning for heat and water stress risk of DOC prosecco was able to discriminate different levels of heat and water stress in the DOC Prosecco,

highlighting different features about grape quality and productivity depending on risk levels. This will provide the possibility of planning differentiated management strategies to safeguard the productivity and quality of the grapes for the different DOC areas. The analyses of high-resolution in field monitoring of nitrous oxide emission allowed to have a better picture of nitrous oxide emission in vineyard soil and to calculate robust and specific annual EF for $N_2O$ emissions for different soil and fertilizer management. It showed that standard EF included background fluxes, while organic fertilization was responsible for about half of emissions. We presented the first multi-annual study on comprehensive C balance in vineyards that shows net negative C balance of viticultural phase in organic conduction in four years with high variability in its components even for the same site, confirming that soil management is crucial for increasing carbon uptake and soil stock in orchards. Sustainable practices were shown to be potentially effective in helping the field phase to be climate neutral or even positive in tree crops. This kind of study and results are essential to drive management of tree crops trough future climate changes, with a perspective of climate neutrality thanks to low carbon agricultural models.

# Chapter I:

# GENERAL INTRODUCTION

Viticulture is the third most worldwide valuable horticultural sector (Alston and Sambucci, 2019) with a global wine export value of 36.0 billion EUR in 2023, the second highest ever recorded, despite the lowest global wine production since 1961. This decline can be attributed to a confluence of adverse climatic events, including early frost, heavy rainfall, and drought, coupled with widespread fungal diseases (OIV, 2024). Italy, where viticulture is one of the most economically relevant sectors, has the fourth largest vineyard landscape with 10% of the world's vineyard area, and was the world's first largest producer of wine in 2022 (OIV, 2024). Although Italy was the second largest producer in 2023, the country faced historically low production levels, with a decrease of 23.2% than 2022, marking the smallest production since 1950. As reported for the global decrease, also in Italy it was caused by an unusual increase in downy mildew in center and southern regions due to heavy rains and, especially, damage from floods and hailstorms in the north of the country (OIV, 2024), extreme events that will likely become increasingly common in the future due to climate change (IPCC, 2023). Despite the worldwide decline in production and exported bottled, Italy was confirmed the first exporter in volume and the second in value, with exports for 7.8 billion, of which 36% from Veneto Region wineries (ISTAT). Indeed, despite many regions in Italy have seen declining exports due to decrease in production of still wine, the North-East experienced increasing production and sales thanks to sparkling wines (OIV, 2024). Sparkling wines were also the ones with greatest growth in 2022 driven, among all, by Prosecco wine. Indeed, north-east Italy is world famous for its Prosecco DOC wine whose production takes up an extensive area of 28,100 hectares across the Veneto and Friuli Venezia Giulia regions, encompassing 9 provinces with a wide variety of soils and meteorological conditions. Also, the heart of the origin area, the Prosecco DOCG, was recognized by UNESCO as a World Heritage site in 2019, as a cultural landscape, where the traditional work of winegrowers had decisively contributed to create an unique scenario. Indeed, the wines and landscape of the regions with long wine-growing tradition have been shaped by winemakers over the centuries by the interweaving of the most suitable varieties and the most appropriate viticultural techniques for their territory, finding an optimal balance between productivity and quality for that specific wine produced in that specific territory.

Furthermore, local climate and seasonal patterns of the region where the grapes are grown have a significant influence on wine features. The phases of the plant cycle are accurately determined by seasonal temperature fluctuations and temperature plays a major role in fruit ripening, which affects the balance and organoleptic qualities of wine. As an example, Alessandrini et al. (2017) found Glera variety, the primary grape used to produce Prosecco DOC and Prosecco DOCG, very sensitive to different altitudes and even small differences in temperatures between sites of the same locality significantly affected grape ripening and volatile concentration and composition at grape maturity, resulting in differences in the ripening process and in aromatic evolution that were perceived in the wines. In effect, wine style and quality are highly dependent on local microclimate, which is one of the elements of the terroir and would seem even more important than soil (van Leeuwen et al., 2004; van Leeuwen and Darriet, 2016). And last but not least, optimal wine production also depends on water availability, that can influence grapes productivity and quality and can limit, if available, or exacerbate, in case of water stress, negative effects of high temperatures (Belfiore et al., 2024; Leng et al., 2022).

However, that particular and unique balance in grape quality features can be destroyed by ongoing climate changes, which impact not only vine productivity but, even strongly, on grapes quality, ripening window and harvest timing (Fraga et al., 2017; Omazić et al., 2020; Van Leeuwen et al., 2019). Indeed, due to its dependance on subtle differences in microclimate, viticulture is especially sensitive to climate change (Metzger and Rounsevell, 2011). As previously mentioned, climate change is already taking place all over the world and also its effects on the productivity and quality of grapes, with significant trends in regional climates and important alterations in grapevine phenology and grape composition observed for most wine growing regions, leading to changed alcohol content and sensory profiles (Neethling et al., 2016; Sodini et al., 2023). Average global temperatures are expected to further increase in future, together with dramatic change in the frequency of extreme events, such as heat-waves, heavy rainfall and drought spells globally (IPCC, 2023) and also in Italy, where very warm climate is expected in the plains for the second part of the century (Alba et al., 2024). In effect, mediterranean wine regions are expected to suffer a particularly pronounced impact by extreme weather events, such as droughts and heat waves (Fraga et al., 2020; Sodini et al., 2023; van Leeuwen et al., 2024).

As a result, future climate changes are very likely to have further key effects on wine quality and style, which over the long term will cause geographical shifts in suitable grapevine varieties and production areas (van Leeuwen et al., 2024), with possible change of suitable growing areas from Mediterranean Europe to Northern Europe (Hannah et al., 2013). It is well known that seasonal temperature changes influence the phases of the vine cycle, due to phases speed up of the vine cycle with increasing temperatures. This can lead, for example, to early budding, which can be an issue in areas at risk of spring frosts. A climate that becomes warmer also involves earlier ripening times of the grapes, potentially leading to a shift with respect to the optimal ripening time window for that specific territory. Studies in France already reported earlier harvest between 18 and 21 days in the period from 1940 and 2000 (Ganichot, 2002) or by 2 weeks between 1972 and 2002 (Duchêne and Schneider, 2005). This trend was confirmed also in northern Italy, where Bagagiolo et al. (2021) identified a significant decreasing trend from 11.6 to 34.2 days in 58 years related to warming temperatures in the Basso Monferrato wine-growing area. Moreover, specifically for the Veneto region, Tomasi et al. (2011) detected an earlier harvest beginning date of 19 days over 1964–2009 for several varieties while Meggio (2022) found significant relationship between weather anomalies, especially if occurred in late-ripening, and shift in ripening timings. The change in ripening window and timing will affect the optimal sugar-to-acid ratios and maximum levels of pigments, aromas, and flavor of berries (Lu et al., 2024), that defines the style and quality of the wine. In particular, high acidity and low levels of sugars are pivotal quality parameters for harvested grapes in high quality wine sparkling production, of extreme interest in north-eastern Italy, as these are the main elements of wine freshness (Morata et al., 2019). Excess heat in summer leads to alterations in metabolic processes during fruit ripening, losing the appropriate fundamental sugar-to-acidity balance in grapes for high quality sparkling wine production, with a marked negative effect on the viticultural sector in Veneto region and, in general, north-eastern Italy. Understanding wine regions and their specificities in terms of local climate and grape quality is essential to prepare for the incoming changes and to develop strategies to cope with them. In this context, it is of strategic importance to outline water and heat stress risks associated with climate change in the region and compare it with the quality of grapes in the different areas recognizing eventual differences, weaknesses and peculiarities of each one. Thus, to plan future rational and specific adaptation strategies that could prevent future impacts on regional viticulture, we need now to have a better knowledge of the actual water requirements of a representative vineyard and identify areas with higher potential risk of water and heat stress in north-east Italy. This will be also useful to increase irrigation precision and limit water waste due to excess irrigation, in face of problems related to increasing water consumption and competition for it.

On the other hand, agriculture influences climate change, as every other human activity, releasing greenhouse gasses (GHG) into the atmosphere during field management. For this reason, and also because organic matter produced through $CO_2$ fixation is removed from the field with the harvest, agricultural lands are considered a net source of carbon released in the atmosphere, for about 12% of the total human emissions (IPCC, 2023). But unlike arable lands, tree crops have been shown to be a good C sinks in the short and medium term, thanks to biological, structural, and management peculiarities, such as perennial structure, abundant pruning debris, limited soil disturbance, and vegetation cover of the alleys, which could potentially lead to the sequestration of a significant amount of $CO_2$ (Gianelle et al., 2015; Tezza et al., 2019; Zanotelli et al., 2018). In effect, the range of annual C net ecosystem exchange (NEE) by vineyard was demonstrated to be quite wide and substantial (Pitacco and Meggio, 2015; Vendrame et al., 2019; Xue et al., 2024). However, although recommended in carbon footprints guidelines (BSI, 2008; OIV, 2015), net ecosystem exchange (NEE) is not considered in the wine carbon footprint (CF), mainly due to the complexity and cost for collection of reliable data. This is primarily due to challenging and highly uncertain C soil measurements (Smith et al., 2020) and no common appropriate methods and standards for C estimations.

Carbon uptake can be more effectively measured by micrometeorological techniques which, however, require high costs and qualified personnel for their application and for data processing and elaboration, limiting their application. These techniques have many advantages: they are in situ and do not disturb the environment around plant canopy; they allow for continuous measurements; time–averaged micrometeorological measurements at a point provide an area-integrated, ensemble average of the exchange rates between the surface and the atmosphere (Baldocchi et al., 1988). Nowadays, the most used technique worldwide to measure vegetation-atmosphere fluxes is the Eddy Covariance (EC) because it allows for direct measurement of energy and matter fluxes from homogeneous and flat surfaces without affecting them, as instruments are placed in free air well above the canopy. Unfortunately, only two studies have coupled CF estimates and actual C uptake measurements with EC (Chiriacò et al., 2019; Marras et al., 2015), presenting one year of data with which is not possible to evaluate the actual variability or stability of field management impact, determined by highly variable elements over time. In addition to high variability as a source of uncertainty which prevents a correct study of GHG flows in agriculture, there are some critical aspects in the calculation of CF in viticulture and, in general, in agriculture.

One pivotal point in CF calculations is nitrous oxide emissions from fertilization, that is one of the primary sources of impact in field management (Bosco et al., 2011; Marras et al., 2015; Nistor et al., 2018; Tomaz et al., 2024). Nitrous oxide presents a global warming potential of 273, but we have very general emission factors for estimation of its emission, that lack in specificity and reliability because non-$CO_2$ GHG fluxes are still little known especially for organic fertilization, despite their high impact and variability with change of management (Nistor et al., 2018). To limit the acceleration of global warming we need to reduce agricultural GHG emissions, also ensuring a lower global warming potential (GWP) impact for viticulture and tree crops in general, optimizing actions both from a product quality and C efficiency point of view. Sustainable agriculture is often proposed as an effective alternative to limit environmental impacts, also from a climate perspective. Unfortunately, there is very little information about the climate effectiveness of these practices through the assessment of the overall GHG balance of the field side and, to be able to intervene effectively on the most critical points of field management, we need to better address GHG fluxes. Thus, in this context we need more information about CF reliability and non-$CO_2$ GHG emissions under organic fertilization. Also, for a better addressing of mitigation strategies and to improve carbon efficiency of viticulture, we should understand whether the yearly vineyard uptake of $CO_2$ could effectively offset the emissions from sustainable field management, possibly turning the viticultural phase of wine production climate neutral.

Resuming, the relationship between viticulture and climate change is bidirectional: climate changes impact viticulture (on grape quality, productivity and growing areas distribution) but viticulture also affects climate changes, with emission of GHG from field management. Moreover, the challenge of agriculture within the climate change context is two-fold, both to reduce emissions and to adapt to a changing and more variable climate (Smith and Olesen, 2010). This duality in the relationship between viticulture and climate was followed in this PhD thesis, with two research lines:

i) focused on the impact of climate change on viticulture, to provide a tool to plan effective and prompt adaptation strategies in preparation of future climate challenges;
ii) focused on the impact of viticulture on climate change and possible mitigation strategies, as lever to stimulate the adoption of sustainable management to improve carbon efficiency of viticulture;

The first research line, developed in the second chapter, responds to the need for better information about water requirements of a representative vineyard of the area and the variability of water and thermal risk related to differences in grape quality in North-East Italy. Using the case study of the wide and varied production area of the Prosecco DOC, that takes up a wide part of North-East Italy, in the first part of chapter II we presented the evaluation of evapotranspiration for a representative vineyard of the area, assessed through the analysis of data derived from its continuous long-term monitoring via EC, presenting the longer time-series of EC data for vineyards (data from 2015 to 2023. Monitoring is still ongoing). Knowing the real water need of the vineyards in the area allowed, in the second part of chapter II, for the zoning of the extensive DOC Prosecco wine region with respect to water and heat stress risk. Afterwards it was evaluated whether this type of zoning could be reflected in different features through characterization of productivity and grape quality in the resulting zones, with a special focus on the levels of malic acid and its possible drivers. Furthermore, the effectiveness of irrigation in supporting acidity levels in high-risk areas was evaluated. The second research line was developed in chapters III and IV, with the first study about $N_2O$ emission in temperate tree crop with organic fertilization and the first coupling of in situ measurements of vineyard NEE and CF on a multi-year scale, respectively. Chapter III, a paper published in 2022 in Journal of Cleaner Production as joint first author and corresponding author, was also the first infield continuous and high-resolution monitoring of $N_2O$ emissions. It was performed installing in the field, for more than one year, a laboratory analyzer connected to 8 dynamic chambers performing measurements every two hours with the aim to have a better understanding on fine dynamics of $N_2O$ fluxes for different soil and organic fertilization management. This allowed to calculate specific emission factors and to evaluate the standard one used in CF calculations. Chapter IV is a manuscript recently submitted to Journal of Cleaner Production, focused on the assessment of climate impact for viticultural phase (grape production) of sparkling wine from an organic vineyard in the renowned Franciacorta DOCG. Starting from the analysis of $CO_2$ fluxes from vegetation measured by EC and the anthropogenic GHG fluxes due to vineyard management and harvest, it finally arrives at the overall C balance which will indicate if sustainable practices in the field can lead viticulture to climate neutrality or not. Fortunately, on current agricultural land, mitigation and adaptation interaction can be mutually reenforcing, particularly for improving resilience to increased climate variability under climate change (Rosenzweig and Tubiello, 2007). Indeed, agricultural soils can now be seen as a reservoir depleted from SOC due to years of intensive management, and now we have the opportunity and the duty to restore this C stock. The opportunity, because cultivated soils have a high C storage potential (Minasny et al., 2017) that can be used to fix atmospheric $CO_2$ and limit the impact of agricultural activities. The duty, because we need to maintain and improve soil healthy and fertility, to support agriculture and associated environmental services for future generations who will likely face further climate change. This type of integrated study can be very useful for planning effective climate change adaptation and mitigation strategies that move in this direction.

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# Chapter II:

# CHARACTERIZATION OF VINEYARD WATER REQUIREMENTS, HEAT AND DROUGHT RISK LEVELS AND GRAPE QUALITY IN THE DOC PROSECCO

# Abstract

Agriculture is a sector that will deeply suffer from the impacts of climate change. Viticulture, in particular, is highly dependent upon climatic conditions during the growing season and climate variations could limit wine quality and vine yield in current wine-producing regions, endangering the current regional viticulture sector. Global warming is altering the timing of ripening with greater risk of loss of freshness and aromatic complexity. This study assessed the actual water fluxes and local crop coefficients (Kc) of a vineyard representative of the region using data measured from 2015 to 2023, with the final aim of building a climate-risk zoning of the DOC Prosecco. The obtained map serves as a base for assessing distinctive quality traits in Glera grapes coming from different areas and to identify the effects of different climatic regimes. Also, two water stress tests have been performed to verify the potential of irrigation in maintaining the acidity levels in the Glera variety, in areas with high thermal and water risks. The average water requirements from April to September was 450 ± 52 mm, and average measured evapotranspiration (ET) during all the growing seasons was 2.46 ± 1.23 mm $d^{-1}$, corresponding to about 59% of the average $ET_0$. Average ET during July and August was 3.30 ± 0.46 mm $d^{-1}$, with maximum exceeding 5 mm $d^{-1}$. Standard Kc reported by FAO has been confirmed for local climate and management conditions from measurement of ET in absence of water stress, once the application periods have been adapted for the local phenological timing. The water and heat stress zoning of DOC Prosecco wine region has elucidated significant regional differences in production quality and yield. The zoning was able to characterize the large area of Prosecco DOC, highlighting two extremes as regards climatic conditions and qualitative parameters of the grapes (zone 1 and 5), a larger, more productive, intermediate-quality area (zone 2, 3 and 4) and particular features of Trieste province in comparison to the original area of belonging. A strong link has been found between the concentration of malic acid in the different sites and the rainfall fallen, both at its maximum and minimum concentration, and higher concentration at harvest was not linked to higher concentration peaks before veraison. Site-specific evaluation revealed a good homogeneity of its degradation rates compared to Huglin Index levels. Zoning and characterization approach could represent an innovative and efficient pathway toward a sustainable future in winemaking, to enhance the capabilities of winegrowers to effectively face the challenges posed by climate change.

Understanding peculiar differences in this extensive wine region is essential for maintaining the quality and consistency of Prosecco DOC wines in the face of a changing climate. Also, it is a forward-thinking strategy that aligns with the principles of sustainable agriculture, promoting efficient resource use, enhances resilience to climate change, and supports the production of high-quality wines that meet market demands.

# 1 Introduction

Global and local climate changes will present a spectrum of ecological and environmental challenges such as increased temperature, altered precipitation patterns, increased frequency of heavy precipitation events, summer heat waves and drought (IPCC, 2023). These have been studied in several scientific domains but, among others, agriculture is a sector that will suffer dramatic changes and viticulture, in particular, will be affected. It is the third most valuable horticultural sector (Alston and Sambucci, 2019) of which Italy owns 10% of the world's vineyard area and is the world's second largest producer of wine in 2023 and the first in 2022 (International Organisation of Vine and Wine (OIV), 2024). Despite a decline in production in 2023, Italy has been the first exporter in volume and the second in value, with exports for 7.8 billion of which 36% from Veneto Region wineries (ISTAT), thanks to sparkling wines driven, among all, by Prosecco wine (OIV, 2024).

Viticulture is highly dependent upon climatic conditions during the growing season. Indeed, climate is one of the elements of the terroir and would seem even more important than soil (van Leeuwen et al., 2004; van Leeuwen and Darriet, 2016). The diversity of wine production depends on subtle differences in microclimate and is therefore especially sensitive to climate change (Metzger and Rounsevell, 2011). Local climate variations could limit wine quality and vine yield in current wine-producing regions, endangering the current national and regional viticulture sector. Warmer temperatures affect not only vine productivity but, even more strongly, grape quality and harvest timing (Fraga et al., 2017; Omazić et al., 2020; Van Leeuwen et al., 2019). For example, serious impacts have been known to occur on grape quality and productivity upon air temperature exceeding a given upper threshold (e.g. 35 °C) at critical phenological stages (flowering, fruit-set and veraison) during the season (Ferrini et al., 1995; Fraga et al., 2020). These changes are already affecting grape composition with observed changes in sugar and acidity concentrations (Schultz, 2016). Over the past 40 years there was an anticipation of the harvest by 2–3 weeks due to advances in phenology caused by higher temperatures (Bagagiolo et al., 2021; Ganichot, 2002; Tomasi et al., 2011), shifting grape ripening to a warmer part of the summer and resulting in modifications in grape composition at harvest and change in wine quality and style (Meggio, 2022; van Leeuwen et al., 2024). High acidity and low levels of sugars are pivotal in harvested grapes for sparkling wines production, as these are the main elements of wine freshness (Morata et al., 2019). Indeed, titratable acidity (tA), composed of organic acids, is one parameter used to evaluate grape quality (Xu et al., 2023; Yan et al., 2022). Tartaric and malic are the two main acids found in grape berries, covering from 60% to 90% of the tA (Bigard et al., 2019; Keller and Ste, 2010), thus contributing to the pH of must and wine. Tartaric acid (TA) is the main acid in grapes and is responsible for wine biological stability, while the second main is malic acid (MA), that confers the typical “green tones” to wines (Bakker and Clarke, 2011). In particular, MA concentration is considered to be a central component of berry juice quality at harvest (Michelini et al., 2021). But the decreasing of grape acidity with high temperature - attributed to the net loss of malate due to its oxidation during respiration process of grape berries - is well-known, while sugars concentration tends to rise (Boulton, 1980; Coombe, 1987; van Leeuwen and Darriet, 2016). In this way excessive summer temperatures can lead to changes in accumulation of berry metabolites disrupting the balance between sugar and organic acids composition (Blancquaert et al., 2019; Van Leeuwen et al., 2019).

As a consequence of global warming, a change of suitable growing areas from Mediterranean Europe to Northern Europe is expected in the future and new winegrowing regions will appear in previously unsuitable areas (Hannah et al., 2013; van Leeuwen et al., 2024). In particular, extreme weather events, such as droughts and heat waves, are expected to have a pronounced impact on Mediterranean wine Regions (Fraga et al., 2020) and almost 90% of traditional wine regions in coastal and lowland areas of Spain, Italy, Greece, and southern California may become unsuitable by the end of the century (van Leeuwen et al., 2024). In these originals vine growing areas, long-term adaptation strategies could be represented by changing plant material, using drought-resistant or with different harvest timing, and the adoption of different training systems (van Leeuwen et al., 2024). Medium-term adaptation measures, less difficult to implement, could include pruning techniques, trellis height and soil management (Santillán et al., 2020), while a short-term option is represented by supplementary irrigation, when sustainable freshwater resources are available (van Leeuwen et al., 2024). Indeed, the effects of the medium scale forcing variables can be locally moderated by water availability and the enhancing of irrigation systems, among others, has been proved a reliable strategy to save the harvest by milden the temperature of bunches, maintaining quality levels under heat and water stress (Hannah et al., 2013), preserving the levels of malic acid and titratable acidity (Uriarte et al., 2016) and reducing the accumulation or concentration of sugars (Zufferey et al., 2020).

Therefore, in the context of global increasing water consumption and competition for it, the correct determination of actual evapotranspiration ($ET_a$) is a key factor for improving water-use efficiency in agriculture and establishing efficient irrigation strategies. $ET_a$ can be measured by using lysimeters, Bowen ratio method, or eddy covariance (EC) systems. Among the available techniques to measure $ET_a$, the most worldwide used technique to measure vegetation-atmosphere fluxes is the Eddy Covariance (EC). This method provides a direct measure of scalar fluxes (water vapor, carbon dioxide, heat, etc.) from and towards the surface, without affecting the studied vegetation as instruments are placed in free atmosphere above the canopy. The theoretical basis of the method is the conservation equation, that describes the time rate of scalar concentration change at a fixed point in space. The conservation equation can be simplified under ideal conditions, and the vertical turbulent flux measured at a certain height is equal to the molecular flux at the surface (Baldocchi et al., 1988). Finally, the covariance between the fluctuations of vertical wind component (w') and scalar concentration (c'), $F = \rho\, \underline{w'c'}$ (ρ is air density), allows the computation of the average net flux. Fast (at least 10Hz) and synchronous sampling of $w$ and $c$ is required to correctly sample the turbulent transport, as turbulent fluctuations happen very quickly and changes in concentrations, temperature and air density are very small. Therefore, very fast, sensitive and reliable instrumentation is required. On the other hand, this technique presents many advantages: it is in situ and do not disturb the environment around plant canopy; it allows continuous measurements; time–averaged micrometeorological measurements at a point provide an area-integrated, ensemble average of the exchange rates between the surface and the atmosphere (Baldocchi et al., 1988). The EC measurements should be combined with several additional meteorological measurements in order to process and post-process the data and to reach a whole vision of the system. Also, the method requires steady-state conditions and horizontal homogeneity of the surface to be applicable, therefore a large homogeneous and flat surface is necessary to ensure adequate fetch to the measurements.

Due to the impossibility of applying these methods extensively, in most of the commercial vineyards $ET_a$ is usually estimated empirically by multiplying the reference evapotranspiration

($ET_0$) by a single crop coefficient (Kc, obtaining the ET for the culture without water stress) or a double crop coefficients (Kcb and Ks, obtaining the estimated ET in case of water stress) as described by (Allen, 1998) and following modifications. The causes of the high variability in ET between different geographical areas are climatic conditions (expressed by $ET_0$), leaf area index (LAI, influencing Kc and Kcb) and water stress (influencing Ks), where the latter two have been shown to be the main drivers within the same climate homogeneous geographical area (Ferreira et al., 2012). For commercial purposes, ET is usually estimated through the use of the most simplistic method, i.e. the use of the Kc. Thus, the major uncertainty of this estimating approach is that local conditions, such as vineyard architecture (influencing LAI) or soil type and wetting (influencing Ks), can alter Kc values from that reported in literature (Jagtap and Jones, 1989; Annandale and Stockle, 1994; Carrasco-Benavides et al., 2012). As reported, irrigation, which can help mitigate the impacts of rising temperatures on grape quality and yield, is increasing in northern Italy viticulture. With the increasing irrigation demand and the increasing problems related to water shortage, estimating the correct actual water consumption with a correct Kc (in an area with homogeneous climate and vineyard architecture and management) is crucial to plan impact mitigation strategies in line with real needs, increasing irrigation precision in vineyards and decreasing unnecessary consumption. In addition, the precise knowledge of the water requirements will allow the identification of areas with a higher potential risk of water stress due to insufficient precipitation during the growing season in a specific wine-growing area. Knowing the grape production area and its peculiarities about local climate and grape quality is fundamental to prepare for incoming changes and to develop strategies to deal with. In this context, to prevent the impacts of the current climate change on regional viticulture with future specific and rational policies, it becomes strategic to have an overview of the risks related to climate change in the area and to connect the quality of the grapes with the dynamics of temperatures and water stress experienced during ripening in the different zones.

This study was inserted in the context of a project financed by Denomination of Controlled Origin (DOC) Prosecco Consortium, that targets the Glera variety - the primary grape used to produce Prosecco DOC status - and focuses on the extensive DOC Prosecco area, which spans 28,100 hectares across the Veneto and Friuli Venezia Giulia regions, encompassing 9 provinces with a wide variety of soils and meteorological conditions. Using the case study of the Prosecco DOC area, the final aim of this research was to evaluate the water and heat stress risk in this wide and varied wine growing area with the production of a zoning that reflects different levels of climate-related risk. This was possible thanks to the mapping of historical means of temperatures and precipitations compared to a solid understanding of the water needs of a representative vineyard in the area. The latter was obtained through the analysis of the longest EC time-series for a vineyard, that monitored the water requirements of a vineyard in the center of the Prosecco DOC from 2015 and is still ongoing. Furthermore, the fine resolution monitoring of vineyard ET allows for the calculation of local crop coefficients ($Kc_{EC}$), enabling for an evaluation of the standard Kc used for commercial estimation of water requirements. Finally, the water and heat stress risk zoning of the Prosecco DOC area has been used as reference for characterizing the productive and qualitative traits of the grapes in the resulting zones, evaluating how this type of zoning is reflected in different grapes features, with a special focus on the levels of malic acid and its drivers. Furthermore, the effectiveness of irrigation in supporting acidity levels in high-risk areas was evaluated.

In the context of climate change, a water and heat stress risk zoning could offer an innovative and efficient pathway toward a sustainable future in winemaking, to enhance the capabilities of winegrowers to effectively face the challenges posed by climate change. For an extensive and varied appellation like Prosecco DOC, which spans different altitudes, soil compositions, and microclimates, zoning is particularly important to identify areas that are more resilient to these changes and those that are more vulnerable. This approach can enable winegrowers to understand the specific strengths and weaknesses of their vineyards, facilitating more informed decisions regarding cultivation practices, grape selection, and resource management. Also, zoning can support market competitiveness by enabling winegrowers to optimize their production processes and improve wine quality (Dominici et al., 2024). By tailoring vineyard management to the specific conditions of each zone, growers can produce grapes that best express the terroir, leading to wines with distinctive and desirable characteristics.

# 2 Methods

## 2.1 Evapotranspiration monitoring

**Site description:**

In order to study the impacts of climate change on the viticulture of DOC Prosecco area, it was firstly necessary to quantify grapevine water requirements a representative vineyard of the area, during all the growing season and at field scale for several years. Among the available techniques to measure ET, the ones that best meet our project requirements of continuous infield monitoring was the EC method. The site has been selected to be well representative of the DOC Prosecco area and meet the needs for the applicability of EC. It is an extensive vineyard of about 33 ha located in Lison di Portogruaro (VE) (45°44'25.80"N 12°45'1.40"E), approximately at the center of the DOC. The vineyard is a flat site with wine rows oriented to 35-125 °N, spaced 2.2 m apart and 2 m of canopy height at full development. Inter-rows are covered by permanent grass, mowed according to the season, while the soil below the rows was mechanically weeded for a strip of about 0.5 m. The higher vines LAI values of 1.75±0.30 were usually reached around mid-June or early July, depending on the seasonal trend, and is kept constant until the end of the growing season with green pruning and trimming. The 5 m high self-supporting lattice tower was located in the southern part of the field, in order to maintain the EC footprint within the limits of the vineyard and free from a tree area within the vineyard. It was surrounded by 10 rows of cv *Sauvignon Blanc* grafted on 3309C while *Glera* cultivar grafted on 3309C was planted in all the rest of the vineyard, representing the majority of the observed area (Figure 1). Soil texture is 54% clay, 22% silt and 24% sand, with 1.53% of organic carbon, wilting point 16%, field capacity of 33.2% and saturation at 59%. Climate is characterized by warm summers and relatively cold winters (Köppen climate classification: Cfa), with average annual temperature of 13 °C and total annual average precipitation ranging between 800 and 1100 mm, distributed over the year with a winter minimum in January and a summer minimum in July (Figure 2). The average cumulated precipitation during the growing season is greater than 50% of the total annual rainfall, and the occurrence of water stress during summer was considered exceptional. The period from July to September presents high rainfall but this is mainly attributable to storm phenomena which give very irregular contributions in space and time. The vineyard was rainfed until 2020, when an underground drip irrigation system was installed. More details on site characteristics can be found in (Tezza et al., 2019; Vendrame et al., 2019).

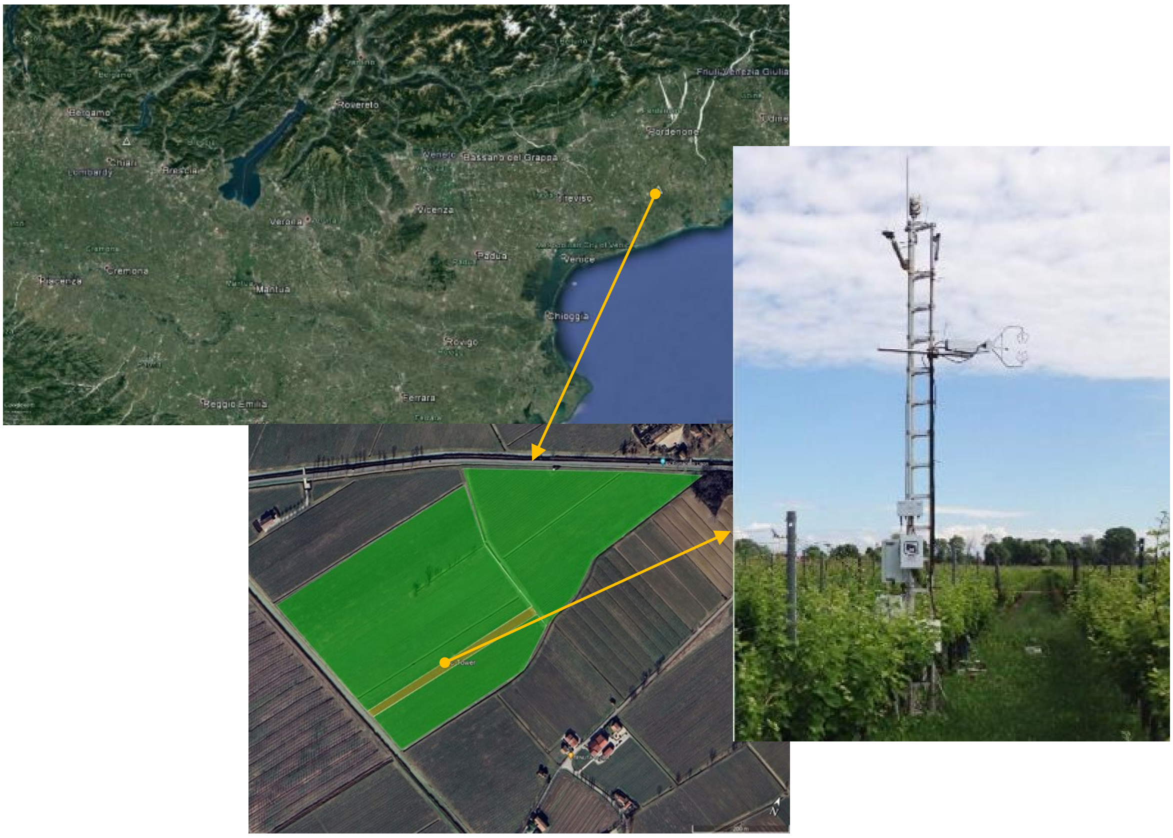

*Figure 1– Position of the site selected for ET monitoring (above) with a zoom over the vineyard (below; rows with cv. Glera are highlighted in green, while cv. Sauvignon blanc is highlighted in yellow) and a picture of the EC station (right).*

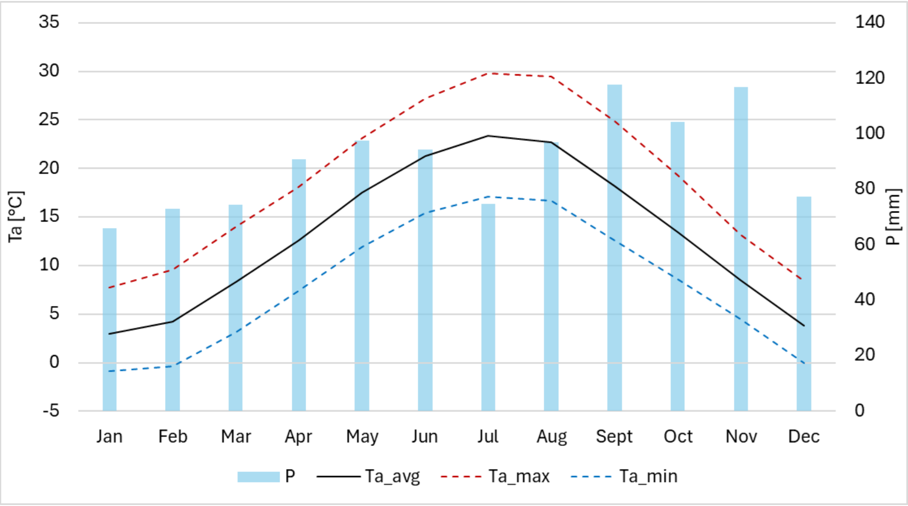

*Figure 2 - Last 20 years monthly averages of precipitations (P), mean (Ta_avg), maximum (Ta_max) and minimum (Ta_min) air temperatures at Lison di Portogruaro site;*

**Instrumentation and data processing:**

Water fluxes has been measured with a CPEC200 closed-path system (Campbell Scientific, Inc., Logan, UT, USA), which is composed of a CSAT3A sonic anemometer and EC155 closed-path IRGA (Figure 3). Sonic and IRGA measurements have been synchronously polled and collected by a CR3000 datalogger (Campbell Scientific, Inc., Logan, UT, USA) with a sampling frequency of 10 Hz. The instrumentation was placed at 4 m height, and sonic anemometer was pointed towards East, to maximize the number of periods with good data according to local wind regime. In addition, several ancillary meteorological variables have been monitored: a CNR4 net radiometer (Kipp & Zonen) was placed at 4.5 m on the top of a row, to measure short-wave and long-wave radiation; meteorological variables (air temperature, humidity and pressure, wind speed and direction, rainfall) have been collected using Vaisala WXT536 meteorological station placed at the top of the tower; soil moisture was monitored at 0.10 and 0.20 m using CSI CS616 probes (Campbell Scientific, Inc., Logan, UT, USA). All meteorological variables have been collected every 1 s and soil variables every 15 s, whereas statistics have been saved every 30 minutes.

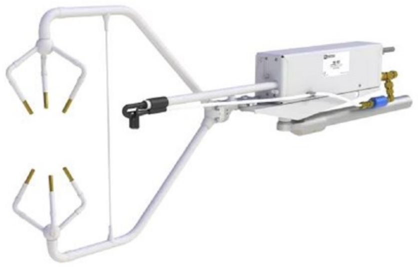

*Figure 3 – EC closed-path system used at the site (CPEC200, Campbell Scientific, Inc., Logan, UT, USA)*

The latent heat flux measured (LE) was transformed into evapotranspiration ($ET_{EC}$) dividing by latent heat of vaporization (λ). The EC raw data processing has been performed using Li-Cor EddyPro® open-source software. Standard processing and corrections for EC measurements have been applied, calculating statistics, quality parameters and fluxes over 30-min time intervals. Periods with rain, wind blowing from behind the sonic anemometer (225-315°N) and with quality flag of 2 (Mauder and Foken, 2006) were excluded. The REddyProc package (Wutzler et al., 2018) was used to perform the gap filling of missing data due to filtering, sensor calibration or sensor failure, applying the approach described in (Reichstein et al., 2005). This approach considers both the co-variation of fluxes with meteorological variables and the temporal autocorrelation of the fluxes.

Reference evapotranspiration ($ET_0$) was calculated half-hourly, and after integrated to daily step, from a modified version of Eq. 53 from FAO 56 (Allen, 1998) as reported by (Allen et al., 2006):

$$ET_0 = \frac{0.408\Delta(R_n - G) + \Upsilon \frac{C_n}{T_{hh} + 273} u_2 \, (e_s - e_a)}{\Delta + \Upsilon \, (1 + C_d u_2)} \qquad \text{Eq.1}$$

where *Rn* is the net radiation at the canopy surface (MJ $m^{-2}$ half-hour$^{-1}$), *G* is soil heat flux density (MJ $m^{-2}$ half-hour$^{-1}$), *γ* is the psychrometric constant (kPa °C-1), $T_{hh}$ is the mean half-hourly air temperature (°C), *RH* is the relative humidity and $u_2$ the half-hourly average of wind speed (m $s^{-1}$) at a height of 2m. The saturation vapour pressure at $T_{hh}$ ($e_s$, kPa), the slope of the saturation vapour pressure temperature relationship at $T_{hh}$ (Δ, kPa °$C^{-1}$), and the average half-hourly actual vapour pressure ($e_a$, kPa) were calculated based on the relative standard equations (Allen et al., 1998). *Cn* is set to 18.5 for half-hourly calculation, while *Cd* assumes a value of 0.24 when *Rn* >0 and 0.96 when *Rn* <0, to consider a different bulk surface resistance ($r_S$) for the day (50 s $m^{-1}$) and the night (200 s $m^{-1}$) as suggested by Allen et al. (2006) when the equation is applied at hourly (or lower) time step. In order to compare with FAO standard Kc ($Kc_{\_FAO}$) from Allen et al. (1998), the local single crop coefficient ($Kc_{EC}$) was calculated as the 10 days average of the ET/ET0 ratio, and after integrated at monthly time scale. The Kc is intended for periods with dry soil surfaces and substantial changes may be due to wet soil due to rain or irrigation, especially during the early growing season, when soil is more exposed. Thus, for $Kc_{EC}$ calculation, values were filtered for optimal soil water availability levels for the site (35%<SWC>55%), to avoid water stress influence and saturated soil conditions after rainfall. This thresholds were empirically obtained comparing measured ET trend and soil water content. The tabular Kc values for temperate vineyards considered for comparison were not modified as the climatic conditions were not different from those assumed as reference, while an adaptation of application timing according to local phenological conditions was applied after data evaluation (see section 3.1) as suggested by Allen et al. (1998) and emphasized by (Allen et al., 2007; Pereira et al., 2015).

## 2.2 Zoning and grape monitoring

To analyze space variability of water and temperature in the area, GIS layers relating to average precipitation and Huglin index (Huglin, 1978) obtained from the processing of weather data from the last 20 years, were used. A third layer, containing information about the available soil water content was obtained from the national soil map. Weather data were obtained from the weather stations networks of regional environmental agencies, spatialized by ABACO company and provided as vector layers, used for the analyses. Huglin Index is a specific bioclimatic heat index for vineyards developed by Huglin (1978). Among others, it was chosen because it considers the maximum daily temperatures, thus being potentially able to better track a thermal risk. It was obtained from the 20 years temperatures layer, converted according to:

$$HI = K \sum_{01.04}^{30.09} \left(\frac{T_{mean} + T_{max}}{2} - 10\right) \tag{Eq.2}$$

Where $T_{mean}$ and $T_{max}$ are respectively the daily mean and maximum temperatures and $K$ a parameter dependent on location latitude. After the zoning (section 3.2), a total of 20 vineyards were selected for the experimental design (Figure 4). In each vineyard, an experimental plot of 50 plants was identified, where identical management of agronomic practices were applied for all the resulting plots. The farmers were responsible for the nutrition, irrigation and pest management of the vines. During the winter pruning, the buds were equally regulated in order to moderate the variability and homogenize the production, thereby reducing the effects of unequal management. Green pruning was then performed in the middle of spring, and finally, the harvest was conducted on 10 vines per three replicates within the experimental plots. The monitoring on the phenological trend and grape qualitative parameters (brix, titratable acidity, tartaric acid, malic acid and pH) was conducted during the growing season until the harvest in 2022 and 2023. The analyses were performed by CREA-VIT laboratory in Conegliano (TV). Grape harvest occurred when the grapes reached technological maturity, which is defined as having a total soluble solids (TSS) content of at least 14 °Babo, approximately 17 °Brix (Benucci et al.,

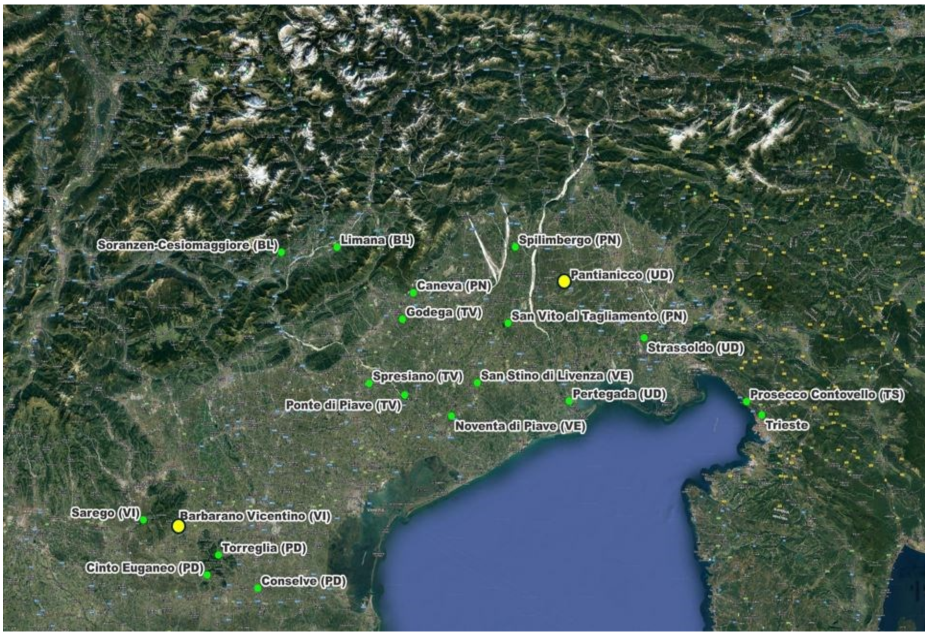

*Figure 4 - Distribution of the 20 available sites for monitoring during the projects. Yellow points remark the two sites where water stress trials were performed.*

2022). Yield per vine and average cluster weight were measured with a hanging scale (Kern CH, KERN & Sohn GmbH, Germany). Temperature and precipitations were monitored at each site, through regional agencies stations if close to the site and in homogeneous environment, otherwise with site specific meteorological stations.

### 2.3 Water stress trials

Two different water stress trials have been implemented with the aim of studying the effect of water stress on grape quality of cv. *Glera*. A first trial was settled in an irrigated vineyard (12.5 mm per week in one turn) in Barbarano Vicentino (Figure 4), province of Vicenza, with a no-irrigated plot of 50 vines. To monitor local conditions, a weather station (Vaisala WXT536) and soil humidity sensors (CSI CS610), were installed. Soil sensors have been installed both in the non-irrigated and in the irrigated (control) blocks. The test began in the early July and measurements of pre-dawn leaf water potential with daily hourly curve and mid-day stem potential (model 670, PMS Instruments, Albany, USA, Figure 5) were made in the two plots during the summers of 2022 and 2023, to monitor the water stress trend.

The second trial was set in an irrigated vineyard at Pantianicco, in the province of Udine (Figure 4). The irrigation was differentiated in three distinct adjacent plots of about 80x120 meters. Since the beginning of July, the irrigation was set with 10, 20 (standard of the company) and 30 mm per week respectively (dispended in 5 days per week), until 25 July. Since then, irrigation has been in one turn every week with around 24 mm at each plot until the end of the season, due to problems with the company's irrigation system. During August, in both trials, malic and tartaric acids concentrations and total titratable acidity were monitored weekly, until the harvest.

*Figure 5 - Scholander pressure chamber used to measure leaf and stem water potential (model 670, PMS Instruments, Albany, USA);*

# 3 Results and discussions

## 3.1 Vineyard multi-year ET monitoring

During the monitoring period, temperatures tended to be warmer than the twenty-year average, with generally higher average monthly temperatures (Figure 6). Monthly minimum temperatures had mainly positive anomalies (up to +4°C) with rarely negative anomalies and only in spring (average seasonal anomalies from +1.3 to +2.9 °C). Instead, the monthly maximums tended to be around the historical average but with spring drops up to -4.5 and summer peaks up to +3 °C (average seasonal anomalies from -0.7 to +1.4 °C). As a result, average temperatures also tend to have positive anomalies, with average seasonal anomalies ranging from +0.5 to +2.2 °C. Precipitation anomalies for the overall growing season ranked from -103.57 mm (2021) to +191.23 mm (2019), with frequent negative monthly precipitation anomalies during summer and exceptionally strong monthly positive anomalies especially in spring (Figure 6).

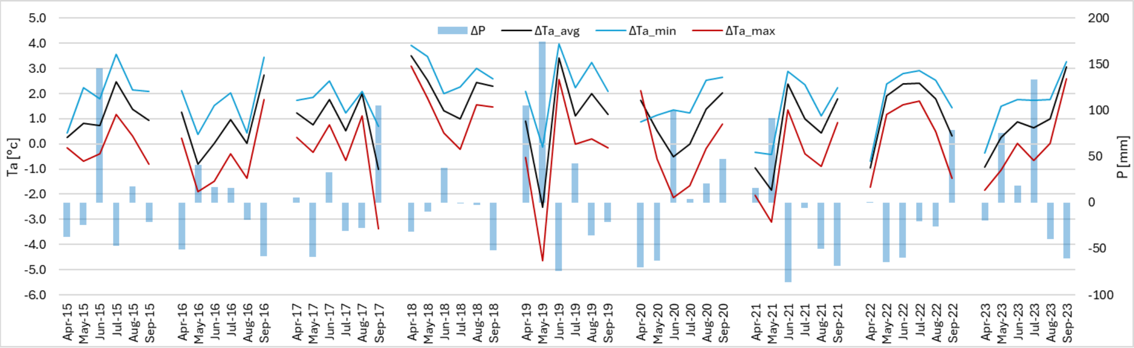

*Figure 6 - Montlhy anomalies respect the 20 years mean for precipitations (ΔP, bars), mean (ΔTa_avg, black line), minimum (ΔTa_min, blue line) and maximum (ΔTa_max, red line) air temperatures at Lison Site;*

This trend was reported also in (Sodini et al., 2023) that analyzed summer temperatures in four vineyards of northern Italy (including Lison), finding that the average summer temperature has been always above the average historical series from 2016 on, with an increase of heatwaves frequency in the last decade (the period presented in our study), where they took place almost every year. As a consequence of this temperature trend and erratic precipitation patterns in summer, the company installed an underground drip irrigation system in 2021. In this year only 50 mm of water was provided in July while in 2022, a very dry year compared to the historical average (-42% of precipitation from march to august), irrigation was used from June to the beginning of September for a total of 233 mm. In 2023, a rainy year compared to the 20-years average (+37% of precipitation from march to august), it was used to a lesser extent, with a total of 92 mm from July to September (Figure 9).

Latent heat fluxes measured with the eddy covariance technique were transformed into quantity of evaporated water, obtaining the evapotranspiration flux in mm for every half hour (Figure 7). In the figure, warmer colors indicate greater ET values than colder colors, and it is possible to appreciate the differences both for magnitude and beginning of the growing season between vintages. Intense drops in ET were noted in the summer period due to periods with scarce water availability or severe thunderstorms, including two heavy hailstorms in July 2019 and 2023 that damaged the vineyard. These flows were subsequently integrated at daily scale.

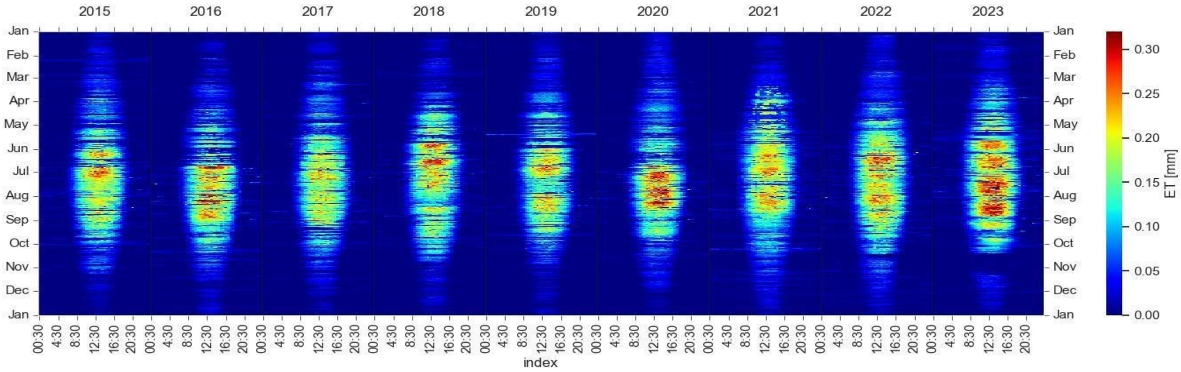

*Figure 7 - Heatmap of water fluxes at Lison site: each pixel indicates half-hourly ET measured in mm;*

Figure 8 shows the daily integrals of ET, $ET_0$ and precipitations and the course of average, minimum and maximum air temperatures together with average soil water content (SWC). The average measured ET during all the growing seasons was 2.46±1.23 mm $d^{-1}$ corresponding to about 59% of the average $ET_0$ (4.16±1.45 mm $d^{-1}$). In particular, the average daily evapotranspiration, during the months from April to August, respectively was 1.19±0.64 mm, 1.98±1.06 mm, 3.09±1.19 mm, 3.45±0.87 mm and 3.14±0.88 mm. The higher daily ET over the years has had a range between 4.6 and 5.7 mm, while maximum daily $ET_0$ ranged between 6.6 mm and 7.5 mm (Figure 8). The maximum temperatures varied between 33.3 °C in 2016 and 37 °C in 2015.

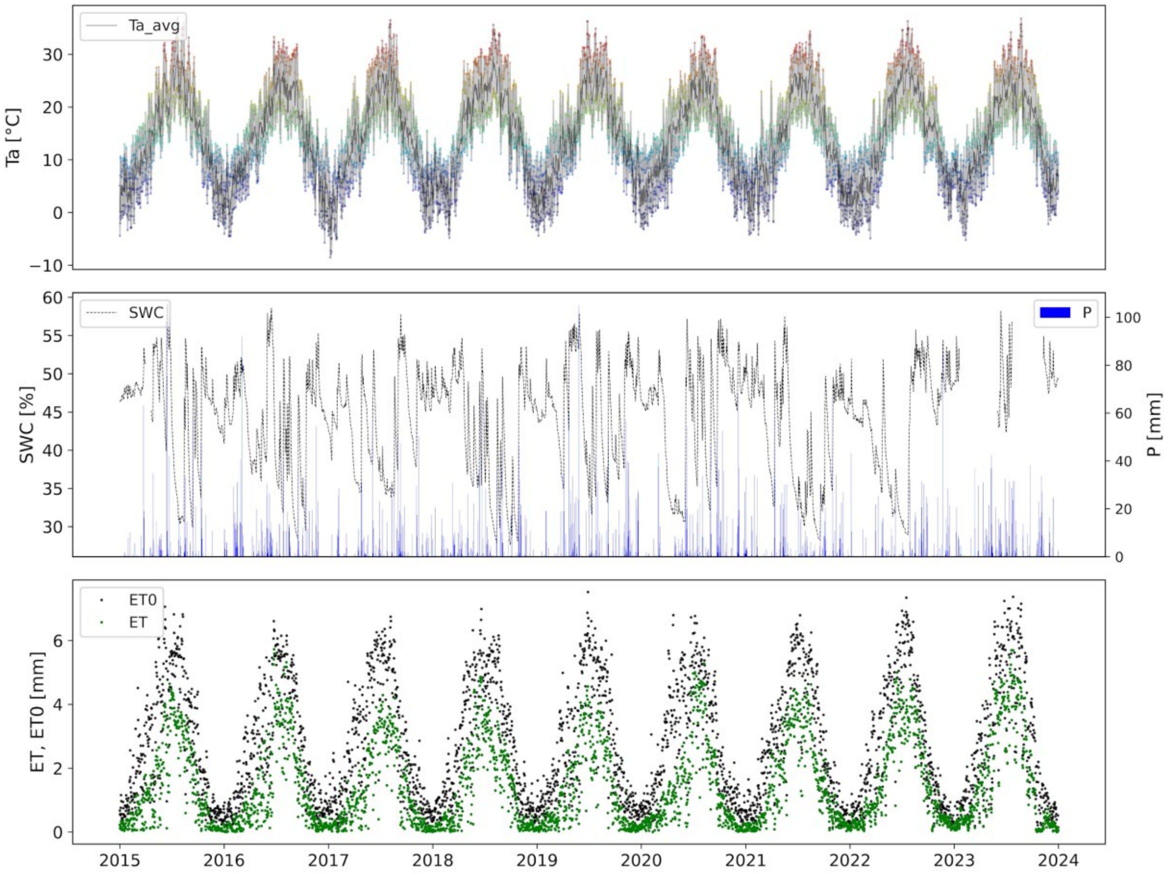

*Figure 8 – Above: Daily mean, minimum and maximum temperatures; Center: average soil water content between 10 and 20 cm (dotted line) and daily precipitation (blue bars); Below: daily measured evapotranspiration (green dots) and reference evapotranspiration (black dots);*

The average ET values reported here are consistent with other studies of water use in vineyards, although literature presents high variability in measured ET values, depending on the pedoclimatic conditions of the context and vineyard management. In irrigated vineyards of semi-arid climate conditions, where soil cover is limited or absent, (Zermeño-González et al., 2017) measured average ET of 2.96+-0.82 mm $d^{-1}$ from May to July, while other authors measured a range between about 1 and 3 mm $d^{-1}$ during July and August in not irrigated vineyard (Paço et al., 2011). In a study where transpiration was measured during the growing season (with negligible evaporation from the soil) in several rainfed or deficit irrigation vineyards in Portugal in Csa and Csb climate according to Koppen classification, (Ferreira et al., 2012) found values from 4 to less than 2 mm per day in no stressed period. In a non-irrigated vineyard in Sardinia, during 3 years of monitoring, (Marras et al., 2014) found a mean ET from

June to September of 1.8, 2.26, 2.97 and 2 mm $d^{-1}$, respectively. Under more similar conditions, in a commercial furrow-irrigated Sultana vineyard in Australia with herbaceous cover variable from 35% to 85%, (Yunusa et al., 1997) during two seasons reports average ET generally around 2.0 mm day $^{-1}$ in spring and 3.5 mm day $^{-1}$ in summer, although it could exceed 4.0 mm day$^{-1}$.

From Figure 9 it is possible to note that the maximum monthly ET before 2020 exceeded 100 mm only a few times, while after the installation of the irrigation system this value was always exceeded from June to August, also with higher intensities in spring. The monthly averages of ET and $ET_0$ and the consequent $ET/ET_0$ ratio were calculated from the original monthly integrals, distinguished for irrigated and non-irrigated years (Figure 10). Significative ($p\_value<0.05$) higher ET was found in irrigated years respect non-irrigated, while no difference in $ET_0$ was present (Figure 10left). As a consequence, a higher $ET/ET_0$ ratio was measured in irrigated years, but especially outside the months where irrigation was applied (Figure 10). This is probably due to more favorable conditions during the beginning of the growing season, which increased the development of grass cover and, therefore, its transpiration. Also, during the period of vines full development the ratio was higher than previous years, demonstrating the greater availability of water thanks to a combination of more abundant rainfall in July (+40 mm on average) and irrigation application that sustained ET in irrigated years. However, the ratio

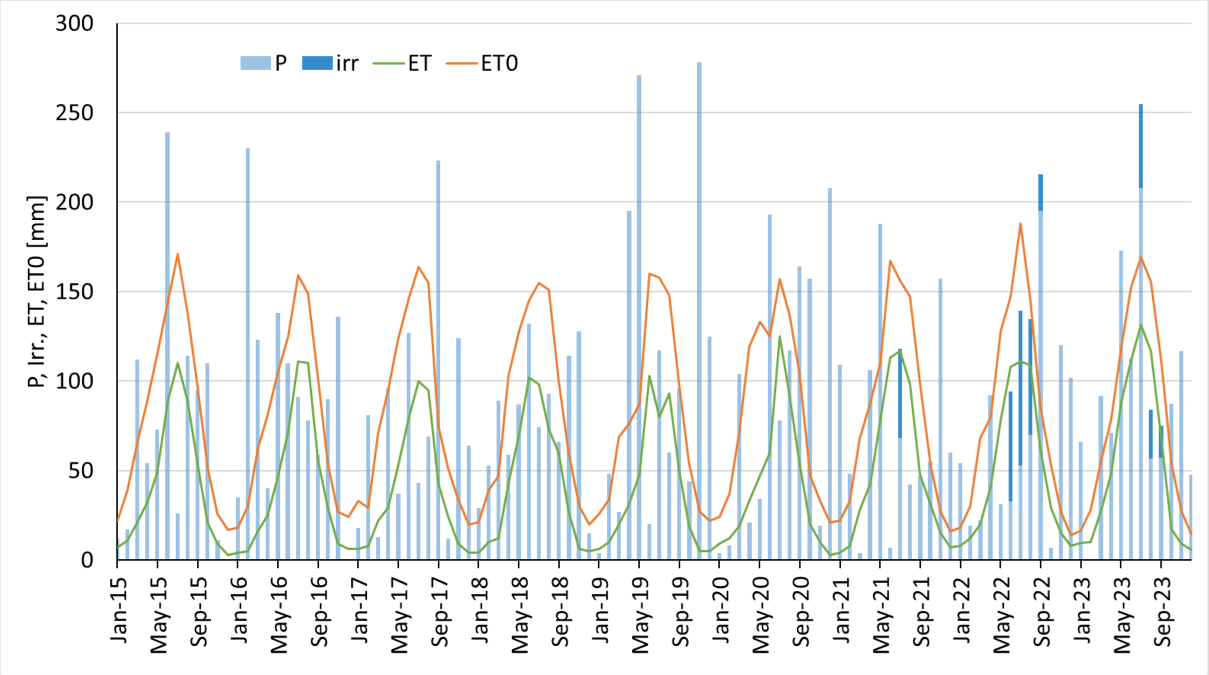

*Figure 9 – Monthly cumulated amount of rainfall (light blue) plus irrigation (dark blue), ET (green curve) and ET0 (red curve) in mm;*

highlighted a smaller difference in summer compared to the spring period. This could indicate that the difference in average evapotranspiration measured between irrigated and non-irrigated years could be mostly attributable to the spring months until June, rather than summer months. In other words, on average, the greater ET could be not only due to irrigation but, to a large extent, to more favorable conditions in spring. All in all, the water requirements for this vineyard over the total growing season (April to September) ranged between 417±15 mm and 523±29 mm in non-irrigated and irrigated years respectively (Figure 10right), showing significative difference ($p\_value<0.05$) between the two means. The founded cumulated ET over growing season was similar to that found by (López-Urrea et al., 2012) in a drip-irrigated vineyard in a semiarid continental climate, where seasonal grapevine evapotranspiration measured with lysimeter was 550 mm in 2007, 377 mm in 2008 and 505 mm in 2009, with the minimum in 2008 related to smaller a canopy caused by late frost. Also, (Teixeira et al., 2007) found an accumulated ET from pruning to harvest of 438 and 517 mm for the first and second growing in a Brazilian semiarid climate that allows 2.5 production cycles per year. In a tree year study Marras et al. (2014) showed a cumulated ET from June to September variable from 302 mm to 325 mm, slightly lower than our average of 365 mm for these 4 months. The monthly $ET/ET_0$ ratio, presented a minimum of about 0.3 and 0.4 in March-April and a maximum of about 0.65 and 0.7 in summer, for non-irrigated and irrigated years respectively. The average seasonal Kc was 0.59±0.06. From the previously cited studies, the $ET/ET_0$ ratio was very variable, due to differences in climate and vineyard conduction. For rainfed or deficit-irrigated vineyards in semi-arid contexts, several authors found a Kc variable from 0.2 to 0.8 (Ferreira et al., 2012;

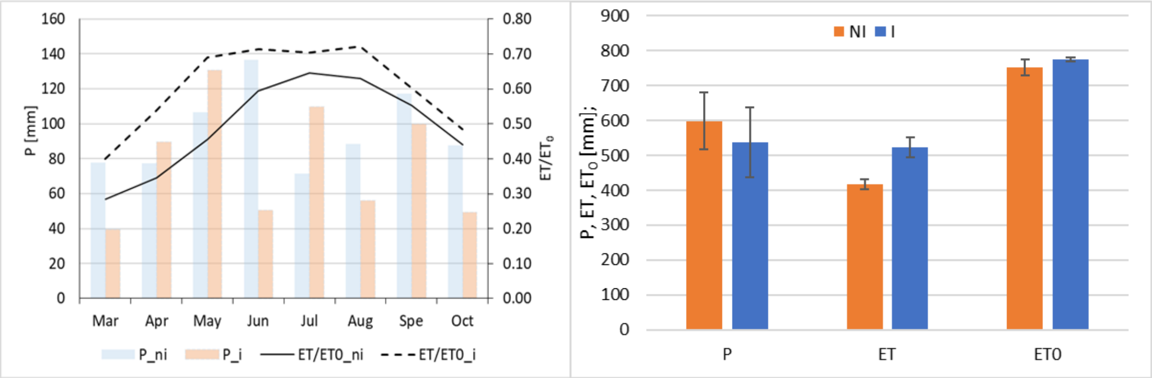

*Figure 10 – Left: Mean monthly precipitation and $ET/ET_0$ ratio calculated as averages of irrigated (P_i; $ET/ET_0$_i) and non-irrigated (P_ni; $ET/ET_0$_ni) years; Right: Cumulated precipitations (P), evapotranspiration (ET) and reference evapotranspiration (ET0) during growing season (April-September) for irrigated (I) and non-irrigated (NI) years;*

Paço et al., 2011; Semmens et al., 2016; Zermeño-González et al., 2017), but usually with higher values in the first part of the growing season. Yunusa et al. (1997) also showed a highly variable Kc during the season, between 0.30 and 0.60 with values close to 0.90 during rainy or irrigated periods, and a seasonal Kc average of 0.46 and 0.49 for two growing-cycles. More similarly to our conditions, (López-Urrea et al., 2012) presents Kc variable from 0.32 to 0.75, with higher values from veraison to harvest, and Marras et al. 2014 found an average $ET/ET_0$ from June to September of 0.63, which in our case was 0.65. The daily $ET/ET_0$ ratio was used to extrapolate actual Kc seasonal course, to be compared with the FAO standard Kc for mid latitudes (Allen et al., 1998). For the application of standard Kc, FAO has identified four development stages for crops (Figure 11left): initial stage, crop development stage, mid-season stage and late season stage. Each development stage corresponds to a different Kc to be used ($Kc_{ini}$, $Kc_{mid}$ and $Kc_{end}$) except for the crop development stage, where the Kc is the interpolation between $Kc_{ini}$ and $Kc_{mid}$, and the late season stage, where the Kc is the interpolation between $Kc_{mid}$ and $Kc_{end}$ (Figure 11, Table 1). Depending on latitude and local conditions, the application periods and the length of the standard FAO stages may vary. Thus, an adaptation of the Kc application timing, according to local conditions, was suggested by Allen et al. (1998). From the seasonal course of the average actual $ET/ET_0$ at daily scale (Figure 11right), an adjustment of stages starting and length was applied, as shown in Table 1 (FAO_adj). From here on Kc_FAO will indicate the tabular Kc with adjusted application periods.

*Table 1 - FAO* Kc *values with original length (FAO) of application and the periods and length of application adjusted to local conditions (FAOadj)*

| | | **FAO** | | **FAOadj** | |
|---|---|---|---|---|---|
| | | **Value** | **lenght** | **Period** | **lenght** |
| **Kcini** | Initial stage | 0.3 | 30 | From 01/03 to 31/03 | 31 |
| | Crop development stage | | 60 | From 01/04 to 30/06 | 90 |
| **Kcmid** | Mid-season stage | 0.7 | 40 | From 01/07 to 20/08 | 52 |
| | Late season stage | | 80 | From 21/08 to 31/10 | 72 |
| **Kcend** | End season | 0.45 | - | 01/11 | - |

Daily ET/$ET_0$ ratio presents characteristics of non-homoscedasticity, as visible in Figure 11, and high variability even in short periods of time. The points cloud, representing the daily ratios, is less dense during the summer period due to the filtering for the optimal SWC conditions, and presents high variability as several factors contribute to its determination, not only the water content of the soil, representing the real ratio conditions in the field. The ten-day average of this ratio can represent the high-resolution seasonal trend of $Kc_{EC}$, which in Figure 11 is indicated by the red line with the related interquartile range as red area. Once synchronized with the inflections of the reference curve of $Kc_{EC}$, the $Kc_{FAO}$ corresponds quite faithfully to the trend of local Kc course (Figure 11). As crop coefficient varies with cultural practices and local conditions, in the last years FAO updated the Kc value with a range depending on different conditions. For clean cultivated conditions and infrequent irrigation or precipitation as in our case, the $Kc_{FAO}$ have been updated on FAO site (https://www.fao.org/land-water/databases-and-software/crop-information/grape/en/#c236018), indicating a monthly range. The range for mature grapevines in areas with light frost, initial leaves early April, harvest late August to early September and ground cover 30-35% at mid-season is reported in Figure 12 (Kc_FAO_act),

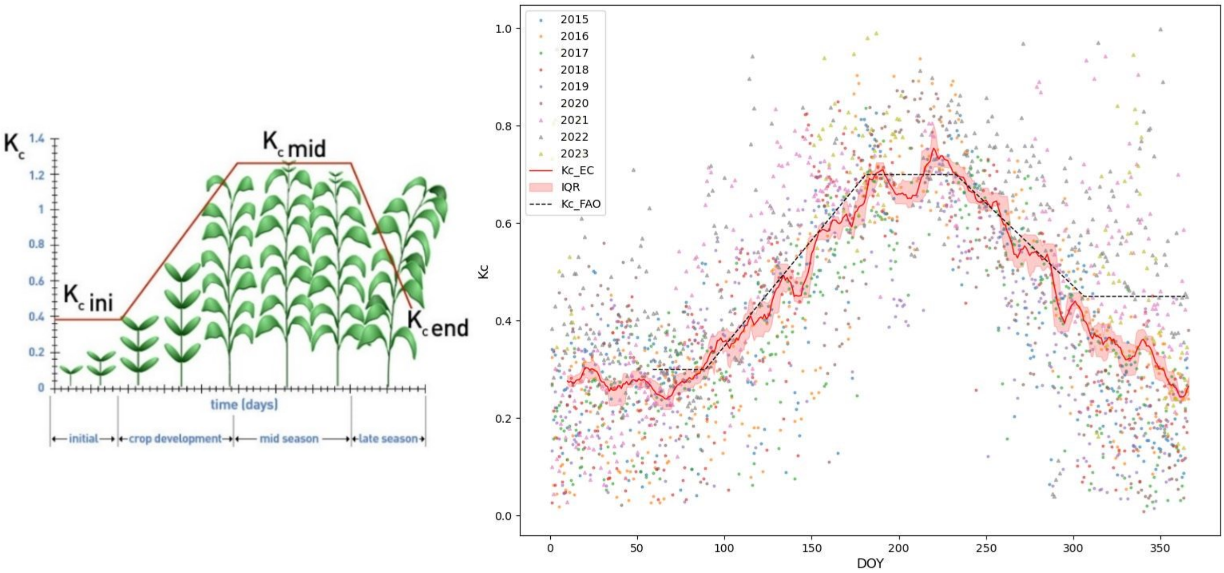

*Figure 11 – Left: Schematic representation of* Kc *distribution during the growing season of a generic crop (from Allen et al 1998); Right: The figure depicts the daily ET/$ET_0$ ratio in non-irrigated (dots) and irrigated (triangles) years and its 10 days average, that represents the average season course of actual* Kc *(red curve,* $Kc_{EC}$*) with its interquartile range (red area, IQR).The black dashed line indicates tabular* Kc *with adjusted periods of application (KC_FAO)*

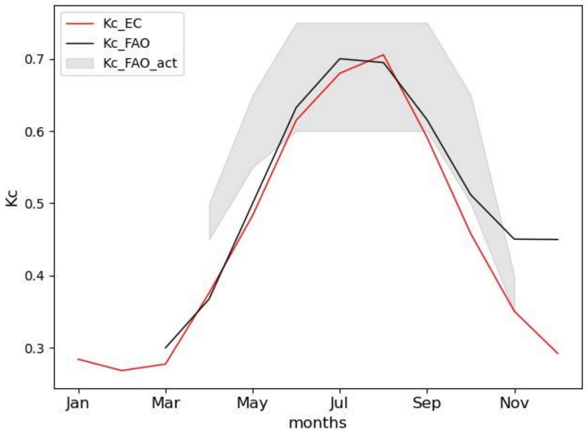

*Figure 12 – Monthly average of actual* Kc *(*Kc*_EC, red line), originals FAO* Kc *(*Kc*_FAO, black line) and updated Kc range from FAO site (Kc_FAO_act, grey area);*

together with monthly average of adjusted $Kc_{FAO}$ and $Kc_{EC}$, whose monthly courses during the season were extremely consistent ($R^2$= 0.98, p_value<0.01). The range of Kc_FAO_act roughly takes as its average the local Kc values in the months from June to August, while it remains above these in the others months. Actually, this vineyard, as the majority of vineyards in North-East Italy, present a higher ground cover than the considered by Kc_FAO_act. Indeed, the vineyard is fully green covered except for the under row, which is often kept free in this context. This brings the herbaceous cover from a minimum of 50%, in case of tillage, up to a maximum of 73% at full development (Tezza et al., 2019). Therefore, the Kc_FAO_act may be considered overestimated for a vineyard with less coverage while could represent the highest levels of the $ET/ET_0$ ratio of a vineyard in our context. Finally, the actual monthly measured $Kc_{EC}$ essentially confirms the originals FAO standard Kc for northern Italy context, once these have been synchronized with the local season timing, however remaining overestimated for different climatic and vineyard management conditions, for example semi-arid areas or with little or no soil herbaceous cover.

### 3.2 Zoning and characterization

The DOC Prosecco zoning of water and thermal risk starts with the classification of key variables essential for defining the characteristics of the territory and the criteria with which to characterize them. Seasonal rainfall (from March to September) has been classified considering the average seasonal water consumption (450±52 mm) of the representative vineyard for the area and its average (3-4 mm $d^{-1}$) and maximum (5 mm $d^{-1}$) daily consumption during summer. The classification considers that temporal distribution of precipitation during the growing season is not uniform. Therefore, the lower the precipitation, the higher the risk of long periods of drought. As a result, the risk of water stress and the need for irrigation will be increased. Seasonal precipitation was ranked in four classes from a minimum of less than 550 mm to over 900 mm. This subdivision highlighted a rainfall gradient in the Prosecco DOC area, which increases from south to north or, in other words, from the plain towards the Alps (Figure 13). Available Water Capacity (AWC) was classified according to the average daily water consumption in the summer period during sunny days, reflecting the potential number of days in which soil can provide water for plants during the warmer and drier season. Of course, the lower the AWC, the shorter the time that the soil can provide water to the plants and, as a result, the greater the risk of water stress and the need for irrigation. The AWC was ranked in four classes of 50 mm span, from a minimum of less than 80 mm to over 180 mm.

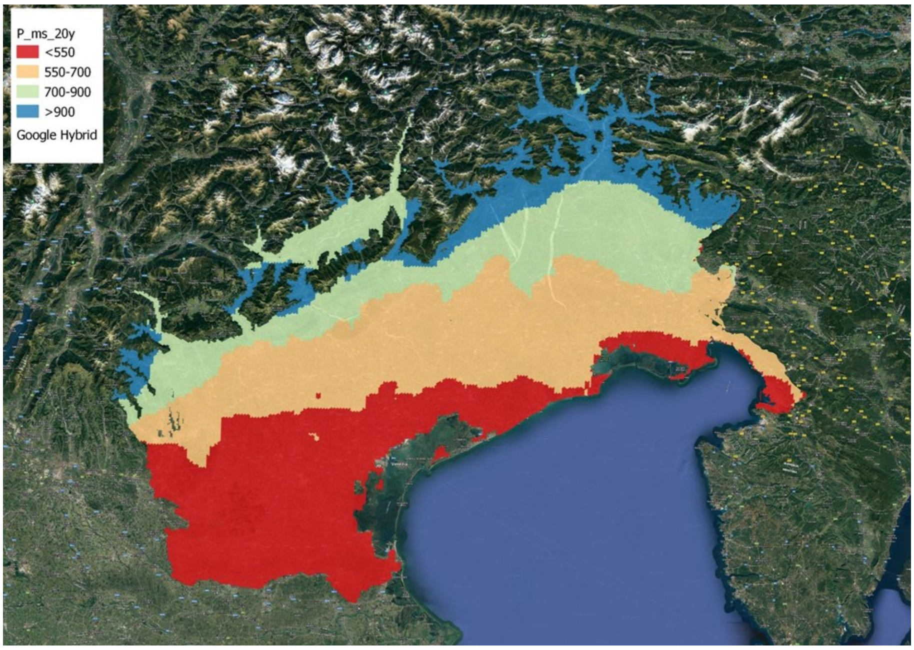

*Figure 13 - Historical seasonal (March-September) precipitation distribution in DOC Prosecco area, according to the adopted classification;*

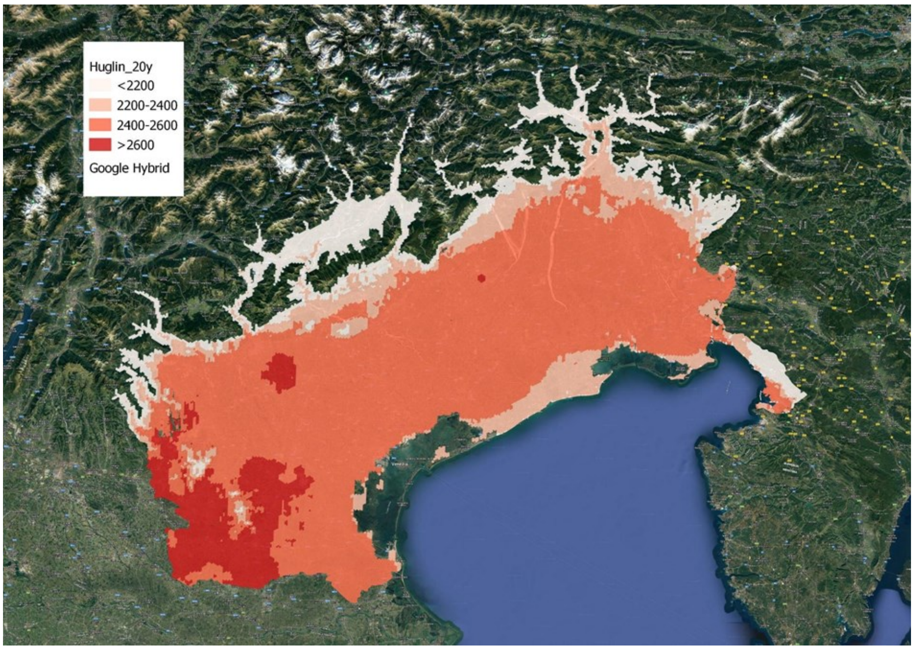

*Figure 15 - Huglin Index distribution in the DOC Prosecco Area, according to the adopted ranking, calculated from the 20 years mean and maximum temperatures;*

Figure 14 shows that the areas with the lowest AWC (indicated by lighter colors) correspond to areas of river screes or the karst province of Trieste, while those with the highest AWC correspond to areas of former peat bogs or deep soils with finer alluvial deposits. Most of the analyzed area is located in intermediate AWC zones: medium-low levels in the part of the plain closest to the mountains, and medium-high levels moving towards the sea in the lower plain. The Huglin Index (HI) has been categorized according to regular thermal levels, identifying steps of potential local thermal risk. It was ranked in four classes with a span of 200, from a minimum of less than 2200 to over 2600 (Figure 15). The Huglin Index ranking shows that the highest levels are in the innermost part of the plain (to the west) while most of the plain area is at a medium-high level of thermal risk. Areas with lower levels correspond to mountain or foothill areas, and/or with a strong influence of the sea (Figure 15). After the classification of the individual variables, these were combined to create aggregated "risk" classes. The AWC was combined with precipitation levels in a new variable (P_AWC), ranked in five classes based on drought risk and potential water reserves, which are related with precipitation and represents decreasing levels of water stress risk (Table 2 and Figure 16). In this context, vineyards located in areas with lower P and AWC classes are more susceptible to water stress due to prolonged drought periods and limited soil water reserves, potentially requiring irrigation support.

*Table 2 - Risk ranking for P_AWC, the aggregate variable indicating drought risk obtained combining P and AWC classes;*

| | | | **P [mm]** | | | |
|---|---|---|---|---|---|---|
| | | | <550 | 550-700 | 700-900 | >900 |
| | | | 1 | 2 | 3 | 4 |
| **AWC [mm]** | <80 | 1 | 1 | 1 | 2 | 3 |
| | 80-130 | 2 | 1 | 2 | 3 | 4 |
| | 130-180 | 3 | 2 | 3 | 4 | 5 |
| | >180 | 4 | 2 | 3 | 4 | 5 |

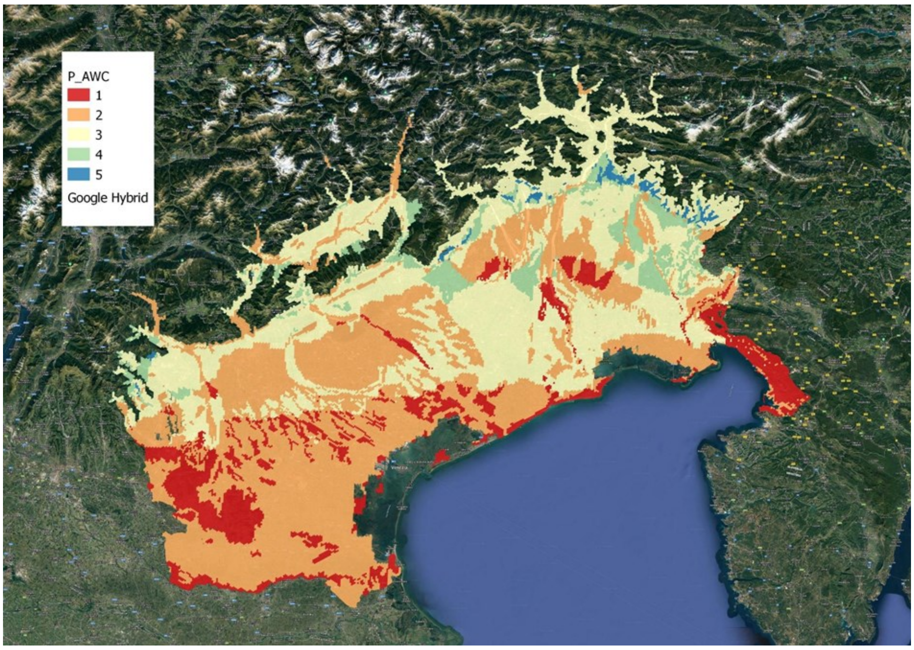

*Figure 16 - Distribution of the five risk classes of P_AWC in the DOC Prosecco area;*

The water stress risk represented by P_AWC was integrated with thermal risk by considering that Huglin Index below 2200 can still be beneficial for grape quality despite possible vines water stress, and also that in this class the probability of water stress should be minimal. Above this threshold, higher Huglin Index values increase the potential risk for both grape quality and overall plant health caused by water stress. The final zoning classification follows the ranking system reported in Table 3 and results in the pattern of areas showed in Figure 17. The map in Figure 17 shows the final results of the DOC Prosecco zoning, together with the position of the 20 vineyards selected for the grape quality monitoring. The map is clear in its delineation of the various zones, each reflecting a specific geographical feature, and identifies areas potentially requiring varying levels of irrigation support and management strategies to maintain grape quality and plant health:

*Table 3 - Final classification based on combined risk factors of water (P_AWC) and thermal (Huglin Index) risk;.*

| | | **Huglin Index** | | | |
|---|---|---|---|---|---|
| | | <2200 | 2200-2400 | 2400-2600 | >2600 |
| | | 1 | 2 | 3 | 4 |
| **P_AWC** | 1 | 2 | 1 | 1 | 1 |
| | 2 | 3 | 2 | 2 | 1 |
| | 3 | 4 | 3 | 3 | 2 |
| | 4 | 5 | 4 | 3 | 3 |
| | 5 | 5 | 5 | 4 | 3 |

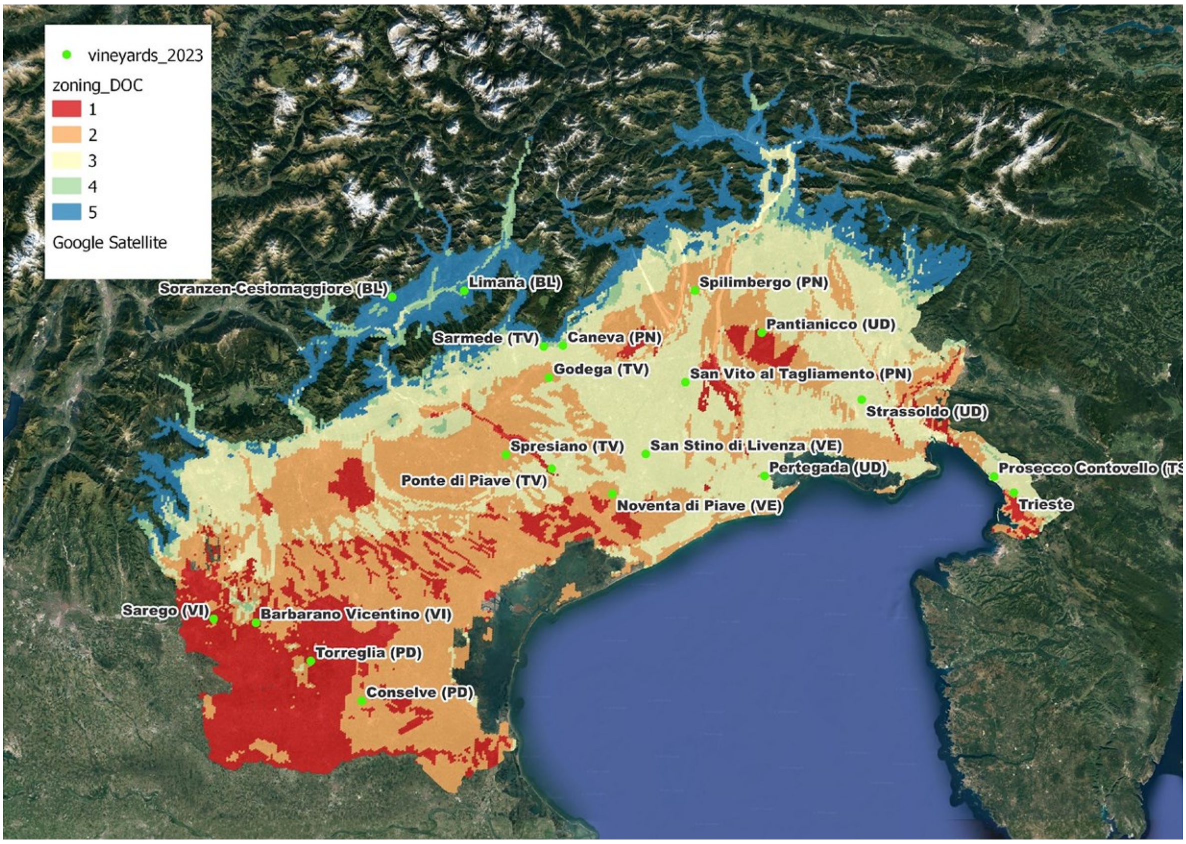

*Figure 17 – Map of the five classes of the final zoning, represented by their respective colours, indicate varying levels of water and thermal risk. The geographical zones can be categorized as follows: Zone 1 Euganean and Berici Hills, Zone 2 Gravel Plain, Zone 3 Loose Clay Soil Plain, Zone 4 Foothill Area, Zone 5: Pre-Alp;*

- Class 1: zone from medium to high Huglin Index and low P_AWC, indicating a significant need for irrigation due to both low water reserves and high temperatures. It covers about 16% of the total DOC and consists mainly of Euganean and Berici Hills;
- Class 2: zone with medium-low P_AWC and high temperatures, indicating a high risk of water stress, potentially requiring supplemental irrigation. It covers about 35% of the total DOC and consists mainly of the Gravel Plain;

- Class 3: zone with a balance of medium P_AWC and temperatures, presenting a low to moderate risk of water stress, with potential risk only during specific periods or in case of high Huglin Index. It covers about 35% of the total DOC and consists mainly of low plain with clay soils;
- Class 4: represents minimal water stress risk due to moderate to high water supply and non-elevated Huglin Index. It covers only 3% of the total area and consists in the foothill transition zone;
- Class 5: represents zones with no water stress risk, abundant water supply, and optimal Huglin Index. It covers about 11% of the total area and is situated in the northern part of the DOC and consists mainly of pre-alpine hills and valleys;

An additional zone, Zone 3T, representing the province of Trieste, has been added. This zone has been created to distinguish its geographical and anthropic characteristics, which are so distinctive to differentiate it from standard Zone 3 to preserve its unique features, discussed in the next paragraphs. Table 4 shows the sites selected for the monitoring, the zone to which they belong and some descriptive characteristics.

Glera is the primary grape variety for Prosecco production and is known for being a particularly productive variety and for exhibiting quite high vigor (Belfiore et al., 2024). The two main cordon training systems in use for its cultivation are Sylvoz and Doppio Capovolto, also found in the vineyards selected for the study. These are two traditional training systems of northern-east Italy, both used with vigorous varieties, where the fruiting shoots are pulled downwards in a vertical or arched position and attached to a wire, to encourage more vigor in the middle of the cane, as opposed to the tips. Doppio Capovolto is a traditional vine training system that can be found in Veneto and in older Tuscan vineyards. The technique involves folding a cane down on each side of the plant and tying them to a lower wire. The result is a “double arch," that almost resembles a heart. Sylvoz was named after the Italian winemaker Carlo Sylvoz from Conegliano (TV). It is a variant of the reverse training system, where long fruiting shoots with around ten eyes are cut as fruiting wood, pulled downwards in a vertical direction. Between 24 and 25 July 2023 a strong hailstorm fell in the area, destroying many vineyards. The sites names underlined in Table 4 are those that have been affected and therefore not available for monitoring in 2023.

*Table 4 - List of available sites for the characterization of grape quality in the different zoning areas, with related characteristics. Underlined sites were unavailable for analysis in 2023 due to the heavy damage caused by the hailstorm.*

| Site | Zone | Lat. (N) | Long. (E) | Year of planting | Vine Training | Spacing (m x m) | Position |
|---|---|---|---|---|---|---|---|
| Barbarano Vicentino (VI) | 1 | 45°24'28.7" | 11°33'28.4" | 2012 | Sylvoz | 2.8 x 1.2 | plains |
| Torreglia (PD) | 1 | 45°19'43.8" | 11°43'07.5" | 2013 | Doppio capovolto | 3 x 1 | hills |
| Sarego (VI) | 1 | 45°24'57.4" | 11°26'01.0" | 2008 | Sylvoz | 2.8 x 1 | hills |
| Conselve (PD) | 2 | 45°14'47.4" | 11°52'06.0" | 2010 | Sylvoz | 2.7 x 1.3 | plains |
| Noventa di Piave (VE) | 2 | 45°40'23.1" | 12°36'15.3" | 2016 | Doppio capovolto | 2.8 x 1 | plains |
| Pantianicco (UD) | 2 | 46°00'09.9" | 13°02'35.1" | 2011 | Doppio capovolto | 2.7 x 1.3 | plains |
| Spresiano (TV) | 2 | 45°45'12.4" | 12°17'31.1" | 2012 | Sylvoz | 2.8 x 1.2 | plains |
| Godega (TV) | 2 | 45°54'40.2 | 12°25'05.8" | 2010 | Doppio capovolto | 3 x 1 | plains |
| Spilimbergo (PN) | 2 | 46°05'18.5" | 12°50'48.1" | 2011 | Doppio capovolto | 2.5 X 1 | plains |
| San Stino di Livenza (VE) | 3 | 45°45'17.3" | 12°42'09.2" | 2012 | Sylvoz | 2.5 x 1.3 | plains |
| Ponte di Piave (TV) | 3 | 45°43'28.3" | 12°25'34.6" | 2009 | Sylvoz | 2.8 x 1.5 | plains |
| Pertegada (UD) | 3 | 45°42'35.5" | 13°03'04.3" | 2008 | Doppio capovolto | 3 X 1.1 | plains |
| Strassoldo (UD) | 3 | 45°51'57.2" | 13°20'11.6" | 2012 | Doppio capovolto | 2.3 X 1.2 | plains |
| San vito al Tagliamento (PN) | 3 | 45°54'05.6" | 12°49'08.2" | 2010 | Sylvoz | 3.1 x 1.3 | plains |
| Trieste | 3T | 45°40'32.0" | 13°47'00.4" | 2013 | Sylvoz | 1.8 x 0.8 | hills |
| Prosecco (TS) | 3T | 45°42'30.3" | 13°43'31.4" | 2013 | Doppio capovolto | 2.6 x 1 | plateau |
| Caneva (PN) | 4 | 45°58'34.8" | 12°27'33.2" | 2012 | Doppio capovolto | 2.5 x 1 | hills |
| Sarmede (TV) | 4 | 45°58'29.0" | 12°24'10.6" | 2010 | Sylvoz | 1.2 x 3 | hills |
| Limana (BL) | 5 | 46°04'11.2" | 12°07'32.7" | 2010 | Doppio capovolto | 3 x 1.1 | hills |
| Cesiomaggiore (BL) | 5 | 46°04'32.2" | 11°57'28.8" | 2010 | Doppio capovolto | 1 x 3 | valley |

### 3.3 Grape quality monitoring

Figure 18 shows the Huglin Index (HI) and seasonal (March to September) precipitation in the 20 monitored sites, confronted with the relative 20-years average. The sites in the plots were positioned from class 1 at left to class 5 at right, and it is possible to see a decreasing trend in HI values and an increasing, but less clear, trend in precipitations values. This indicates that the zoning correctly reflects the climatic peculiarities of the areas involved, especially with regards to the Huglin Index, while rainfall presents higher variability between sites, as expected. The two years presents opposite trends. The season 2022 has been particularly hot, with an average temperature anomaly for the northern Italy of +2.32 °C in the trimester June-August (https://www.isac.cnr.it/climstor/DPC/climate_news.html). The analysis of weather data for the vineyard involved in the projects shows that for all the sites HI was higher than the 20 years mean, with an HI anomaly range from + 4.2% to +18.4%. At the same time, precipitation during the 2022 growing season was lower than the historical mean (-38.2±11.8% on average) except for the two vineyards in the Province of Trieste (zone 3T). The meteorological data of 2023 showed an opposite trend compared to the previous year. In fact, it has been characterized by lower temperatures, and therefore lower Huglin indices, than the twenty-year average (up to -13.2%), except for the zone 3T, that has slightly higher temperature trend. Precipitation anomaly has been quite variable between sites, ranging between -28.3% up to +45.5%. Most of the cumulated precipitation values were in line with the historical means, others presented negative anomalies and, for sites at extreme East (Strassoldo and Trieste province) and extreme west (Barbarano and Sarego) of the area, there has been positive anomaly (Figure 18).

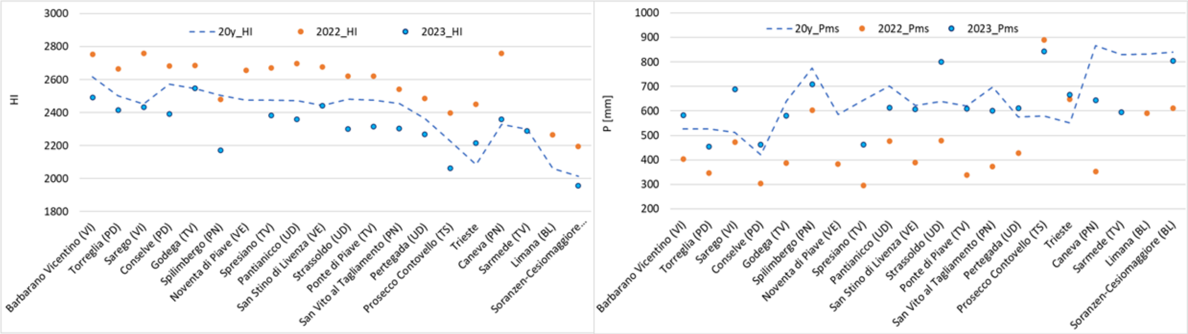

*Figure 18 - left: Huglin index (HI) for the seasons 2022 (orange circles) and 2023 (blue circles) and 20 years mean (dashed line); right: Precipitations during 2022 (orange circles) and 2023 (blue circles) seasons, compared with 20 years mean (dashed line).*

The differences in seasonal climate conditions resulted in a shift of harvest timing in the sites for the two years, in line with shift reported in literature (Meggio, 2022; Tomasi et al., 2011). In 2023 harvest was from 1-2 two weeks later than 2022, depending on sites, up to 30 days at Prosecco site (Figure 19, in 2023 there was a lower number of harvested sites due to the aforementioned hailstorm). Table 5 presents the average quantity and quality data of the two years per zone with the results of ANOVA analyses, where different letters express significant differences between the groups ($p<0.05$). Yield results highlight a notable trend: Trieste area was significantly less productive compared to other regions, except for the Pre-Alps. The two-year average yield in these areas was adversely affected by the severe impact of powdery mildew in Trieste and downy mildew in the Pre-Alps (Gessler et al., 2011; Rienth et al., 2021). In contrast, the other regions performed consistently with the planned bud load, showing significantly higher fertility in the plains compared to the hilly areas, which produced slightly less. This is consistent with common situations in hilly areas due to low fertility and better drained soils. Table 5 and Figure 20 shows the two-years averages for pH, titratable acidity and malic acid at harvest, the key quality parameters for Glera, the primary variety used to produce Prosecco DOC sparkling wine. These are particularly noteworthy across different zones. The results of the sugar content analysis (Table 5) highlight the timeliness and efficiency of the harvest over such a vast area that allowed for the identification of differences in other parameters by region.

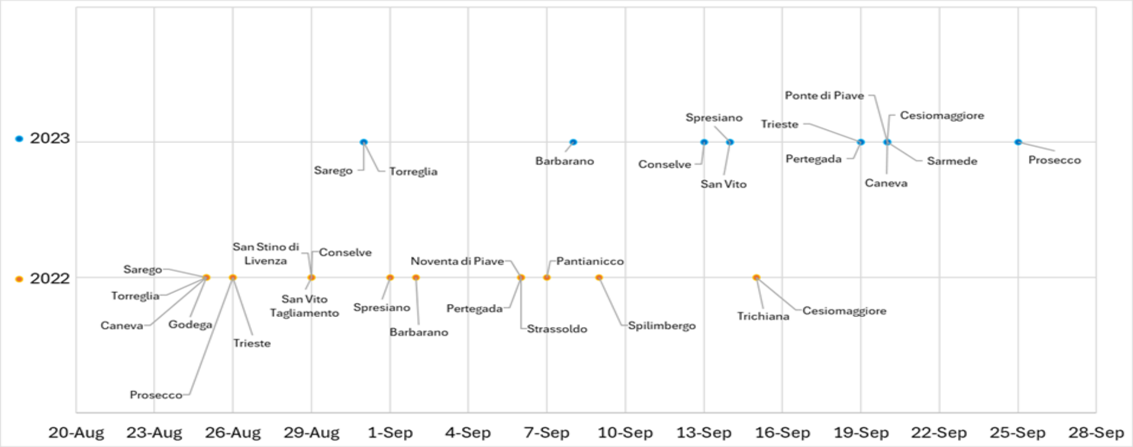

*Figure 19 – Harvest dates for the season 2022 (orange dots) and 2023 (blue dots) for the available sites;*

*Table 5 - Statistical analysis of quantitative and qualitative data by zone (two-years averages). A Tukey Post Hoc was conducted following Analysis of Variance with n=5 (p<0.05), and different uppercase letters express significant differences between groups. ABW stands for average bunch weight. Actual fertility is the quantity of clusters produced per bud remained after winter pruning;*

| | | **Zone** | | | | | |
|---|---|---|---|---|---|---|---|
| | | **1** | **2** | **3** | **3 T** | **4** | **5** |
| | | **Euganean and Berici Hills** | **Gravel Plain** | **Clay Plain** | **Trieste** | **Foothill** | **Pre-Alps** |
| **Buds** | **(n°)** | 24 | 24 | 25 | 21 | 24 | 24 |
| **Bunches** | **(n°)** | 26 $^{b}$ | 29 $^{ab}$ | 35 $^{a}$ | 16 $^{cd}$ | 25 $^{bc}$ | 12 $^{d}$ |
| **Yield per vine** | **(kg)** | 6.1 $^{bc}$ | 9.1 $^{a}$ | 10.6 $^{a}$ | 3.4 $^{cd}$ | 6.0 $^{c}$ | 1.7 $^{d}$ |
| **ABW** | **(kg)** | 0.23 $^{b}$ | 0.33 $^{a}$ | 0.31 $^{a}$ | 0.20 $^{b}$ | 0.24 $^{b}$ | 0.15 $^{b}$ |
| **Actual fertility** | **-** | 1.1 $^{ab}$ | 1.2 $^{a}$ | 1.4 $^{a}$ | 0.8 $^{bc}$ | 1.0 $^{ab}$ | 0.5 $^{c}$ |
| **Sugar** | **(°Babo)** | 15.3 | 14.6 | 14.4 | 15.2 | 14.4 | 14.3 |
| **Titratable Acidity** | **(g/L)** | 5.4 $^{c}$ | 6.0 $^{bc}$ | 6.3 $^{bc}$ | 7.0 $^{b}$ | 6.0 $^{bc}$ | 10.6 $^{a}$ |
| **pH** | | 3.26 $^{ab}$ | 3.28 $^{ab}$ | 3.30 $^{a}$ | 3.18 $^{b}$ | 3.30 $^{ab}$ | 3.00 $^{c}$ |
| **Tartaric Acid** | **(g/L)** | 5.45 $^{ab}$ | 5.40 $^{ab}$ | 5.32 $^{b}$ | 6.30 $^{a}$ | 5.65 $^{ab}$ | 5.96 $^{ab}$ |
| **Malic Acid** | **(g/L)** | 1.46 $^{c}$ | 2.47 $^{b}$ | 2.82 $^{b}$ | 2.52 $^{b}$ | 2.42 $^{b}$ | 6.02 $^{a}$ |
| **Yeast Assimilable Nitrogen** | **(mg/L)** | 97.2 $^{b}$ | 109.2 $^{b}$ | 90.0 $^{b}$ | 100.7 $^{b}$ | 72.3 $^{b}$ | 188.7 $^{a}$ |

The highest average value for tartaric acid (TA) content was found in the Trieste area (Table 5), compared to the clay plain, which is its sub-region, underscoring the unique characteristics of the Karst area (Bastianich and Lynch, 2012). Yeast assimilable nitrogen (YAN) is a crucial parameter for natural fermentation during the vinification process. It is closely related to the nutritional status of the vineyard. In this study, the selected vineyards exhibited similar YAN levels, with the notable exception of the Pre-Alps region. Specifically, only the Pre-Alps region reached a level considered optimal for the vinification process (Butzke, 1998). Generally, YAN levels are significantly influenced by nitrogen fertilization. It can be hypothesized that the Pre-Alps region was less prone to nitrogen loss in its various forms, thereby maintaining higher YAN levels. The box plot (Figure 20 central) shows the two years average of pH, titratable acidity (tA) and malic acid (MA). Titratable acidity level in grape musts tends to increase from warmer and low-rainfall, water-stress-prone areas to cooler, wetter, and rainier areas. This trend is

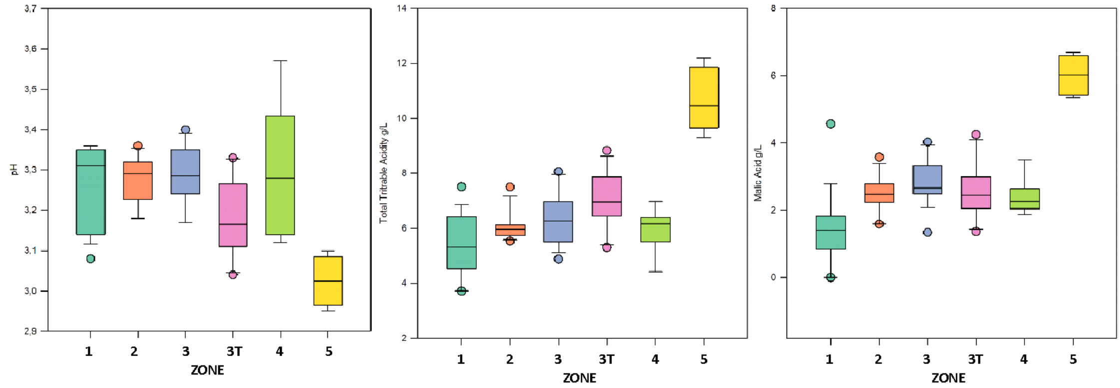

*Figure 20 - The figure shows the box plots the principal quality parameters (two-years averages): pH (left), titratable acidity (center), and malic acid (right) for each zone. The values represent means with standard deviation as the whiskers of the boxes. The graphs depict the results of a one-way analysis of variance (ANOVA) with Tukey's Post Hoc Test (p<0.05). Each replicate consists of a minimum of 5 samples;*

corroborated by corresponding pH levels (Figure 20left). A significant aspect in this context is the role of malic acid (Figure 20right), which drives the acidity levels of Glera at harvest depending on the sampled zone ($R^2$= 0.95, p_value<0.01), rather than tartaric acid ($R^2$= 0.29, p_value>0.1). These results indicate that the zoning was able to distinguish the different quality of the grapes in the area, with two clear extremes about tA and MA, corresponding to zone 1 with the highest risk of water and heat stress and the cooler and rainier zone 5. In between there is the most productive area of the intermediate classes, the plain area. The latter does not show clear intra-variability in the qualitative parameters highlighting that, despite possible AWC or climate differences, the plain zone is a quite homogeneous area. An exception is observed in the foothill area (zone 4), which behaves more like a warm and dry region. This area is the smallest and has very variable characteristics within. Its resulting trend was most likely due to the non-representativeness of founded vineyards, which were probably far from the ideal situation described by the zoning. On the contrary, Trieste area has demonstrated a behavior closer to zone 5 than zone 3, with lower pH levels and higher acidity compared to the other intermediate zones and, in particular, with acidity driven by TA levels, demonstrating a peculiar feature. The trend described was highly significant, and these results underscore the substantial impact of climate on influencing quality parameters in vineyards. As previously described (in section 1 - Introduction) TA of grapes is one of the main factors affecting the winemaking process determining wine quality and stability while, especially in fresh sparkling wines, malic acid (MA) concentration is a central quality parameter of grape at harvest

(Michelini et al., 2021) due to its influence on wine freshness. The decrease in titratable acidity (tA) at higher temperatures is in line with literature studies and it could be possibly attributed to the role of MA in the respiration process of grape berries (Ford, 2012; Rienth et al., 2016; Ruffner et al., 1976). While TA concentration is relatively constant during berry ripening (Cholet et al., 2016; de Oliveira et al., 2019) and unaffected by temperature (Coombe, 1987; de Oliveira et al., 2019; Rienth et al., 2016), MA concentration is variable, because it is transformed to fructose and glucose or used as a source of carbon and energy for respiration (Conde et al., 2007). MA is sensitive to warm temperatures both before and after veraison, when process linked to its biosynthetic pathway and respiration take place (Kliewer et al., 1967; Ruffner et al., 1984) and literature reported a negative correlation between high temperatures and MA content after veraison (Blank et al., 2019; Rienth et al., 2016; Sweetman et al., 2014). Indeed, confronting tA and MA between the two vintages at harvest, this trend was confirmed: if there were significant differences between the vintages, higher acidity and malic concentrations were found in 2023, the cooler season (Figure 21). Once again, the significant differences between vintages occurred in the extreme areas (1 and 5) and in the Trieste area (3T). On the overall average, with the same sugar level between the two years (mean variation of 0.5°Babo between years, no significative difference between means) in 2023 there was 20% higher concentration of MA and 15% of tA. The described trend in higher acidity and lower sugar content for cooler zones and years is in agreement with a study of Alessandrini et al. (2017) that found Glera variety very sensitive to different altitudes, where even small differences in temperatures between sites significantly altered grape ripening speed and affecting sugar to acidity ratio, with double ripening time, lower sugar and higher titratable acidity in sites with

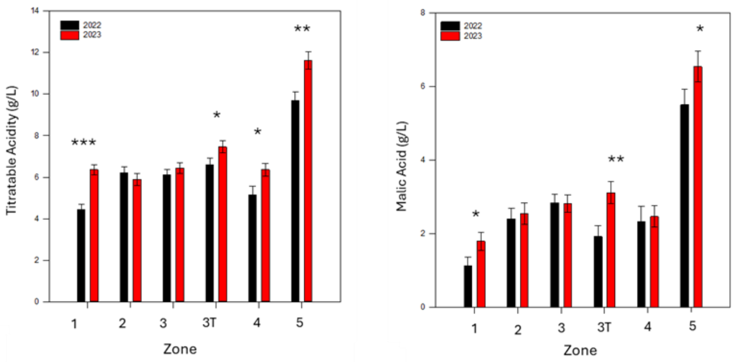

*Figure 21 - Values of titratable acidity (left) and malic acid concentration (right) at harvest for the year 2022 (black bars) and 2023 (red bars). Asterisks indicates significance levels: "***" < 0.01 < "**" < 0.05 < "*" < 0.1;*

lower minimum temperatures. Also, these small differences in temperatures altered volatile content at grape maturity, resulting in differences in the ripening process and in aromatic evolution that were perceived in the wines. To have a better picture of the malic concentration trend in the different areas of DOC Prosecco and its relationship with climate conditions, in 2023 we carried out a weekly monitoring of its concentration starting before veraison. For this analysis, some vineyards that had been partially affected by the hail were also used. In Figure 22 is possible to note that the initial concentration on 20 July does not differ significantly except for zone 5 and, to a lesser extent, zone 4 which is slightly lower than the others. It is interesting to note the phase shift of the maximum concentration peak in the different zones (Figure 22), where it had probably occurred at the first sampling (20-July) for zone 1 (or just before), while both zone 2, 3 and 3T presented the same trend with maximum peak of concentration on 27 July. The lower risk areas have more delayed peaks, 10 August for zone 4 and 17 August for zone 5, confirming literature results for this specific cultivar and growing area (Alessandrini et al., 2017; Meggio, 2022). Maximum concentrations in zones 1, 2, 3 and 4 did not differ significantly from each other (mean of 28.4±0.4 g $L^{-1}$) while the higher concentration was in zone 3T (32.9 g $L^{-1}$) and the minimum in zone 5 (21.6 g $L^{-1}$). Interestingly, zone 5 presented the lower MA concentration at peak but had the higher concentration at harvest. It presented a maximum concentration peak much later than the others, so the degradation phase began later. The slope of the curves, which represents the speed of degradation of MA, presents a steep slope immediately after the maximum peak and subsequently a slowdown in all the curves. The degradation speed indicated by the slopes of the curves were quite similar to each

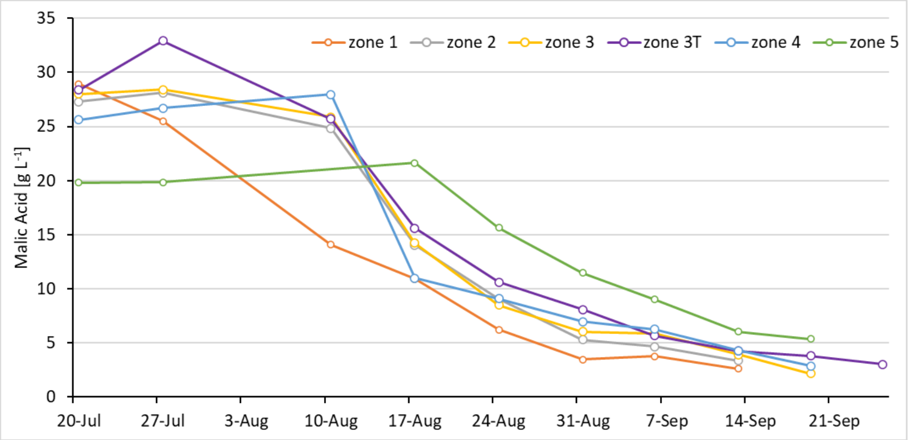

*Figure 22 - – Malic acid concentration course from before veraison to harvest (site data aggregated according to belonging area)*

other, indicating that probably the timing of the peak was crucial for the conservation of MA. Thus, the higher final concentration at harvest found in zone 5, probably was not due because of a higher starting point (i.e. higher acid production) or higher degradation rates in warmer zones, but because there was less time for MA degradation. It was more conservative than warmer areas which, on the contrary, started from higher concentrations but presented earlier peaks and therefore more time for MA degradation. Indeed, the comparison of the initial and final concentration of malic acid with precipitation and HI reported some interesting results. MA concentration peak seems to have a negative relation with the amount of rain fallen from the beginning of the season ($R^2$=0.63, p_value<0.01, Figure 23above) while no correlation was found with Huglin Index of the relative site ($R^2$=0.06). This finding, and the fact that zone 5 started from lower levels of MA (Figure 22) could be linked by the role of water stress before veraison, during malic acid biosynthesis, as reported by Zhan et al. (2023) that founds significantly higher level of malic acid in berries with moderate water stress treatment before veraison, when the berry was still hard and green. Minimum malic concentration at the harvest was positively correlated with the cumulated precipitations during the season ($R^2$= 0.70, p_value<0.01, Figure 23 center) and showed fairly good negative correlation with cumulated Huglin Index from the season starting ($R^2$= 0.44, p_value<0.05, Figure 23 below). It could be

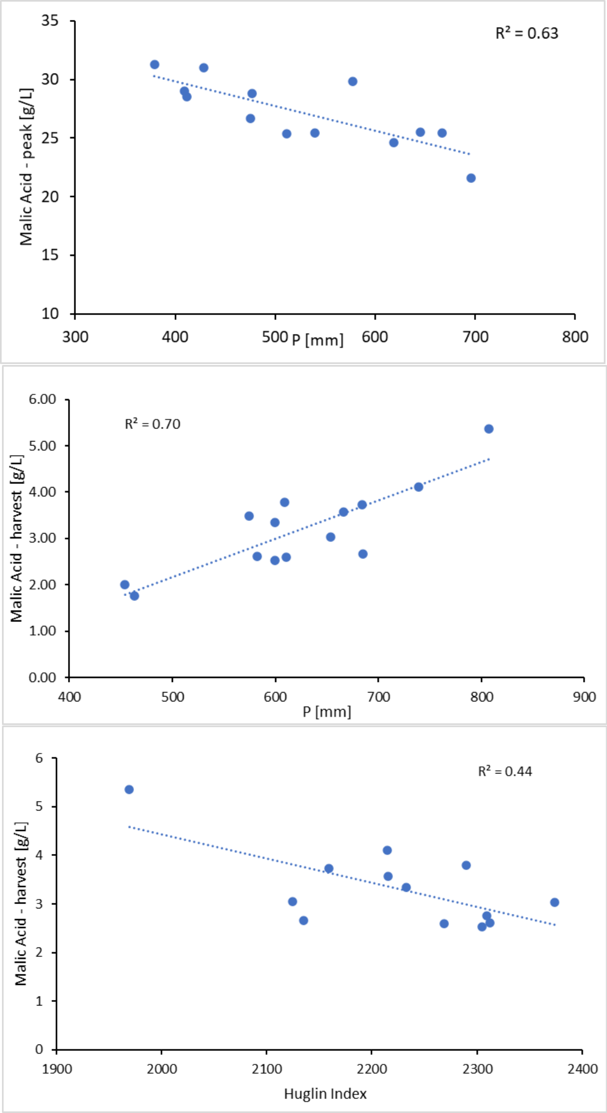

*Figure 23 – Above: scatterplot of cumulated precipitation and* peak of malic acid concentration in 2023 ($R^2$=0.63, *p_value<0.01); Center: scatterplot of cumulated precipitation and minimum malic acid concentration (harvest) in 2023 ($R^2$= 0.70, p_value<0.01); Below: scatterplot of cumulated Huglin Index and minimum malic acid concentration (harvest) in 2023 ($R^2$= 0.44, p_value<0.05);*

speculated that malic acid degradation was related with temperatures experienced by the berries as reported in literature, but rainfall and water availability could have had a major role in determining the timing and the maximum MA concentration level and in the mitigation of its degradation. This is consistent with other studies in literatures, where the amount of rainfall was positively correlated with the titratable acid content of grape berries (Du et al., 2013; Mira de Orduña, 2010; Yan et al., 2022). Going deeply into malic degradation rate and HI, we searched for a relationship between the MA concentration from the maximum value and the accumulation of HI, between one sampling and the next one. Figure 24 shows scatterplot and regression line of cumulated HI and difference of malic concentration at each sample date. The regression presents $R^2$ of 0.61 with $p\_value<0.01$, indicating a strong relation between cumulated daily temperatures and malic concentration decreasing. Confronting cumulated HI and malic concentration in cv. *Pinot blanc* in Tyrol, (Michelini et al., 2021) found good relations and similar slope values ranging between 0.0093 and 0.02, depending on vineyards altitudes. But this relationship may mask an effect due to season progression. To exclude this possibility and better characterize the relationship between HI and MA concentration, an analysis was performed on the delta HI and delta malic concentration between one sampling and another, for each site. In this way it was possible to obtain the degradation rate for each site from the slope of the regression line. Table 6 shows the degradation rate, $R^2$, $R^2_{adj}$ and p_value for the regression between these two parameters for each site. In Figure 25 a scatterplot shows the linear regression for those sites where the relationships have significance at least for $p\_value<0.1$. The lines had an average slope of $-0.10 \pm 0.035$ $gL^{-1}HI^{-1}$, indicating similar degradation rates, and the average slope values calculated for each zone were not in

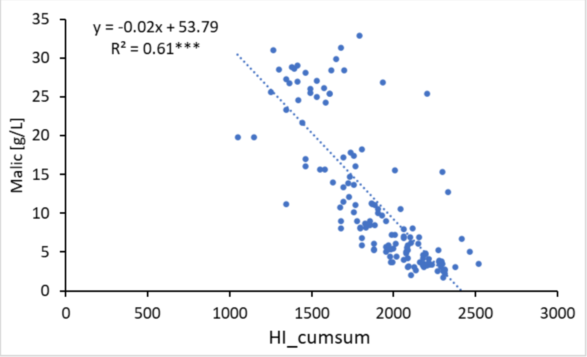

*Figure 24 - Scatterplot of the cumulated HI and MA concentration between sampling date, from the peak of concentration to the harvest ($R^2$=0.61, $p\_value<0.01$);*

accordance with the respective risk level. This could confirm that the decrease in MA concentration had variable but rather similar speed in the sites. Therefore, the hypothesis for future studies is that the final malic concentration depends more on malic peak timing and value, rather than the temperatures experienced, and timing could be very important: later peak leave less time for malic degradation.

*Table 6 - Degradation rate obtained from the slope of the regression line of ΔHi vs. ΔMA concentration between one sampling and another starting from the peak concentration, and related regression quality parameters ( $R^2$, $R^2$adj and p_value) for each monitored site;*

| zone | site | degradation rate ($gL^{-1}HI^{-1}$) | $R^2$ | $R^2_{adj}$ | p_value |
|---|---|---|---|---|---|
| 1 | Barbarano | -0.04 | 0.72 | 0.66 | 0.016 |
| | Sarego | -0.11 | 0.91 | 0.88 | 0.012 |
| | Torreglia | -0.06 | 0.56 | 0.12 | 0.461 |
| 2 | Conselve | -0.11 | 0.43 | 0.24 | 0.228 |
| | Spilimbergo | -0.16 | 0.70 | 0.63 | 0.036 |
| | Godega | -0.14 | 0.81 | 0.75 | 0.036 |
| | Spresiano | -0.06 | 0.83 | 0.78 | 0.031 |
| 3 | Pertegada | -0.13 | 0.69 | 0.62 | 0.021 |
| | Strassoldo | -0.11 | 0.91 | 0.88 | 0.012 |
| | San Vito al Tagliamento | -0.06 | 0.46 | 0.32 | 0.141 |
| | Ponte di Piave | -0.07 | 0.41 | 0.29 | 0.125 |
| 3T | Prosecco | -0.08 | 0.53 | 0.42 | 0.099 |
| | Trieste | -0.05 | 0.26 | 0.11 | 0.241 |
| 4 | Caneva | -0.02 | 0.32 | -0.35 | 0.615 |
| | Sarmede | -0.06 | 0.35 | 0.22 | 0.159 |
| 5 | Cesiomaggiore | -0.09 | 0.84 | 0.79 | 0.028 |

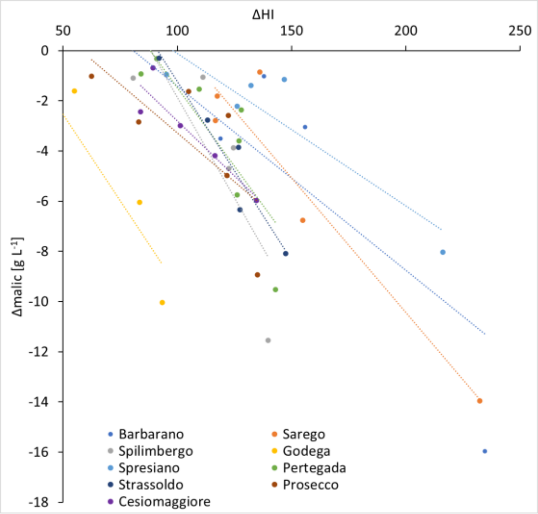

*Figure 25-Scatterplot and regression lines of sites presented in table 6, where the relationships have significance at least for p_value<0.1*

## 3.4 Water stress and grapes quality

To better understand the role of irrigation in mitigating temperature effect on berry acidity in higher risk zone of DOC Prosecco, two water stress trials have been conducted. At Barbarano site, starting from 1st July 2022, some precipitation event occurred during the stress test. Non-irrigated (NI) plot received about 50% less water than the irrigated one during the experiment (Figure 26). This results in no differences between irrigated and non-irrigated plots for pre-dawn leaf water potential, with no water deficit during august and moderate to severe stress

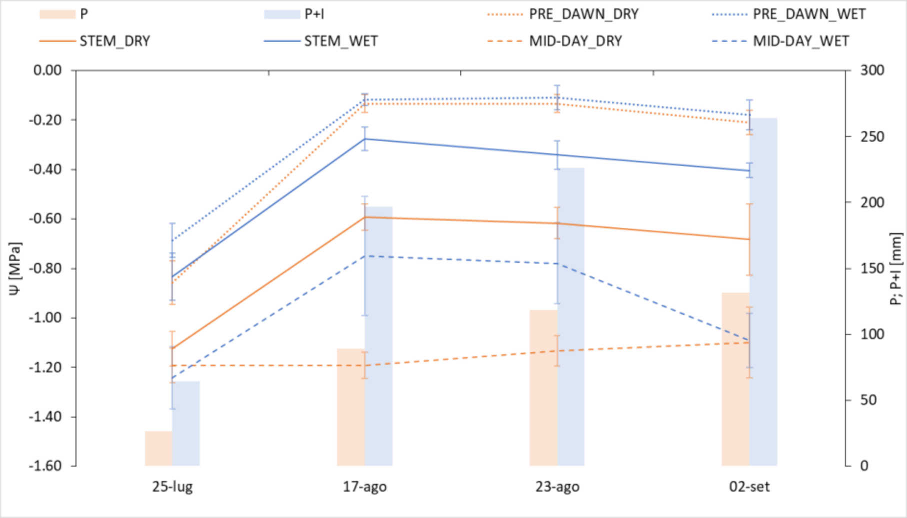

*Figure 26 – Water stress test at Barbarano site in 2022: bars indicate cumulated precipitation from 1st July for non-irrigated (orange) and precipitation plus irrigation for irrigated plot (blue). Dotted lines indicate pre-dawn leaf water potential, continuous lines indicate stem water potential and dashed lines mid-day leaf water potential (orange stand for non-irrigated, blue for irrigated plots);*

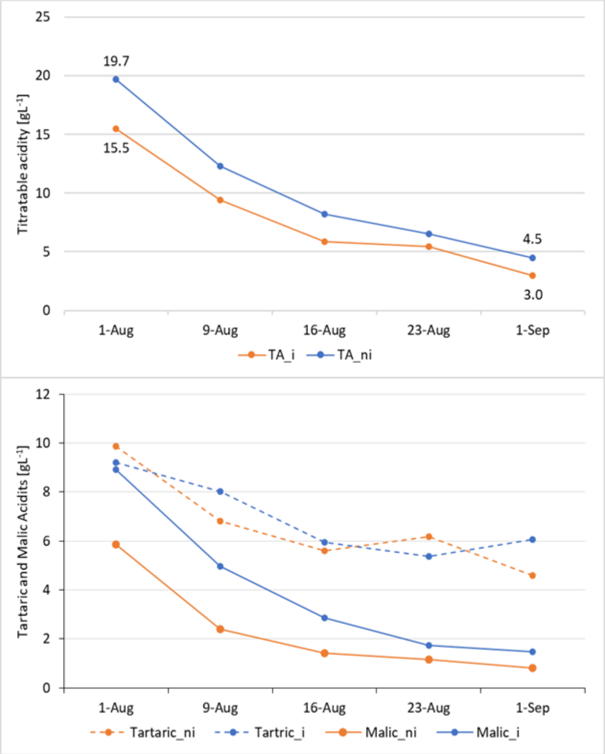

*Figure 27 - Results of monitoring of titratable acidity (above) and tartaric acid and malic acid concentrations (below) in irrigated and non-irrigated plots (orange stands for non-irrigated and blue for irrigated plots) at barbarano site in 2022;*

at the end of July, before veraison (Figure 26). On the other hand, leaf and stem water potential measured at mid-day demonstrated different levels of stress in the two plots (Figure 26). In particular, stem potential presents lower values in all the measurements dates for no-irrigated plots, detecting a mild stress level, while mid-May leaf water potential were different after veraison, reporting mild to moderate stress levels in NI plot. Even if stress levels after veraison were mild to moderate, these differences translated into different tA levels as early as August 1st (ΔtA= 4.2 gL$^{-1,}$ Figure 27 above). The difference shrunk during vintage but remained until harvest (ΔtA= 1.5 gL$^{-1}$). Although TA is the main acid in quantity, the trend of tA seems to be more determined by MA. Tartaric acid levels decreased in the first half of August and then remained constant and did not present constant trend or differences between plots. On the contrary, MA had a rapidly decreasing trend in the first half of August and then slowed down, in both plots (Figure 27 below). As for tA, the difference in MA quantity between plots on August 1st was greater (ΔMA= 3.1 gL$^{-1}$) than at the harvest (ΔMA= 0.7 gL$^{-1}$). The same trend in tA concentrations was visible at the Pantianicco site, with a rapid initial decrease which then slowed down (Figure 28 above). The three plots presented a concentration gradient concordant with the irrigation levels on first sampling while only the 10 mm plot presented a lower tA level at harvest than the other two. A slight difference was visible in the decrease in TA in the first half of August in the three plots, which on 23rd August settled at an identical concentration, stable until the harvest (Figure 28 below). Again, tA seemed to be driven by MA concentration trend also at this site. In fact, malic acid presented three distinct levels of concentration on August 2nd and a rapid initial decrease (Figure 28 below). The slight increase in MA concentration in all three plots (greater in 30 mm irrigated plot), visible also in tA concentration, corresponded to the fall of 30 mm of rain in the previous week, while no drop in temperatures were detected. Subsequently the concentration decreased again, and at harvest only the 10 mm thesis differs in lower tA levels than the other two. The results indicated that irrigation before veraison is crucial in this zone and insufficient water supply, even for short time, can cause lower MA concentration ant tA at harvest while overirrigation did not bring any benefit. As consequence of the described courses, in both trials tA presented very high and significative regressions with the two organic acids, but slightly better between tA and malic than tA and tartaric acid (Table 7). The slightly finer tuning found between tA and MA, confirmed the role of MA in defining the course of tA during vintage founded in literature (Ford, 2012; Rienth et al., 2016) and founded in harvest levels between zones (Table 5). On the other hand, the higher R$^2$ between titratable acidity and TA in non-irrigated than irrigated plots, seems to indicate that in case of water stress the role of tartaric acid becomes more important, probably due to the increase in MA degradation and consequent higher TA/MA ratio. The results confirmed the potential role of irrigation in preserving titratable acidity and malic acid during warmer months before the harvest for Glera cultivar. In the two trials, sugar levels presented different behaviors at harvest: at Barbarano site, no significative differences between plots were detected during august, but only at harvest with 18.25 and 15.80 °Babo in irrigated and non -irrigated plots respectively.

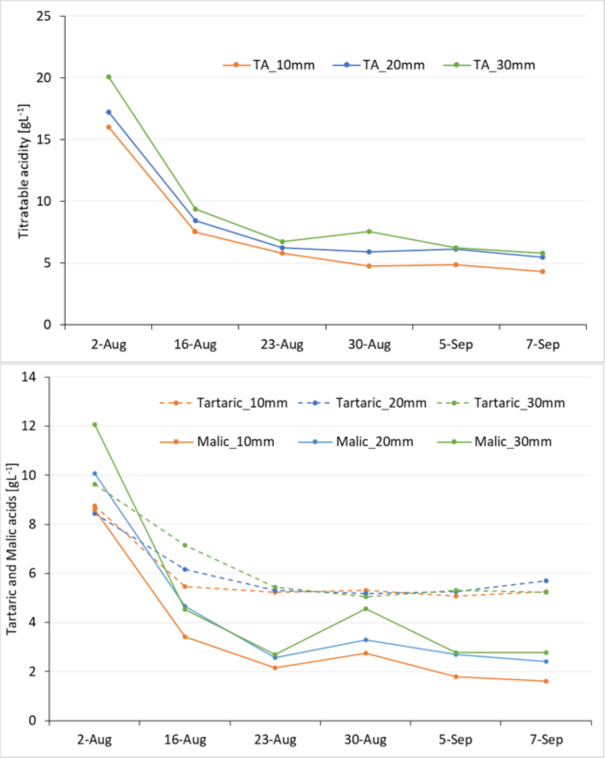

*Figure 28 - Results of monitoring of titratable acidity (above) and tartaric acid and malic acid concentrations (below) in 10 mm (red), 20 mm (blue) and 30 mm (green) irrigated plolts at Pantianicco site in 2022;*

*Table 7 – $R^2$ and significance level ("***" < 0.01 < "**" < 0.05 < "*" < 0.1) of linear regression between titratable acidity (tA) and tartaric acid (TA) and malic acid (MA) in the two test sites;*

| | **Barbarano** | | **Pantianicco** | |
|---|---|---|---|---|
| | **Irrigated** | **Non irrigated** | **30 mm** | **10 mm** |
| **tA vs. TA** | 0.88** | 0.97*** | 0.93*** | 0.96*** |
| **tA vs. MA** | 0.99*** | 0.96*** | 0.98*** | 0.98*** |

Lower levels of malic acid in stressed treatment are coherent with studies on mechanisms affecting malic acid biosynthesis and degradation in grape berries, where water stress reduced the malic acid content after grape veraison (Berdeja et al., 2014; Cooley et al., 2017; Leng et al., 2022; Uriarte et al., 2016; Zhan et al., 2023; Zufferey et al., 2020) in respect to no stressed plants; at Pantianicco site, the 10 mm plot presented higher °Babo from mid-august to the harvest than the 20 and 30 mm plots that had similar sugars level. At harvest, plot with less irrigation presented higher sugar levels (14.30 °Babo) than higher irrigation plot (12.65 °Babo). The opposite results in the two trials probably depended on differences in the processes of sugar production and their concentration or dilution into the grapes between the two sites.

# 4 Conclusions

This study focused on the analysis of critical issues with respect to water and heat stress risk in the DOC Prosecco area. We identified the water requirements of a vineyard representative of the area and analyzed the pedo-climatic conditions of the region, through an innovative procedure based on a combined variable ranking, that considered Huglin Index, seasonal rainfall and soil water capacity. The derived map has been the basis for the identification of the most critical areas in relation to grape quality in the perspective of climate change. For two years, we monitored grape maturation dynamics in 20 vineyards, selected to represent a broad range of different pedo-climatic conditions, and focusing especially on acid metabolism of berries, in relation to grapevine water status and thermal conditions.

We found a quite stable yearly water consumption by the vineyard, well below the total annual precipitation. Nonetheless, stress periods have been observed, raising the issue of irrigating vineyard also in Veneto region. All in all, the average water consumption over the growing season (April to September) for a vineyard in the DOC Prosecco area was 450±52 mm, with significant different values in non-irrigated and irrigated years (between 417±15 mm and 523±29 mm respectively) but very stable values inside these two periods. The average measured daily ET during all the growing seasons was 2.46±1.23 mm corresponding to about 59% of the average $ET_0$ while average ET during July and August were 3.30±0.46 mm $d^{-1}$, with maximum exceeded 5 mm per day. Standard Kc reported by FAO (Allen et al., 1998) has been confirmed for this geographical zone and this common vineyard conduction type, once the application periods have been adapted for the local seasonal timing. However, they are probably overestimated for different climatic and vineyard management conditions, for example semi-arid areas or with little or no soil herbaceous cover.

The zoning of Prosecco has elucidated significant regional differences in production quality and yield. The developed zoning was able to characterize the large area of Prosecco DOC, highlighting two extremes as regards climatic conditions and qualitative parameters of the grapes (zone 1 and 5), and a larger, more productive, intermediate-quality area (zone 2, 3 and 4). Indeed, plains, with their fertile soils, stable microclimate, and consistent water availability, demonstrated higher fertility and productivity. In contrast, foothill and pre-alps areas (zone 1, 4 ,5), characterized by rocky soils, variable climate, showed lower yields. Higher levels of total acidity and malic acid, and lower pH, were found in the pre-Alps area (zone 5) compared to the plains, especially the more internal, warmer and less rainy area (zone 1). Surprisingly, the Trieste area (zone 3T) differed in its peculiarities, coming closer to the pre-Alps, with lower pH levels and higher acidity, in particular for tartaric acid, compared to the other intermediate neighboring zones.

A strong link has been found between the concentration of malic acid in the different sites and the rainfall fallen, both at its maximum and minimum concentration, while the relationship with Huglin Index levels was less strong and present only at the minimum concentration of MA. Furthermore, a site-specific evaluation revealed a good homogeneity of MA degradation rates. The maximum concentration values (the highest peak was in 3T and the lowest in 5) were not linked to the zoning distribution of the areas, while were found a coherent distribution of the dates on which they were reached, as first zone 1 and last zone 5, suggesting the hypothesis that the timing and the value of maximum concentration peak may be more important than the temperatures experienced, with timing becoming very important. These aspects relating concentration of malic acid and precipitations, or the timing of MA peaks are a very interesting aspect to explore in future studies.

The results confirmed the crucial role of irrigation in preserving titratable acidity and malic acid during warmer months after veraison in Glera variety, in the DOC Prosecco area. Irrigation can help in high HI areas, mitigating the impacts of climate change in the short term but up to a certain level. In fact, over-irrigation did not bring improvements compared to the company standard. Zone 1 is already irrigated and demonstrates lower levels of acidity. Medium-term adaptation strategies could also include earlier harvesting and using shading but, given the future increase in heat waves and temperatures in general, this area probably will have to face more drastic strategies in future, such as changes in plant material (later berry ripening) or different training models (e.g. pergola). Intermediate zones will be less susceptible to higher temperatures and in the short-term irrigation will be useful (and already applied in some cases) but, again, deeper changes to vineyard management will probably be useful in the future, especially if temperature increases will be in the high range of IPPC estimates. On the contrary, zone 5 has proven to have the best potential for high quality of Glera production and an expansion of Glera vineyards in this area can be expected, where today is little practiced, confirming the possibility of shifting the wine-growing areas towards the north. Also, the Trieste area has shown good potential for the production of Glera, better than plain areas, with peculiar features. These findings underscore the importance of tailored vineyard management practices to optimize production and resources use, in particular water, in each zone. Understanding these zonal differences is essential for maintaining the quality and consistency of Prosecco DOC wines in the face of a changing climate. By tailoring vineyard management to the specific conditions of each zone, growers can produce grapes that best express the terroir, leading to wines with distinctive and desirable characteristics. The detailed zoning map provides a valuable tool for producers to enhance vineyard performance by leveraging regional strengths and addressing specific challenges, ultimately improving the overall quality and consistency of Prosecco DOC wines. Also, it is a forward-thinking strategy that aligns with the principles of sustainable agriculture, promoting efficient resource use, enhances resilience to climate change, and supports the production of high-quality wines that meet market demands.

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# Chapter III:

# EVALUATION OF NITROUS OXIDE EMISSIONS FROM VINEYARD SOIL: EFFECT OF ORGANIC FERTILIZATION AND TILLAGE

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## Abstract

It is well known that the largest source of $N_2O$ is the agricultural sector, where fertilization represents the main source of this GHG. Monitoring $N_2O$ fluxes for different soil management and crops type is essential to define better N management practices in agro-ecosystems. However, scarce studies have been published about field measurements of organic fertilizers effects in orchards and vineyards. In this paper we present the first long-term high-resolution study on $N_2O$ emissions in a vineyard, in temperate climate. The use of dynamic chambers connected directly to an IRGA, allowed to collect one year (from May 2018 to May 2019) of measurements at time resolution of 2 hours. The aim of the study was to assess the effect of organic fertilization (compost) and tillage on $N_2O$ emissions. Emission factors of uncorrected ($EF_t$) and corrected for no-fertilizer induced emissions ($EF_f$) were calculated. Results showed a seasonal trend in $N_2O$ fluxes, with higher base fluxes and peaks during the warm season and in correspondence of rainy events. Emission peak linked to fertilizer application occurred during the first 6-7 days after treatment followed by a decrease in $N_2O$ fluxes. Cumulated annual emissions varies between 0.54 and 1.38 kg $N_2O$-N $ha^{-1}$ $y^{-1}$, depending on treatment and level of soil total organic carbon (TOC) content. The uncorrected EF was between 0.4 and 0.9 % of N input, in line with the IPCC value for organic fertilizers in wet climate, while the $EF_f$ (referred only to direct fertilizer application) were in range of 0.02 – 0.4% of N input. We found $EF_f$ of fertilized not tilled treatments from 17 to 79% lower than fertilized tilled, with different magnitude of reduction depending on soil TOC. Our $EF_t$ were in line with disaggregated EFs from IPCC 2019 but $EF_f$ were 43% lower on average, confirming the IPCC reference value to be a good estimator of the overall $N_2O$ emissions from organic fertilized soil, but too high for the estimation of only fertilizer application emissions. These results are of remarkable importance to direct organic fertilization management and related policies towards more environmentally sustainable approaches.

# 1 Introduction

Agriculture, forestry, and other land use (AFOLU) are one of the largest producers of direct and indirect emissions of carbon dioxide ($CO_2$), methane ($CH_4$) and nitrous oxide ($N_2O$), that represent the three major Green House Gases (GHGs). AFOLU are responsible for 23% (12.0 ± 2.9 $GtCO_2eq\ yr^{-1}$) of the global human-generated emissions of these GHGs, while about 50% of this value (6.2 ± 1.4 $GtCO_2eq\ yr^{-1}$) is agriculture related $N_2O$ emissions in viticulture. Agriculture, and especially intensive agriculture, is one of the largest contributors to non-$CO_2$ GHG anthropogenic emissions (IPCC, 2014). Livestock production systems are one of the main producers of $CH_4$ emissions (Heiling, 1994), while agricultural soils are the largest source of human related $N_2O$ emissions (IPCC, 2019). In viticulture $CH_4$ emissions are insignificant (Carlisle et al., 2010), and the main non-$CO_2$ GHG produced is $N_2O$ (Nistor et al., 2018). Beyond being the dominant ozone-depleting substance emitted in the 21st century (Ravishankara et al., 2009), the critical issue regarding $N_2O$ lies in its high global warming potential (GWP), that is about 300 times more effective than $CO_2$ in trapping heat in Earth's atmosphere (Solomon et al., 2008). $N_2O$ emissions from soil are directly related to fertilization and tillage. N inputs applied in the field as fertilizers are released through nitrification and denitrification processes, or immediate volatilization (Linton et al., 2020), while impact related to soil tillage in terms of $N_2O$ emissions is contradictory (Gregorutti and Caviglia, 2017).

The improvement of fertilizer applications efficiency will be essential to reduce N-related emissions (Solomon et al., 2008) and pollution of ground and surface waters, increasing soil N availability for the crop. The reduction of the nitrogen footprint (NF) through better N management practices in agro-ecosystem have become an increasingly hot topic in global climate change and agricultural research (Xue et al., 2016), and it represents an important mitigation opportunity (Paustian et al., 2014) for the agricultural sector, especially for viticulture. Wang et al. (2021) found more than 2000 published papers about $N_2O$ in agriculture from 1990, with the number of papers steadily increasing per year since 2005. Going more specifically to organic fertilizers and tree crops, the studies are scarcer, as shown by the reviews of Charles et al. (2017) and Gu et al. (2019). Very few papers are available for non-$CO_2$ GHG emissions in vineyard (Nistor et al., 2018), with a significant variability in the data (Longbottom and Petrie, 2015), despite being an agro-ecosystem distributed all over the world with remarkable ecological and economic importance (OIV, 2022). In these studies, soil $N_2O$ emissions are commonly measured through static-chamber (non-steady state chambers) method, a relatively economic but time and labor consuming technique where manually collected samples are subsequently analyzed on a gas chromatograph in laboratory. The closure time of this type of chamber is variable, generally a few hours. This could address problems about representativeness of soil under the dome (mainly about irradiation and soil moisture) and altering gas production and transport processes (Rochette, 2011). Another limitation of this method is temporal resolution of monitoring. Often the total duration of these monitoring was the vegetative season of the crop, and the frequency of data collection is, at most, once a day for 7-10 days after the event of interest (fertilization and, in some studies, rain). It is well known that $N_2O$ fluxes present high temporal variability at different scales (hours, days, seasons, years) that respond to climate and agronomic events (Laville et al., 2011; Hénault et al., 2012), but measurements normally are discontinuous in time (weekly to monthly measurements) and often realized over short periods (Hénault et al., 2012). These types of monitoring are unable to follow the diurnal flux pattern, as the daily (or longer periods) emission calculations are based on the extrapolation of a single daily measurement (Alves et al., 2012; Cowan et al., 2014).

Although efforts have been made to identify the best time to do the single daily measurement, Francis Clar and Anex (2019) affirm that $N_2O$ fluxes cannot be accurately measured with infrequent measurements. The discontinuous nature of the measurement strongly impacts the estimation of cumulative emissions (Hénault et al., 2012), where individual management events have a significant impact on seasonal $N_2O$ emissions (Longbottom and Petrie, 2015). Annual estimation of cumulative $N_2O$ emissions based only on growing-season measurements, could also presents important bias (Gregorich et al., 2005). On the other hand, the quantification of $N_2O$ emissions throughout the year presents some technical difficulties, in addition to the wide spatial and temporal variability in the field (Laville et al., 2015; Laville et al., 2017). The use of an automatic survey systems associated with flow-through dynamic chambers during infield measurement is preferable than manual static chambers, especially during long-term studies, and suggested as standard method in international GHG monitoring network (Pavelka et al., 2018). This technique is widely used in various application fields such as soil respiration (Parkinson, 1981), volcano monitoring (Hernández et al., 2001), and contaminated sites (Centioli et al., 2019). This method decreases the need for manual operations, allowing minimal disturbance to soil surface (Pavelka et al., 2018), and allows to have a high time resolution of $N_2O$ effluxes for extended periods of time (Cowan et al., 2014), able to catch daily and seasonal trends, as well as momentary peaks (Kostyanovsky et al., 2018). However, dynamic chambers connected to a $N_2O$ analyzer directly in the field, with shorter measurement times (3- 10 minutes), better performance and less environment disturbance, rarely have been used for soil $N_2O$ fluxes monitoring (Cowan et al., 2014; Volpi et al., 2018). This is because the technology associated with this system is currently very expensive and energy demanding, limiting the possibility to gain detailed information about $N_2O$ fluxes directly and continuously in the field. This makes it hard to define the amount of annual $N_2O$ emissions from specific fertilizers, soils, and crop management type.

For nitrogen footprint (NF) scope, annual $N_2O$ emissions are usually estimated using an average emission factor (EF) derived from literature. In case of N from fertilizers, default EF to estimate direct $N_2O$ emissions from managed soils has been quantified in an average value of 0.01 kg $N_2O$–N (kg N)$^{-1}$ (ranging from 0.001 to 0.018 kg $N_2O$–N (kg N)$^{-1}$) (IPCC, 2019). This aggregated value has been further disaggregated in the IPPC document, reporting higher value in case of synthetic fertilizer inputs (0.016 kg $N_2O$–N (kg N)$^{-1}$) and lower value for other N inputs. This latter refers to organic amendments, animal manures (e.g. slurries and digested manures), N from crop residues and mineralized N from soil organic matter (SOC) decomposition. This default value is further differentiated between dry and wet climates with 0.006 and 0.005 kg $N_2O$–N (kg N)$^{-1}$, respectively (IPCC, 2019). Emission factors provide a useful shortcut in NF, avoiding the need for detailed calculations or direct measurements of emissions. However, the EF does not consider differences in soil properties and agronomic management between different cases studies (Garland et al., 2014; Gu et al., 2019). The effect on $N_2O$ emissions of soil tillage and fertilizer incorporation into the soil have not been fully clarified (Baggs et al., 2000; Bosco et al., 2019), as contrasting results could be found in literature due to different environmental conditions and management time (Hassan et al., 2022). EFs suffer from the gaps that exist in literature with respect to measurements technique and type of crops, soil management and climate. For example, in their review Gu et al. (2019) highlights that no EFs were determined in orchards with temperate or continental climates. Several authors underline that the high variability of EFs and the wide variety of factors that can influence $N_2O$ emissions makes it difficult to correctly assess the effects of agricultural practices (Cowan et al., 2020). For these reasons, continuous advances in research and knowledge are important to enhance EF assessment associated with N input in agriculture, and to achieve EF as specific as possible about agricultural practices and climate condition.

This study presents a one-year dataset of high frequency measurements of $N_2O$ fluxes from soil with different management, in a temperate vineyard (North-eastern Italy), with the intent to acquire new information regarding the relationship between $N_2O$ emissions, organic fertilization and soil tillage. Very few high-time resolution monitoring has been done on $N_2O$ fluxes (Francis Clar and Anex, 2019), and none of these were made in orchards or vineyards. To our knowledge, this is the first study reporting this type of measurement in tree crops and, especially, in temperate climate. The aim of the present work is to i) to increase the knowledge about dynamic of $N_2O$ fluxes in different soil conditions (tillage and not tillage application) and organic fertilization management (incorporated and not incorporated in the soil); ii) to calculate a robust and specific annual EF for $N_2O$ emission related to each practice. Monitoring the impact of fertilizers distribution on the environment is of fundamental importance to promote good agricultural practices, improving the environmental performance in viticulture and agriculture in general. Non-$CO_2$ GHG emissions are still little known but are the most directly and promptly influenced by a change of management. To take this effective mitigation opportunity for agriculture, it is of crucial importance to deepen the knowledge of N related emissions and their dynamics in relation to the different types of agronomic practices and achieve a more conscious management.

# 2 Material and methods

## 2.1 Study sites

The study was carried out in a central portion of a commercial vineyard (*Vitis vinifera*, cv. Sauvignon Blanc grafted on 3309C) located in North-eastern Italy (Figure 1). It was established in 2001 and it is trained to Vertical Shoot Position trellis system and Guyot pruned. Rows are 2.2 m apart and oriented to 35–215 °N, while plant spacing is 0.9 m. Canopy height at full development is around 2 m. The vineyard is rainfed, and alleys are covered with resident herbaceous vegetation (dicots like Taraxacum officinale, Trifolium spp., Plantago spp. and graminoids) mowed once or twice per year (according to summer rainfall), except for a strip about 0.6 m wide on the row that is chemically weeded. Soil is ripped in winter. A more detailed description of pedological and climatic characterization of the vineyard is reported by Tezza et al. (2019).

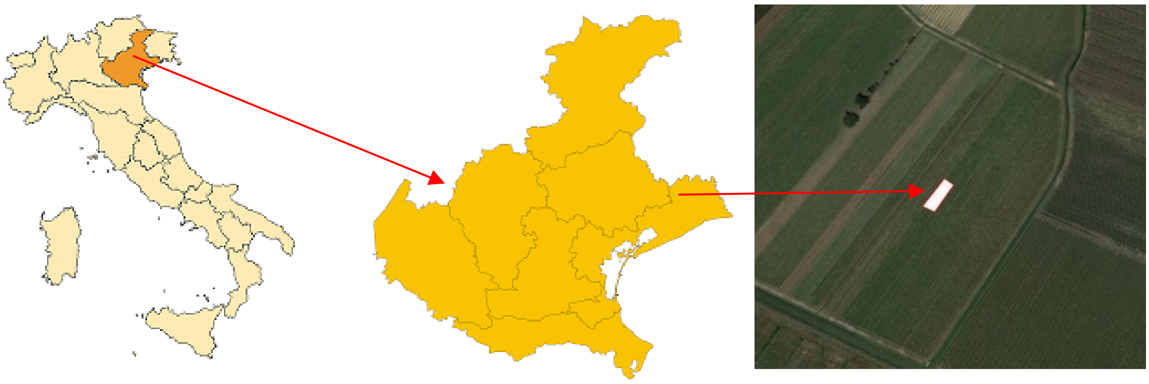

*Figure 1 - Location of the wine-growing area included in the study*

## 2.2 Meteorological and soil sensors

During the study period, air temperature, humidity and rainfall were monitored using a WXT520 weather station (Vaisala, Helsinki, Finland) placed at 5 m of height from the ground. Soil water content and soil temperature were measured at 0.04 m depth with a 5TM soil sensor (Decagon Devices, Inc., Pullman, WA, USA), while soil water content at 0.10 m and 0.20 m depth were measured using two CS616 water content reflectometer (Campbell Scientific, Logan, UT, USA). Meteorological and soil variables were collected every 1 s and 15 s respectively, with statistics calculated every 30 min. The data for the construction of the historical average (1994-

2017) were taken from the nearest Regional Meteorological Agency (ARPAV) station, 1 km away from the experimental plots.

## 2.3 Experimental plan

With the aim to monitor soil $N_2O$ fluxes related to organic fertilizers addition, 4 treatments were set up on October 19th 2017 and repeated in October 8th 2018: i) untreated (not fertilized) and no-tilled control (UNT); ii) untreated (not fertilized) and tilled control (UT); iii) Treated (compost addition) no-tilled (without incorporation into the soil) - TNT; iv) compost addition immediately incorporated into the soil (TT). The compost was analyzed before the distribution and characterized for Density (589±1g $L^{-1}$), Dry weight (73.90±0.13 %), pH (7.02±0.04), Total Organic Carbon referred to Dry weight (298±11 g $kg^{-1}$), Total Nitrogen referred to the fresh weight (19.90±0.57 g $kg^{-1}$), Ammonia Nitrogen referred to the fresh weight (3.27±0.02 g $kg^{-1}$), Carbon nitrogen ratio C/N equal to 20. Four 1m x 1m plots were dedicated to a specific treatment and replicated twice; each plot was maintained associated to the same treatment both in 2017 and 2018. In the treated plots, a total of 17,6 g $N/m^2$ (equal to 9 t/ha of compost) were distributed manually in each year. The soil of tilled plots was manually worked with a shovel to about 15 cm deep and, in TT plots, the fertilizer was incorporated into the soil during this operation. From October 2018 the growing of herbaceous vegetation in the plots was avoided by manual uprooting and soil cleaning. The two replicas were placed in two different areas of the portion of vineyard dedicated to the experiment: i) Zone A where infield activities have been carried out by hand without access to agricultural machineries since 2015 and where no fertilization had been done in the period 2015- October 19th 2017; ii) Zone B where the soil, before the start of the experiment, had been fertilized with traditional (solid fraction of digestate and chemical fertilization) products according to enterprise schedule (Figure 2). One accumulation chamber was installed in the central part of each plot for a total of 8 chambers installed. Each chamber was numbered and associated with the specific treatment applied on the plot monitored as reported in Table 1.

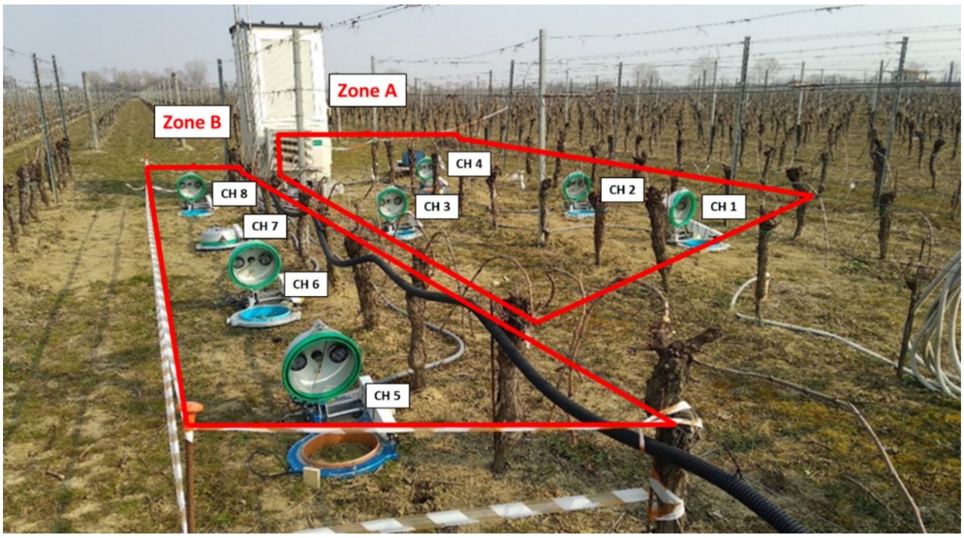

*Figure 2 - Experimental area where Continuous Monitoring Station of N2O emissions were installed*

*Table 1 - Experimental plan: treatments associated with each chamber and chamber code*

| Zone | Chamber code | Treatment description |
|---|---|---|
| Zone A | CH1_UT | Untreated (not fertilized) and tilled control |
| | CH2_TT | Treated (compost addition) immediately incorporated into the soil |
| | CH3_TNT | Treated (compost addition) no-tilled (without incorporation into the soil) |
| | CH4_UNT | Untreated (not fertilized) and no-tilled control |
| Zone B | CH5_UNT | Untreated (not fertilized) and no-tilled control |
| | CH6_TNT | Treated (compost addition) no-tilled (without incorporation into the soil) |
| | CH7_UT | Untreated (not fertilized) and tilled control |
| | CH8_TT | Treated (compost addition) immediately incorporated into the soil |

## 2.4 Physical and chemical soil characterization

A soil sample was collected at each plot before the treatment application, to perform physical and chemical characterization of the soil (Table 2). The soil sample was taken at a depth of 0-20 cm and mixed uniformly, excluding the leaf litter layer and dried at room temperature in two to three days. Subsequently, they were sifted to <20 mm. The prepared samples were then analyzed following the European UNI EN methodology. The analyses carried out were: - pH in water (ISO 14254:2001) - Total Organic Carbon (ISO 14235:1998) - Total Nitrogen (ISO 11261:1995; ISO 13878:1998) - Available Phosphorus (ISO 11263:1994) - Cationic exchange capacity (ISO 11260:1994; ISO 13536:1995) - Calcium Carbonate Content (ISO 10693:1995) - Soil Texture (USDA).

*Table 2 - Chemical and physical soil characterization of each plot before treatment application*

| | Zone A | | | | | Zone B | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | CH1_UT | CH2_TT | CH3_TNT | CH4_UNT | Avg±Std.Dev. | CH5_UNT | CH6_TNT | CH7_UT | CH8_TT | Avg±Std.Dev. |
| pH (in $H_2O$) | 8.15 | 8.04 | 7.98 | 7.88 | 8.01±0.11 | 7.94 | 8.03 | 8.01 | 7.94 | 7.98±0.05 |
| TOC (g $kg^{-1}$) | 5.8 | 9.0 | 10.6 | 7.0 | 8.1±2.1 | 11.5 | 11.2 | 13.0 | 14.0 | 12.4±1.3 |
| Ntot (mg $g^{-1}$) | 0.7 | 0.9 | 1.1 | 0.8 | 0.9±0.2 | 1.0 | 1.2 | 1.0 | 1.2 | 1.1±0.1 |
| C/N | 8.3 | 9.9 | 10.0 | 8.7 | 9.2±0.9 | 11.0 | 9.2 | 12.9 | 12.1 | 11.3±1.6 |
| $P_2O_5$ (mg $kg^{-1}$) | 17.3 | 25.8 | 19.5 | 18.9 | 20.4±3.7 | 33.5 | 19.2 | 24.4 | 29.4 | 26.6±6.2 |
| CSC ($cmol^+$ $kg^{-1}$) | 28.4 | 36.2 | 34.9 | 29.9 | 32.4±3.8 | 30.3 | 30.1 | 34.5 | 29.9 | 31.2±2.2 |
| CaCO3 (g $kg^{-1}$) | 336 | 499 | 231 | 296 | 341±114 | 221 | 268 | 231 | 148 | 217.±50 |
| Silt (%) | 50.3 | 46.1 | 50.5 | 49.8 | 49.2±2.1 | 47.7 | 49.2 | 50.3 | 46.7 | 48.5±1.6 |
| Clay (%) | 46.1 | 42.0 | 46.3 | 45.7 | 45.0±2.0 | 43.5 | 45.0 | 46.1 | 42.5 | 44.3±1.6 |
| Sand (%) | 3.6 | 11.9 | 3.2 | 4.5 | 5.82±4.09 | 8.8 | 5.8 | 3.6 | 10.8 | 7.3±3.2 |

## 2.5 $N_2O$ Data collection

The continuous monitoring of soil $N_2O$ fluxes was carried out from October 19th 2017 till the end of October 2019. In the present paper, we present the results obtained during one year from May 1st2018 to May 1st2019. The methodology used to measure GHG emission was the automated closed dynamic accumulation chamber (a non-steady-state through-flow system). The measurement system was a West Systems “CM-HWR11”, continuous monitoring unit that allowed the monitoring of $N_2O$ fluxes from soil over time. This is a multi-chamber system developed during the IPNOA project (LIFE+ IPNOA, LIFE11 ENV/IT/000302), that uses a Thermo i46 $N_2O$ analyzer, based on Filter Correlation technique (Laville et al., 2017). The gas analyzer was located in a shelter together with auxiliary equipment. The detection limit of the system for nitrous oxide flux was 0.5 $\mu mol\ m^{-2}\ d^{-1}$. The station was equipped with eight multiplexed automatic chambers, operating sequentially in turns. Each chamber closes its dome for 10 minutes to make the measurements. After the measuring time, it opens the dome, and stay opened until the next measure, leaving the soil expose to weather condition. The domes were positioned to not overshadow the ground inside the chamber collars. The monitoring system was set to perform a measure every 2 hours, therefore 12 measurements per day were available for each chamber, for a total of 4187 annual $N_2O$ measurements. The soil inside the chamber remains exposed to the same weather conditions as the uncovered soil for 92% of the day. The measurements were stored in a SD card in ASCII format file, the station was remote controlled and raw data were processed with the West Systems Supervisory Control and Data Acquisition (WS-SCADA) software.

## 2.6 Data processing

The diffuse emissions of gasses from soil generate an increase in concentration in the accumulation chamber, which can be measured directly in the field using appropriate instrumentation. The flux of $N_2O$ was calculated starting from the concentration gradient over time (dC/dt) and considering volume (V) area (A) of the accumulation chamber, according to the formula below (Chiodini et al., 1998):

$$Flux = \frac{dC}{dt} \, .ACk \qquad \text{Eq.1}$$

where $AC_k$ is a function of pressure (p), temperature (T) and the size of the accumulation chamber (R is the universal gas constant):

$$ACk = \frac{p \cdot V}{R \cdot T \cdot A} \qquad \text{Eq.2}$$

The calculated $N_2O$ fluxes was expressed in mol $m^{-2}d^{-1}$. The data collected from treated and untreated plots were analyzed to highlight the temporal pattern of fluxes during one year for each plot, and the related cumulative emissions. For each chamber the main statistical parameters have been calculated (Rstudio software Version 1.4.1103). The confidence interval of $N_2O$ fluxes mean value of each chamber was evaluated using a t test with a significance level of $P > 0.05$. A one-way ANOVA was used to analyze the differences in $N_2O$ emissions from treated and untreated chambers ($P < 0.05$). Total $N_2O$ emissions calculated from each plot, expressed in $gN_2O$ $m^{-2}$ $d^{1}$, were converted in $N_2O$ –N, using equation:

$$N_2O - N = N_2O * \frac{28}{44} \qquad \text{Eq.3}$$

The obtained data were used to estimate two $N_2O$ specific emission factor, one related to the overall emission of $N_2O$ (background + fertilization, $EF_t$) and one for fertilizer induced direct $N_2O$ emissions ($EF_f$), to be compared with IPCC EFs (IPCC, 2019). We have chosen to present both calculations because in literature the distinction is not always clear and both ways are used according to the type of experiment conducted, as lot of studies did not include an unfertilized control (Gu et al., 2019). The same IPCC EF is calculated from studies in literature, which do not always present the results related to emissions from control plots (IPCC, 2006). The $N_2O$-N total annual emission was divided by the total nitrogen distributed as fertilizer in each plot to obtain $EF_t$, while $EF_f$ were calculated subtracting emissions from untreated plots as control plots according to the equation:

$$EF_f = \frac{(Et - Eu)}{N} \qquad \text{Eq.4}$$

Where $E_t$ is the $N_2O$ emission from treated (fertilized) plot, $E_u$ is the $N_2O$ emission from corresponding untreated control plots and N the annual amount of N applied to soils as organic fertilizer.

# 3 Results and discussion

## 3.1 $N_2O$ time series

The descriptive statistics for $N_2O$ daily fluxes are reported in Table 3. Values range from a minimum value of 0.002 mg $m^{-2}$ $d^{-1}$ in CH5_UNT and a maximum of 14.508 mg $m^{-2}$ $d^{-1}$ in CH8_TT. This value is similar to the value obtained by Lazcano et al. (2022) that reported, in a Mediterranean vineyard, a maximum value of 104 g $N_2O$-N $ha^{-1}$ $d^{-1}$, equal to 16.343 mg $N_2O$ $m^{-2}$ $d^{-1}$.

*Table 3 - Minimum, maximum, median and mean value of $N_2O$ (mg$N_2O$ $m^{-2}$ $d^{-1}$) from each treatment chamber. 95% confidence interval is defined with t-test (p-value >0.05). Data collected from 1st may 2018 to 30th April 2019*

| Zone | Plot | Min. | Max. | Median | Mean $N_2O$ fluxes | 95% confidence interval |
|---|---|---|---|---|---|---|
| | | | | mg$N_2O$ $m^{-2}$ $d^{-1}$ | | |
| A | CH1_UT | 0.027 | 2.318 | 0.169 | 0.242 | 0.218 – 0.266 |
| | CH2_TT | 0.017 | 5.886 | 0.191 | 0.311 | 0.261 – 0.360 |
| | CH 3 TNT | 0.024 | 5.923 | 0.221 | 0.387 | 0.329 – 0.444 |
| | CH 4 UNT | 0.030 | 9.243 | 0.178 | 0.373 | 0.303 – 0.442 |
| B | CH 5 UNT | 0.002 | 1.866 | 0.210 | 0.296 | 0.266 – 0.326 |
| | CH 6 TNT | 0.033 | 7.640 | 0.38 | 0.540 | 0.460 – 0.620 |
| | CH 7 UT | 0.007 | 3.964 | 0.238 | 0.325 | 0.288 – 0.361 |
| | CH 8 TT | 0.022 | 14.508 | 0.353 | 0.618 | 0.502 – 0.734 |

The time series of daily $N_2O$ fluxes measured for each chamber, average air temperature and daily precipitation are shown in Figure 3. Mean daily air temperature shows the expected seasonal variation with highest maximum Ta during summer (35.9 °C on August 1st) and lower minimum Ta in winter (-4.8 on January 6th). The number of rainy days and total amount of rainfall peaked in spring (29 days with a total of 410.6 mm) while dipping during the cold season (12 days with precipitations, with a total of 79.2 mm). During summer and autumn, the amount of precipitations was similar (21 days with a total of 231.7 mm and 18 days with a total of 255.9 mm, respectively), with some drought periods occurred during summer. The average annual Ta and total annual precipitation were 14.7 °C and 977.5 mm.

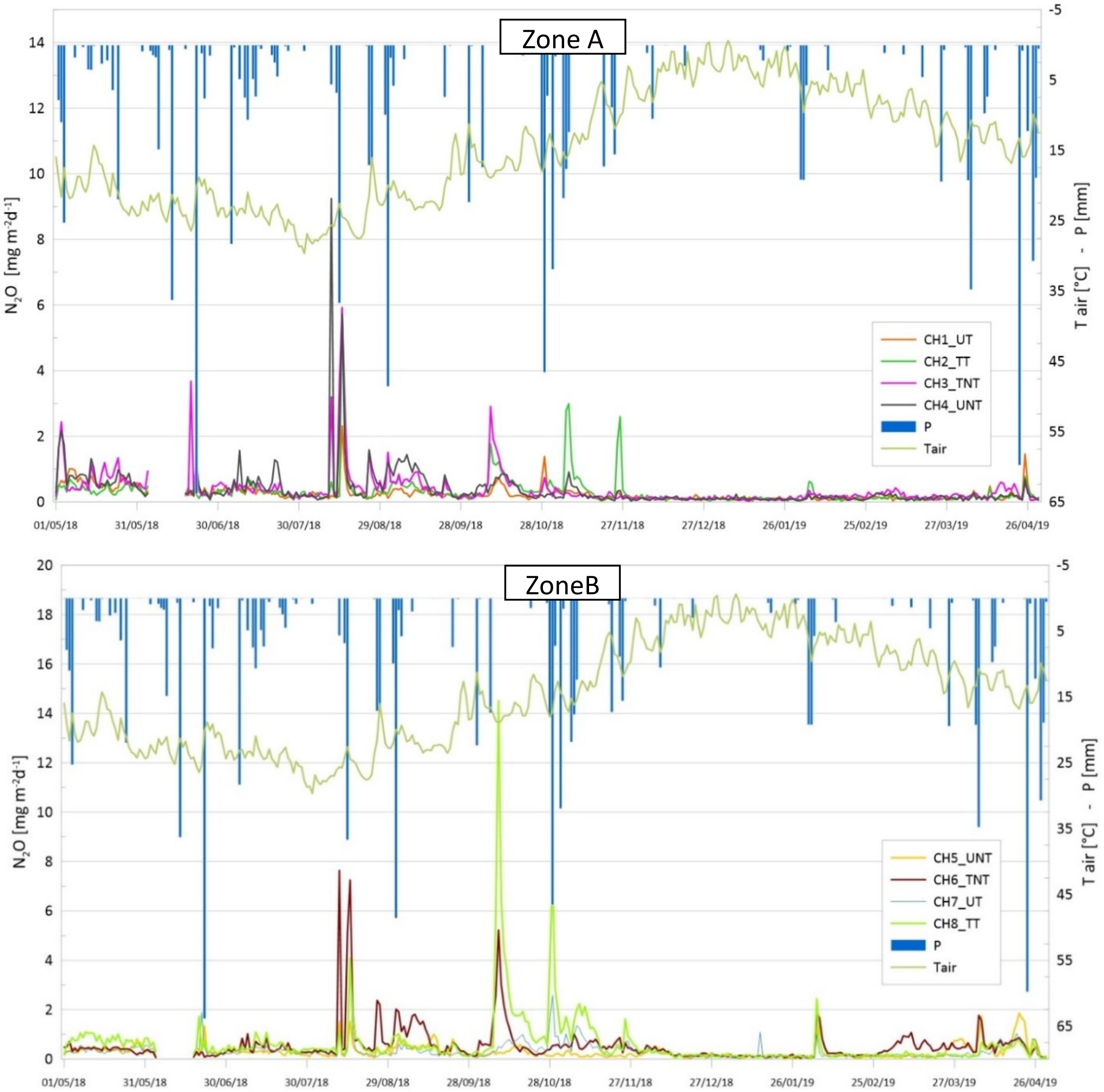

*Figure 3 - Time series of $N_2O$ emissions in mg $m^{-2}d^{-1}$ measured in each plot of zone A (above) and zone B (below). Detailed chambers fluxes are represented with different colors on left y-axes. Daily precipitation and average air temperature data are plotted on the right axis;*

Total annual precipitation is not very different from historical mean (-9%) but the distribution pattern was quite unusual for this area, with periods with lack (Dec-18 - 81%, Jan-19 - 94%) and periods with overabundance (Apr-18 +115%, Jun-18 +37%) of rains. Higher base fluxes and peaks were shown during the warm season, while during the cold and dry period the baseline fluxes were the lowest of the study time. Average background $N_2O$ emission, not related with fertilization or rain events, was 0.23±0.10 mg $m^{-2}$ $d^{-1}$ (1.48±0.62 g $N_2O$-N $ha^{-1}d^{-1}$) during summer while during wintertime it was 0.11±0.04 mg $m^{-2}$ $d^{-1}$ (0.68±0.28 g $N_2O$-N $ha^{-1}d^{-1}$). These values are higher than reported from other authors for Mediterranean vineyards with averages of about 0.5 g $N_2O$-N $ha^{-1}d^{-1}$ (Garland et al., 2014). From Figure 3 is visible the lower background fluxes from late November to spring, in particular when air and soil temperature are below 10 °C. The seasonal effect of temperature on the trend of $N_2O$ fluxes is highlighted by Table 4 where for each chamber the median value of $N_2O$ daily fluxes has been calculated in the warm period (from 1thMay to 8thOctober 2018) and in a colder period (from 1th November 2018 to 30th April 2019). This seasonal trend could be mainly addressed to the dependence of the kinetic reaction and the growth of microbial communities to temperature (Wang et al., 2021) and $O_2$ depletion from soil respiration processes that increase the anaerobic volume fraction (Smith et al., 2003). On the contrary, studies conducted in Mediterranean vineyard (Garland et al., 2014; Lazcano et al., 2022) found the higher fluxes during the cold-wet season, highlighting the dependence of $N_2O$ emissions to climate type (i.e. intertwining of temperature and rainfall).

*Table 4 - Median value of $N_2O$ fluxes expressed in mg $m^{-2}$ $d^{-1}$ for two different period: warmer (from 1th may to 8th October 2018) and colder (from 1thNovember 2018 to 30thApril 2019). Period from 9th to 30th October has been excluded because related with the treatment;*

| | **$N_2O$ fluxes [mg $m^{-2}d^{-1}$]** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **CH1_UT** | **CH2_TT** | **CH3_TNT** | **CH4_UNT** | **CH5_UNT** | **CH6_TNT** | **CH7_ UT** | **CH8_TT** |
| **Warm period** | 0.264 | 0.266 | 0.380 | 0.429 | 0.275 | 0.426 | 0.252 | 0.473 |
| **Colder period** | 0.061 | 0.087 | 0.110 | 0.058 | 0.120 | 0.284 | 0.136 | 0.206 |

Apart from this seasonal response, $N_2O$ fluxes follow the meteorological pattern, and a strong link appears between $N_2O$ emissions peaks and rains. As visible in Figure 3, in general, there are peaks of $N_2O$ emission whenever there was a precipitation. The effect of rains on $N_2O$ emissions has been emphasized by other authors, that found an increase of soil $N_2O$ fluxes after changes in soil moisture derived from irrigation and rainfall (Baggs et al., 2000; Alves et al., 2012; Wang et al., 2021). The effect of soil water content on $N_2O$ fluxes can be related to the effect determined by soil pore space saturation that induces a slower supply of $O_2$ through diffusion, creating anaerobic conditions and favoring denitrification (Khalil et al., 2005; Gregorutti and Caviglia, 2017). As an example of meteorological drivers, the high value of $N_2O$ measured in early August happened in presence of heavy rains, and the same situation occurred at the end of October, where fluxes decrease after the treatment and suddenly rise in conjunction with rains (Figure 4). This behavior is also highlighted in winter, where emissions curves in December and January are very flat due to absence of rains and lower temperatures, and then they suddenly rise after three days of abundant rain in early February. Besides the evident peak of emissions after abundant rains, a lot of smaller variation in fluxes magnitude can be noted in correspondence of precipitation events. Focusing on the period near the treatment application (October 8th2018) three major peaks of $N_2O$ fluxes can be identified (Figure 4).

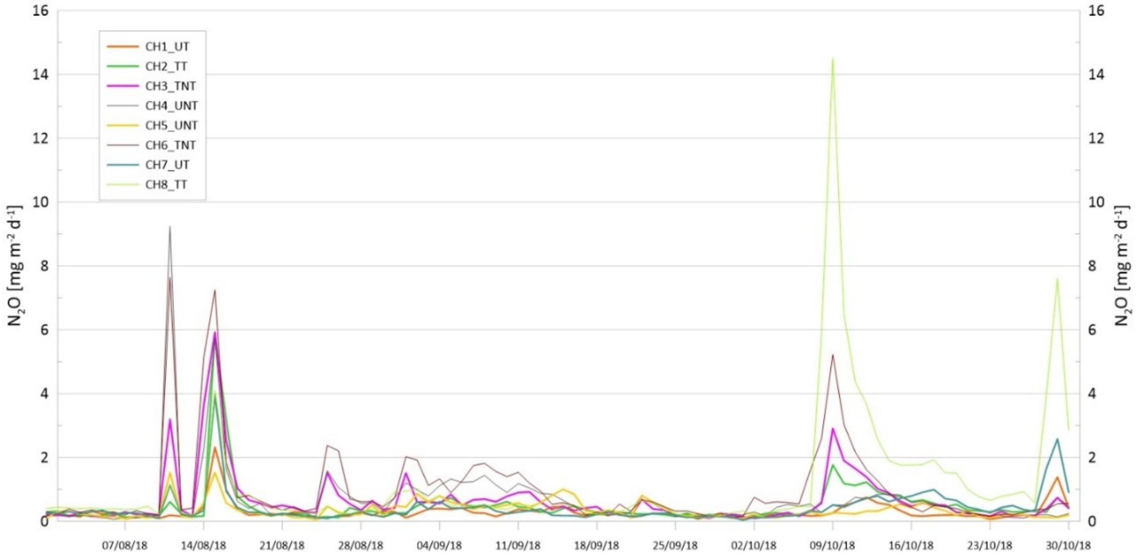

*Figure 4 - Focus on the three principal peaks (August – October 2018) of daily $N_2O$ emissions (mg $m^{-2}$ $d^{-1}$) detected during the study.*

The peak occurring in August 2018, as already described, can be associated to rainy events. No differences between untreated and treated plots can be observed as this peak occurs before treatment application (Figure 5). The most relevant peak is recorded immediately after the treatment application, but in this case only fertilized plots had an increase of emissions (TT and TNT), while the emissions of untreated plots (UT and UNT) remain on the baseline (Figure 5). After fertilization, treated plots tend to have higher peaks related to rains than untreated for the rest of the period (Figure 3 and Table 4). In treated chambers (total annual N input 176 kg $ha^{-1}$) the emissions peak occurred at same time (9th of October) and the daily emissions reach a maximum value in zone B, with of 14.50 mg $m^{-2}$ $d^{-1}$ (92.8 g $N_2O$ – N $ha^{-1}$ $d^{-1}$) in treated and tilled CH8_TT and treated not tilled CH6_TNT 5.23 mg $m^{-2}$ $d^{-1}$ (33.26 g $N_2O$–N $ha^{-1}$ $d^{-1}$). In zone A, CH2_TT and CH3_TNT has lower emissions, respectively 1.76 and 2.91 mg $m^{-2}$ $d^{-1}$ (11.3 and 18.5 g $N_2O$ – N $ha^{-1}$ $d^{-1}$). The study of Marques et al. (2018) in a Mediterranean vineyard with low SOM content, shows daily fluxes after fertilization (50 kg N $ha^{-1}$) and tillage of 10-30 g $N_2O$–N $ha^{-1}$. Similarly, (Garland et al., 2014) report a maximum peak after fertigation (5 kg N $ha^{-1}$) of 23 g $N_2O$-N $ha^{-1}$ $d^{-1}$, in dry climates, but with a maximum peak of 360 g $N_2O$–N $ha^{-1}$ $d^{-1}$ after the first abundant precipitation of the season. In another vineyard of northern California, with a total annual N input of 66.4 kg N $ha^{-1}$, Verhoeven and Six (2014) found a maximum flux rate of 141 g $N_2O$-N $ha^{-1}$ $d^{-1}$ after fall fertilization event, comparable to the major peak of our study. At

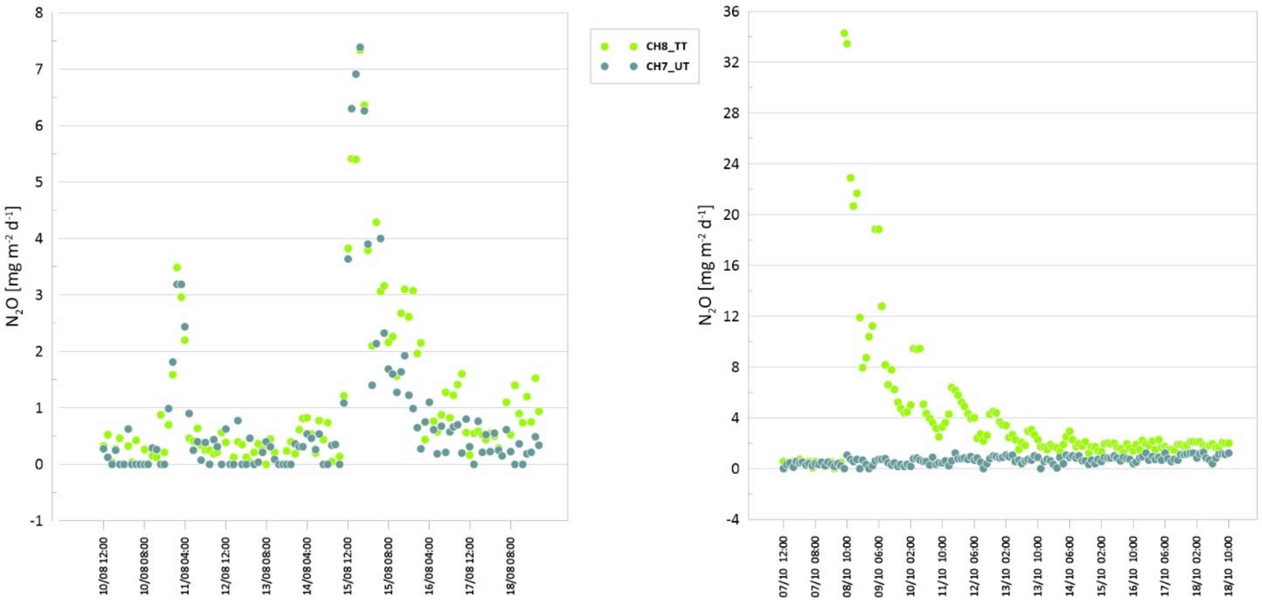

*Figure 5 - Focus on $N_2O$ emissions (data collected every 2 hours) in Chamber 7 (Untreated Tilled) and Chamber 8 (Treated Tilled): from 10 to 18 August the peak after rain (on the left) and from 7 to 18 October the peak after fertilizer application (on the right). Note the difference in scales.*

the opposite extreme, in a highly fertilized (up to 664 kg N ha$^{-1}$) and flooding irrigated vineyard in semi-humid continental climate, Guo et al. (2022) reached daily fluxes of 383 g $N_2O$–N ha$^{-1}$. Most of the emissions in fertilized plots occurred during the first 6-7 days after treatment followed by a decrease in $N_2O$ fluxes. After this, the slope of the curve decreases, until it reaches a baseline value with different times depending on the chambers, at maximum two weeks. This response is comparable to those observed by other authors. Cowan et al. (2020) tested the effect of N fertilizer application (ammonium nitrate and urea) from an intensively managed grazed grassland, and he reported an immediate increase in $N_2O$ emissions after the fertilization, reaching a peak within 7 days, with a return to fluxes near zero after two to three weeks. Bosco et al. (2019) highlighted high peaks of $N_2O$ a few days after fertilization events (4–10 days) in an irrigated vegetable crop rotation in the Mediterranean area. Other researchers (Garland et al., 2014) reported peaks lasting for only four days, in fertigated dry climate vineyard, while (Marques et al., 2018) showed peaks lasting some weeks to return to baseline, depending on treatment.

Considering the emissions immediately after the fertilization event (10 days) the cumulated value ranged between 65.5 and 296.5 g $N_2O$–N ha$^{-1}$, similarly to Verhoeven and Six (2014) where total emissions per fertigation event ranging from 54 to 244 g $N_2O$–N ha$^{-1}$. This means a direct and immediate loss of N supplied with fertilizer from 0.04% to 0.17%, lower than that reported from Garland et al. (2014) with fertigation (0.3-8.2%). A third peak can be highlighted at the end of October (October 29th) (Fig. 5) in correspondence to a rainy event. Differently from the peak of August, this peak is significant only for some plots and it is mostly associated to tillage: the higher fluxes are associated with CH8_TT and secondly to CH7_UT and CH1_UT. This tendency to have high fluxes in tilled plots, for both treated and untreated plots, could be due to the higher possibility of infiltration of water into the soil in tilled plots, that suddenly displace air previously accumulated in soil macropores (Carlisle et al., 2010; Tezza et al., 2019), resulting in a high flux effect.

### 3.2 $N_2O$ cumulative annual emissions

Cumulative emissions for each chamber are showed in Figure 6. The line plot highlights the emission peak (October 9th2018) derived from the treatment application of the day before. This peak is evident in case of Chambers located in Zone B (CH6_TNT and CH8_TT), while less visible in Chambers located in Zone A (CH2_TT and CH3 TNT). Cumulated annual emissions varies between 0.54 and 1.38 kg $N_2O$-N $ha^{-1}$ $y^{-1}$ (Table 5).

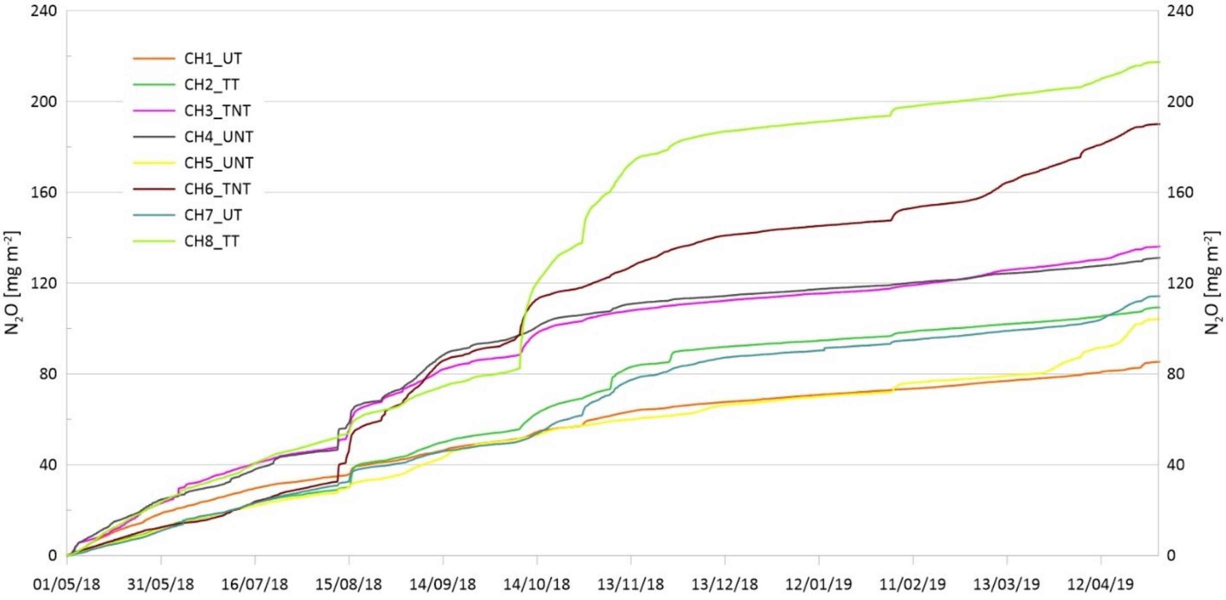

*Figure 6 - N2O cumulative annual emission expressed in mg m-2 calculated for each chamber*

*Table 5 - Total $N_2O$ emissions for each experimental plot in $mgN_2O$ $m^{-2}$ and $kgN_2O$-N $ha^{-1}$ (Different uppercase letters indicate significant difference ($P < 0.05$) between untreated and treated treatment chambers (ANOVA analysis);*

| | ZONE A | | | | ZONE B | | | |
|---|---|---|---|---|---|---|---|---|
| | CH1_UT | CH2_TT | CH3_TNT | CH4_UNT | CH5_UNT | CH6_TNT | CH7_UT | CH8_TT |
| **Total $N_2O$ [$mgN_2O$ $m^{-2}$]** | $85.3^{a}$ | $109.3^{b}$ | $136.1^{c}$ | $131.1^{d}$ | $104.2^{e}$ | $190.1^{f}$ | $114.3^{e}$ | $217.4^{g}$ |
| **Total N [$kgN_2O$-N $ha^{-1}$]** | 0.54 | 0.70 | 0.87 | 0.83 | 0.66 | 1.21 | 0.73 | 1.38 |

As described in relation to peaks, a consistent difference in $N_2O$ emissions from Zone A and Zone B can be observed for cumulated emissions. This is confirmed by ANOVA analysis that shows significative difference in $N_2O$ emissions from Zone A and Zone B for both treatment and untreated chamber, while in Zone B the $N_2O$ fluxes the untreated tilled and not tilled plots are not significantly different. In Zone B the effect of fertilization and fertilizer incorporation is evident. An average increase of 87% of $N_2O$ emission can be observed comparing fertilized treatments and not fertilized treatments (CH8_TT vs CH7_UT and CH6_TNTvs CH5_UNT), while an increase of about 15% and 10% can be highlighted comparing till treatments (CH8_TT vs CH6_TNT and CH7_UT vs CH5_UNT). These results are similar to those obtained by (Zhou et al., 2022) in a citrus orchard. Values of emissions obtained by these authors varied from 0.19 and 1.80 kg $N_2O$-N $ha^{-1}$ $y^{-1}$ and a positive relation to N application rates in chemical fertilizer treatments were also reported. In their meta-analysis, also Cayuela et al (2017) found an averaged emission of 1.2±1.5 kg $N_2O$-N $ha^{-1}$ $y^{-1}$ for perennial crops in Mediterranean climate. Other cumulated emissions from vineyard presented in literature were higher and ranged from 1.6 to 3.92 kg $N_2O$-N $ha^{-1}y^{-1}$ (Verhoeven and Six, 2014; Gardland et al., 2014; Guo et al ,2022). Seasonal (May-September) cumulated emissions ranged between 0.31 and 0.60, similarly to (Verhoeven and Six, 2014; Gardland et al., 2014; Guo et al ,2022) but higher than Garland et al. (2011), respectively 0.24-0.53 and 0.07-0.19 kg $N_2O$-N $ha^{-1}$. The effect of fertilization is less evident in Zone A, where no relevant differences can be observed between fertilized and non-fertilized plots, while treatments where fertilizer was not incorporated (CH3_TNT) recorded higher value compared to that incorporated (CH2_TT). This different behavior of Zone A and Zone B can be related to differences in soils carbon and nitrogen contents (Table 1). Zone A, where infield activities have been carried out by hand without access to agricultural machineries since 2015, and where no fertilization have been done in the period 2015- October 19th2017, was characterized by a lower value of TOC (8.10±2.13 g $kg^{-1}$), a lower value of N tot (0.87±0.15 g $kg^{-1}$) and a lower C/N ratio with respect to Zone B. Zone B where the soil has been fertilized with traditional products (solid fraction of digestate and chemical fertilization) according to company schedule until the start of this trial, shows higher average values for TOC (12.43±1.31 g $kg^{-1}$), N tot (1.10±0.09 g $kg^{-1}$) and C/N ratio. Previous studies suggest high C/N ratio as the optimum condition for the aerobic denitrification (Zheng et al., 2012). This is because, in addiction to soil temperature and dissolved oxygen concentration, another crucial factor affecting the activity of denitrification-related enzymes is soil organic carbon, resulting in bacteria often failing to fully denitrify under low C/N ratio conditions (Tong et al., 2014). Our results reflect the dependency of $N_2O$ fluxes to available soil organic carbon, for the same quantity of N inputs: total annual emissions of treated plots suggest a linear relationship with TOC ($R^2$ 0.86), where higher $N_2O$ fluxes were registered in the presence of higher level of organic carbon available for microbial activities. No relationships were detected between untreated plots and soil parameters, due to high CH4_UNT emissions, which deviate from the linear trend shown by the other non-fertilized plots in respect with C/N.

### 3.3 Emission Factors calculation

Table 6 shows the $EF_t$ and $EF_f$ for each plot. As a result of the differences founded between Zone A and B, EFs in CH8_TT and CH6_TNT (Zone B) are higher than CH2_TT and CH3_TNT (Zone A). For TT treatments $EF_t$ is between 0.004 and 0.008 g $N_2O$-N g $N^{-1}$, while compost not tilled varied from 0.005 to 0.007 g $N_2O$-N g $N^{-1}$. Average $EF_t$ value from all chambers results 0.006±0.002 Kg $N_2O$-N Kg $N^{-1}$, lower than IPCC $EF_1$ and in line with values reported by IPCC (2019) for N additions in wet climates, quantified in 0.006 Kg $N_2O$-N Kg $N^{-1}$ with an uncertainty range of 0.001 – 0.011 Kg $N_2O$-N Kg $N^{-1}$.

*Table 6 - Emission Factor (EF) for $N_2O$ emissions in vineyard: related to overall soil $N_2O$ emissions ($EF_t$) and related only to fertilizer application ($EF_f$);*

| | **$EF_t$ [kg $N_2O$ – N kg $N^{-1}$]** | **$EF_f$ [kg $N_2O$ – N kg $N^{-1}$]** |
|---|---|---|
| **Zone A - tilled** | 0.004 | 0.0009 |
| **Zone A - not tilled** | 0.005 | 0.0002 |
| **Zone B - not tilled** | 0.007 | 0.0031 |
| **Zone B - tilled** | 0.008 | 0.0037 |

This value of IPPC refers to an average value obtained from many studies (IPCC, 2006) that, based on specific experimental plan, can include or not N mineralized from mineral soil because of loss of soil carbon (i.e., $N_2O$ fluxes not attributable to fertilizer input). As indicated from IPCC (2006) and underlined by Zheng et al. (2004) and Marques et al. (2018), it is important considering emission factors without background emission (i.e. from a no-nitrogen addition control plot), to accurately represent the real emission factor deriving from N input application. In our study, this was represented by plots not fertilized (UT or UNT). Emission factors related only to fertilizer application, $EF_f$, ranged between 0.0002 and 0.0037 g $N_2O$–N g $N^{-1}$, with a mean value of 0.0019±0.0015 kg $N_2O$–N kg $N^{-1}$. These results were from 97% to 38% lower than IPCC EF, respectively for zone A and B. Zone B $EF_f$ were comparable to others presented in literature. In their study, Marques et al. (2018), obtained an $EF_f$ for NoTill+N treatment of 0.23 ± 0.29% and 0.57 ± 0.12% for the Till+N treatment. The average EF found by Cayuela et al. (2017) for perennial crops in Mediterranean climate was 0.54%. In a review of emissions factors from organic amendments additions, Charles et al. (2017) calculates a global mean $EF_f$ for organic fertilizers of 0.82 and describes compost as low risk amendments with a mean $EF_f$ or 0.27, similarly to our results. On the contrary Gu et al (2019), in a review of $N_2O$ emissions from orchards, found an average emission factor uncorrected for control plots ($EF_t$) of 1.76% of the applied fertilizer N, more than double of $EF_t$ found in this study. It must be said, however, that almost half of the data used for that average were between 0 and 1.

Considering Zone B as the most representative of the standard commercial vineyard conditions, as enterprise scheduling for soil management were applied regularly before the experiment, the emissions related only to fertilizer application is about 45% of the total emissions. The $EF_t$ for tillage treatment reacts differently: in Zone A the $EF_t$ decreases with incorporation, while in Zone B $EF_t$ increase with incorporation. Different responses to tillage and fertilizer incorporation were also found by previous studies (Longbottom and Petrie, 2015). In some cases, an increase of $N_2O$ emissions after tillage were reported (Drewer et al., 2017) while, in other context, no relevant effect of tillage on $N_2O$ emissions were underlined (Bosco et al., 2015). Garland et al. (2011) reported no significant differences in $N_2O$ fluxes from till and no-till treatment. In their study, Marques et al. (2018) report no differences for no tilled treatment alone, but 34% reduced emission in fertilized no tilled than fertilized tilled treatment in agreement with (García-Marco et al., 2016) where tillage increased $N_2O$ emissions by 68% compared to no-tillage in fertilized vineyard. Indeed, some authors reported greater emissions from undisturbed soils can be observed despite greater decomposition rates in cultivated soils (Staley et al., 1990). In our case, the exclusion of control plots from EFs clarify the role of tillage inverting the proportion between the EFs of till and no till in Zone A. As can be observed, $EF_f$ of fertilized not tilled plots were 79% and 17% lower than fertilized tilled treatments, respectively for area A and B. Also, these results suggest that low soil organic carbon content could lead to a lower loss of N due to $N_2O$ release to atmosphere.

## 4 Conclusions

Results obtained in the present study represent the first high temporal resolution monitoring of $N_2O$ fluxes from organic fertilization in vineyard, and the first EF calculation presented for a temperate tree crop. The equipment has allowed the collection of a large annual data set (4187 annual $N_2O$ measurements) which allowed the calculation of site-specific emission factors based on a robust time series.

A seasonal related trend was visible for both peak and background $N_2O$ fluxes, with majority of emissions during growing season until early autumn. A strong relationship between meteorology and $N_2O$ emissions can be observed: emissions peak was observed, both in treated and untreated sites, in correspondence of heavy rainfall events through the entire year. The maximum $N_2O$ fluxes were measured in fertilizer plots, where high emissions occurred during the first 6-7 days after treatment, followed by a decrease in $N_2O$ fluxes.

Annual cumulated $N_2O$ emission ranged between 0.54 and 0.73 kg $N_2O$-N $ha^{-1}$ $y^{-1}$ in non-fertilized plots and from 0.7 to 1.38 in fertilized plots. These were lower than other types of organic fertilizers compared to literature for vineyard, indicating compost as a soil amendment with low risk of emissions. Results indicate that $N_2O$ emissions from fertilized plots is linearly related to TOC content and data from unfertilized plots suggesting a tendency to a linear relationship with C/N, confirming other authors findings.

The calculated $EF_t$ was between 0.4 and 0.9 % of N input, in line with the IPCC value for organic fertilizers in wet climate. In case of $EF_f$, referred only to fertilizer application, the values were in range of 0.02 – 0.4% of N input. The high difference between the two types of Emission Factor calculations suggests more studies are necessary to investigate the contribution of background soil emissions to total $N_2O$ fluxes, and to better interpret soil management effects. The calculation of the $EF_f$, corrected with the control plots, allowed an interpretation of the effect of tillage and fertilizer incorporation, finding $EF_f$ of plot where fertilizer was not incorporated 48±31% lower on average than treatments where fertilizer was incorporated, with different magnitude of reduction depending on soil TOC.

These findings highlight the importance of clarifying the computation of EF and their use in literature, as the application of one type of EF instead of the other can give unclear information and great differences during $N_2O$ emissions estimation. IPCC reference value was confirmed to be a good estimator of the overall $N_2O$ emissions from organic fertilized soil, but too high for the estimation of only fertilizer application emissions. These results are of remarkable importance to improve the reliability and specificity of EFs, not only to have more effective estimates but, especially, to reduce impacts from fertilization management. These will be important to address agricultural policies and strategies towards higher environmental sustainability.

## Declaration of competing interest

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

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# Chapter IV:

# CLIMATE NEUTRALITY OF VITICULTURE: A COMPREHENSIVE MULTIANNUAL GHG BUDGET

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## Abstract

To limit the acceleration of global warming we need to reduce greenhouse gasses (GHG) emissions, making our production processes more C efficient, and optimize absorptions. Viticulture, and agriculture in general, is a sector with great and real possibilities of improving its environmental impact, with significant and cost effective GHG mitigation potential. In the last years, vineyards, and in general the orchards, have been shown to be a good C sink in the short and medium term, especially due to the peculiar management of the ground in a life cycle of decades. But are these sinks comparable to the GHG emissions by field management? This was the first multi-annual study combining carbon footprint (emissions of $CO_2$ from field management) and vineyard net ecosystem exchange (NEE - $CO_2$ absorptions) of wine making field phase. The average yearly anthropogenic emission of $CO_2$ was 2.59±0.89 $MgCO_2$-eq $ha^{-1}$, equivalent to 0.41±0.26 $kgCO_2$-eq per kg of yield or 0.47±0.30 $kgCO_2$-eq per bottle, of which major contributor were diesel consumption (about 61%) and $N_2O$ emission from fertilizer application (about 31%). We raised some concerns about carbon footprint (CF) calculation, which could overestimate emissions due to $N_2O$ and does not consider increase in soil organic carbon (SOC) due to organic fertilization. Anyway, the average comprehensive annual C balance was -0.85±1.82 $MgCO_2$-eq $ha^{-1}$, equivalent to -0.28±0.44 $kgCO_2$-eq $kg^{-1}$ yield or -0.32±0.51 $kgCO_2$-eq $bottle^{-1}$, indicating that the field phase of the wine production could be a potentially capable net sink of carbon. The results indicate high variability in the comprehensive annual C balance and its components (anthropogenic emissions, vineyard absorption and harvest), with usually, but not always, negative net balance (i.e. absorptions>emissions). This study suggests that optimizing processes in agriculture, at least tree crops, with strategies focused on C management that minimize emissions and optimize absorption, is a possible, effective and high value option. The calculation of the complete C footprint in the agricultural sector can be very useful in the perspective of carbon farming initiatives and for directing the management of tree crops towards climate neutrality, with a better addressing of environmental issues.

# 1 Introduction

Agriculture is one of the most vulnerable sectors to climate change and, at the same time, it contributes to climate change by releasing large quantities of greenhouse gases into the atmosphere (GHGs). Agriculture, forestry and other land use (AFOLU) are responsible for just under a quarter (12.0 ± 2.9 $GtCO_2eq\ yr^{-1}$) of anthropogenic GHG emissions, of which the agricultural sector was estimated to contribute to about a half (IPCC, 2019). In particular, cropland soils have lost 20–60% of their organic carbon content prior to cultivation, and soils under conventional agriculture continue to be a source of GHGs (IPCC, 2023). On the other hand, agriculture is a sector with great and real possibilities of improving its environmental impact, with significant and cost effective GHG mitigation potential. This would be through different and synergic actions, all referable to the enhancement of removals of GHGs as well as reduction of emissions through management of land (IPCC, 2023; Longbottom and Petrie, 2015; Smith and Olesen, 2010).

Terrestrial vegetation plays an important role in the global carbon budget, absorbing from the atmosphere about 29% of CO anthropogenic emissions (IPCC, 2019). In the accounting of land ecosystems GHG, forest ecosystems are recognized as land carbon sink, while biogenic fluxes from agricultural lands are not considered. Neutrality of vegetation C balance can be true for those agricultural products with an annual cycle where most of the organic matter is removed every year with the harvest. The vineyards, and in general the orchards, have instead been shown to be good C sinks in the short and medium term (Gianelle et al., 2015; Pitacco and Meggio, 2015; Scandellari et al., 2016; Vendrame et al., 2019; Zanotelli et al., 2018), not only for their wood structure but also thanks to the increase in Soil Organic Carbon (SOC) due to the peculiar management of the ground in a life cycle of decades (Callesen et al., 2023; Montanaro et al., 2017; Gianelle et al., 2015; Scandellari et al., 2016; Tezza et al., 2019; Williams et al., 2020; Xue et al., 2024). For this reason, in the last years the focus of grape ecology research has shifted towards the role of vineyards as carbon sinks and their soil carbon sequestration capacity (Xue et al., 2024). But are these sinks comparable to the GHG emissions by field management? The possibility to enhance agricultural sustainability is clearly bound to the actual capability to track and quantify the overall GHG fluxes. Furthermore, it is important to distinguish the different steps in the production chain and assign their relative environmental impact.

The carbon footprint (CF) based on international standards (UNI EN ISO 14064 and 14067) is a common instrument used to assess and report the environmental performance of a production cycle in terms of GHGs emission, able to quantify the direct and indirect GHG emissions (Pattara et al., 2016; Pinto da Silva and Esteves da Silva, 2022; Rugani et al., 2013). However, in contrast to industrial systems, agriculture is characterized by fluctuating GHG fluxes from nonpoint sources and a high degree of system variability (Goglio et al., 2015; Miller et al., 2006). The difficulties encountered by applying CF to the agricultural sector are also linked to the great variability of practices and technologies adopted. This turns into a lack of standardized methodologies and unavailable information and data regarding specific processing steps, especially in field (Adewale et al., 2018; Arcese et al., 2012; Pattara et al., 2016; Rugani et al., 2013). Beside this, consistency of agricultural stage CF is also particularly lacking in the choice of functional units, definition of system boundaries and specificity of emission factors (EFs) (Adewale et al., 2018; Minardi et al., 2022). For its both economic importance and global distribution, the wine making chain is one of the most analyzed within the agro-food sector (Barber et al., 2009; Bosco et al., 2011; Point et al., 2012), and it is under increasing pressure to adopt approaches for environmental assessment and reporting of product-related greenhouse gas emissions (Pattara et al., 2016).

The impact of wine sector on the GHG emissions of worldwide human activities was estimated to be around 0.3%, corresponding to about 2% the CF of the agriculture sector (Rugani et al., 2013). Being aware of the big technological, geographic and managing differences, reviews of literature case studies found values ranging from 0.15 to 3.51 kg $CO_2$ eq. per bottle of wine (0.75 L), demonstrating the high variability in CF estimates (Pattara et al., 2016; Pinto da Silva and Esteves da Silva, 2022; Rugani et al., 2013). The processes with higher impact on winemaking CF are viticulture activities, packaging processes and end- of-life (Bosco et al., 2013; Notarnicola et al., 2003; Pattara et al., 2016; Petti et al., 2015; Rugani et al., 2013). To reduce the overall impact, (Arcese et al., 2012) pointed out that it is necessary to focus on the productive phase more than others. Indeed, improvements can be made on shorter term, and effort pays off more, if applied directly on viticulture and wine making activities rather than on packaging or transport, which are dependent from highly standardized process and location of the market, that are not under winery control (Bosco et al., 2011; Pattara et al., 2016). Viticulture phase presents high variability, averaging typically around 16-40% of total CF (Bosco et al., 2011; Point et al., 2012; Rugani et al., 2013) but often is identified as the most relevant phase, up to more than 90% (Adoir et al., 2019; Johnson et al., 2007; Neto et al., 2013; Pinto da Silva and Esteves da Silva, 2022; Vázquez-Rowe et al., 2013, 2012) proving to be an excellent phase to focus on. The principal agricultural sources of $CO_2$ in viticulture are related to fuel combustion and soil management practices (Bosco et al., 2011; Marras et al., 2015; Tomaz et al., 2024) which includes increasing decomposition of Soil Organic Matter (SOM) and release of $N_2O$, while $CH_4$ emissions in viticulture are insignificant (Brodt and Thrupp, 2009; Carlisle et al., 2010; Gu et al., 2019; Wolff et al., 2018).

However, to have a more complete understanding of the impacts of viticultural activities and improve the accuracy and reliability of field stage CF, an integration of estimating and measuring methods for accounting biogenic fluxes is desirable (Arzoumanidis et al., 2014b; Montanaro et al., 2021; Rugani et al., 2013). This could be a lever to stimulate the adoption of sustainable management and to an optimal and efficient targeting of GHG mitigation actions, clarify the importance of SOC sequestration and protection as a GHG emission mitigation tool (Adewale et al., 2018; Arzoumanidis et al., 2014a; Montanaro et al., 2021; Novara et al., 2020; Rugani et al., 2013). Unfortunately, most of the studies about agricultural CF operate without considering ecosystem services. The need to include all the biogenic C exchanges in CF computations is highlighted in the ISO/TS 14067:2013 and technical specification, and soil C management and absorptions have been included in guidelines for the compilation of CF (BSI, 2008; ISO, 2013, OIV, 2017, 2015). Carbon farming, was also recently adopted as a fundamental strategy in the Union certification framework for carbon removals (COM(2022) 672 final). Despite this, no real consensus has been reached on how to account for these components in the C footprinting (Arzoumanidis et al., 2014a; Marras et al., 2015; Pinto da Silva and Esteves da Silva, 2022). As a result, CF analysis considers anthropogenic C emissions and usually omits biogenic carbon issues (Marras et al., 2015), which are traditionally considered GW-neutral (Arzoumanidis et al., 2014b).

This is primarily due to challenging C soil measurements (Smith et al., 2020) and no common appropriate methods and technical standards for C accounting in agriculture (Adewale et al., 2018; Arzoumanidis et al., 2014a; Brandão et al., 2013; Montanaro et al., 2021). Secondarily, biogenic fluxes are considered part of the short-term carbon cycle (Gianelle et al., 2015; Pattara et al., 2012) or balanced from the oxidation of organic carbon in pruning waste and grapes (Neto et al., 2013). Thus, C balance of the vineyard is assumed to be neutral, with no concern about the timing difference between uptake and release, higher sequestration than $CO_2$ emission by oxidation of organic matter from pruning debris and yield, or SOC increasing during time thanks prolonged turnover period of soil microbial dead residues (Arzoumanidis et al., 2014a; Bosco et al., 2013; Gianelle et al., 2015; Novara et al., 2019; Xue et al., 2024). For these reasons, some authors hope for an harmonization of various frameworks of CF calculation along with the integration of others ecosystem services components for a more comprehensive and detailed mapping of sustainability in agriculture (Montanaro et al., 2021; Williams et al., 2020), as vineyard C sequestration can be essential to mitigate emissions of viticultural stage (Pinto da Silva and Esteves da Silva, 2022).

Effectively, when included, both soil and vegetation C budgets can significantly affect a viticultural CF. Some studies showed that considering estimated C accumulation in soil and vegetation, total emissions of the production phase (i.e. viticulture or field stage) can vary substantially, changing from source to sink of C (Bosco et al., 2013; Martins et al., 2019; Montanaro et al., 2021; Williams et al., 2020; Wolff et al., 2018), with a better addressing of environmental issues (Novara et al., 2020). Unfortunately, very few studies coupled carbon footprint estimated with LCA method with actual measurements of ecosystems C fluxes. These demonstrated that accounting for net vineyard $CO_2$ exchange in viticulture CF (i.e. the production phase) contributed to substantially offset the production phase CF (Marras et al., 2015) or, at least, to its neutrality (Chiriacò et al., 2019). However, to the best of our knowledge, these are the unique studies about the integration of CF with measured $CO_2$ balance, at least for vineyards. Unfortunately, these were studies over a time step of one year where no consideration about time variability and consistency of fluxes were available. Thus, the short time scale can potentially turn into influences of vintages specific trend and issues over both vineyard or anthropogenic fluxes, for example particularly lower yield or NEE due to unusual weather.

Resuming, wine CF presents high variability due to low standardized calculation and processes, and, in addition, presents high variability in viticulture phase and yield, even for the same product in different years. Thus, to evaluate the comparability of biogenic VS anthropogenic fluxes in vineyards, long-term studies are desirable to assess the temporal variability and continuity of fluxes and total C budget. This study aims to contribute to this lack, investigating the vineyard mitigation potential on anthropogenic emissions produced by agricultural activities over a multi-year time span. To attempt at this scope, vineyard C fluxes were measured with eddy covariance (EC) technique and coupled to the classic CF computation for the field phase of wine making during a period of four years. This will lead to a better and more reliable knowledge about carbon balance of the viticulture phase, enabling to evaluate its variability over the years, and to appraise fluctuation in both absorption and emission of $CO_2$. This kind of information, focused on field activities and their improvement, is of remarkable interest to enhance the reliability of CF calculation for the wine sector and to improve viticulture sustainability towards climate neutrality. Results obtained can also be of great utility for policy makers, supporting carbon farming strategy and allowing to plan future agro-environmental policies, or in national or international emission accounting framework.

## 2 Materials and methods

### 2.1 Description of site and study period

Ecosystem net $CO_2$ fluxes have been monitored with Eddy Covariance technique since July 2015 in an organic commercial vineyard property of “Guido Berlucchi & C.” historical winery, and measurements are still ongoing. The vineyard is located in Northern Italy near Erbusco (Brescia) in the Franciacorta DOCG Region, a hilly area between Brescia and the southern end of Lake Iseo. It was planted in 2000, with cv. Chardonnay grafted on different rootstocks (e.g. 41B, SO4, 775P, Fercal), on an area of about 17 ha, in place of a previous vineyard. Vines are vertical shoot position (VSP) trained and spur-pruned cordon, with rows oriented along N−S direction. The distance between rows is 1.2 m and vines are 0.8 m apart, resulting in a planting density of

10 416 vines ha$^{-1}$. Vines height at full development is kept at 1.5 m. Vineyard is rainfed and alleys are covered with spontaneous herbaceous vegetation. Field works (canopy and soil management) are conducted with straddle machinery, while winter pruning and harvest are manual, except for 2015 where a mechanical pre-pruning was carried out before manual pruning. Winter pruning debris are left in the field and shredded. The company is managed in compliance with the European Regulation on organic farming (regulation (EC) no. 2018/848 and subsequent amendments and additions). Therefore, no chemical fertilizers are used. Different quantities of manure fertilization were applied over the years. Specifically, 19 Mg ha$^{-1}$ have been applied in 2016, 17 Mg ha$^{-1}$ in 2017 and 31 Mg ha$^{-1}$ in 2019, while during 2018 fertilization was avoided. An organic pelleted fertilizer (0.8 Mg ha$^{-1}$) was also used in 2016, in addition to manure. According to the organic conduction of the vineyard, spontaneous vegetation control is mechanical. As a consequence, soil management was performed several times during each year of the study period, with irregular schedule and intensity depending on the peculiarity of the season. Franciacorta area is essentially of morainic origin and, according to WRB classification (IUSS, 2022), the vineyard soil is a Cutanic Luvisols (Humic, Endoskeletic, Siltic), with about 10% of skeleton and fraction of clay, silt and sand of about 8.7%, 12.8% and 78.5% respectively. Total organic carbon (TOC) was 3.5%. It is a deep and well-drained soil, with saturation point of 33%, field capacity of 16.6% and wilting point of 7.3%. Franciacorta is in the insubric mesoclimatic region (Cfa climate, according to Koppen classification), with relatively mild winter, warm and moist summer, and moderate daily and annual temperature variations. Average annual daily temperature is 12.3 °C, while average minimum and maximum annual temperature are respectively 7.6 and 17.1 °C. Annual total precipitation is about 900 mm, of which 2/3 generally falls during vegetative season (1982-2012 average data from Climate-data.org). In this study, four years of measurements are presented, from November 1st 2015 to October 31st 2019. Both measurement of vineyard $CO_2$ fluxes and carbon footprint estimation were splitted into 4 sub-periods, from November 1st to October 31st, obtaining 4 years of data (November 1st 2015 - October 31st 2016, November 1st 2016 - October 31st 2017, November 1st 2017 - October 31st 2018, November 1st 2018 - October 31st 2019, referred from here in the text as 2016, 2017, 2018, 2019). Data collection from October 2016 to October 2019 were carried out within the project LIFE15 ENV/IT/000392 LIFE VITISOM.

## 2.2 Vineyard $CO_2$ flux measurements

The monitoring of ecosystem $CO_2$ fluxes was carried out using the micrometeorological method of eddy covariance (EC) that allows for the direct measurement of fluxes between a surface and the atmosphere, based on the principles of fluid dynamics (Baldocchi et al., 1988). The technique requires fast (≥ 10 Hz) measurements of vertical wind velocity (*w*) and simultaneous concentration (*c*) of the gas of interest. The wind velocity components can be measured with a 3D sonic anemometer, while $CO_2$ and $H_2O$ concentration with an Infrared Gas Analyser (IRGA). The EC station (48°37'8.58"N 10°0'8.26"E) was equipped with a USA-1 sonic anemometer (Metek, Elmshorn, Germany) and a LI-7500 open-path IRGA (Li-Cor Biosciences, Lincoln, NE, USA) installed on a steel pole, at 3 m height. Air temperature and humidity have been measured with HMP45C sensor (Vaisala, Helsinki, Finland) and short- and long-wave radiation components with a CNR1 net radiometer (Kipp & Zonen, Delft, The Netherlands). Data have been recorded with a CR1000 Campbell Scientific datalogger (Campbell Scientific, Logan, UT, USA).

Meteorological variables (air temperature and humidity and atmosphere pressure) have been sampled at a frequency of 1 Hz, with statistics computed every 30 minutes. While temperature and global radiation data presented were collected at the station, rainfall data were acquired from regional meteorological service (ARPA) station of Sarnico (45°39'57.4"N 9°57'24.8"E), about 6 km from the experimental site. Fluxes of $CO_2$ were computed as the covariance between *w* and *c* over a 30 minutes time interval. Corrections and quality control of raw data need to be implemented to obtain final fluxes (Aubinet et al., 2000). We used the open-source software EddyPro® version 6.2 (Li-Cor Biosciences, Lincoln, NE, USA) for EC data processing. Gaps resulting from data filtering of periods with rain, distorted wind, or unrealistic values, were filled by modelling NEE fluxes following the approach by (Desai et al., 2005). The 30 minutes time series were aggregated as integrals at daily, monthly and annual time scale.

Ecosystems GHG fluxes can be both emissions in- and removals from- the atmosphere and, therefore, the net $CO_2$ flux (or net ecosystem exchange - NEE) is calculated as the sum of them. In this work we follow the micrometeorological convention that assign positive sign (+) for net transport from the surface toward the atmosphere (emissions), and the opposite (removals from atmosphere due to absorption by the vegetation) with a negative sign (-). It is worth to remember that turbulent flux measurements are strictly bounded to local environmental conditions occurring in the field, especially to wind speed and direction, and one of the fundamental assumptions of this technique is the spatial homogeneity of the source area. For these reasons, the application of EC over spaces with moving point sources - like emissions from tractors that can cross the EC footprint – is very challenging due to the spatially and temporally uneven distribution of these $CO_2$ point sources, as well as the possible distortions of the flow due to the objects themselves and the ephemerality of the occurrence (Felber et al., 2015; Marras et al., 2015). Thus, anthropogenic GHG emissions produced by human or machinery activity in the field were not registered in the EC measurements and, thus, not embedded in NEE, but accounted in CF.

## 2.3 Carbon footprint assessment

Anthropogenic $CO_2$ fluxes produced by field operations were determined through the CF calculator Ita.Ca® - Italian wine carbon calculator. This calculator has been developed in 2009 by Sata Agronomic Study in cooperation with the DiSAA (University of Milan), with the aim of adapting the IWCC (International wine carbon calculator) to the Italian wine context. Compliant with IWCP - International Wine Carbon Protocol and GHGAP - Greenhouse Gas Accounting Protocol (OIV, 2015), Ita.Ca® allows to assess the GHG emissions accounting of wine making process following the standard UNI ISO 14064. The purpose of the present paper is to examine the overall net GHG budget of the field phase of wine making. Therefore, vineyard management has been included in the CF, while wine making and marketing were not considered. Specifically, aspects related to pest management, soil management and canopy management have been accounted. Temporal system boundaries were referred to the time period November 1th 2015 to October 31th 2019; for this reason, land use change and vineyard planting phase were not included.

The inventory data was collected directly on site at the winery through specific questionnaire, interviews and cultivation register. It has been referred to the reference period of one year (from November 1st to October 31st), as for NEE measurements. Specifically, the following information has been required: i) hours worked with agricultural machinery subdivided by agronomic practices (Supplementary material - Table 1); ii) quantity of organic fertiliser used; iii) quantity of pesticides used (i.e. fungicides, insecticides); In the Carbon footprint assessment $CO_2$ and $N_2O$ emissions generated by the following items were considered: i) fossil fuel consumption related to agronomic practices (in this case, for direct emissions included in Scope 1, $CH_4$ emissions were also considered according to product category rules (PCR) for arable crops (PCR 2020:07); ii) organic fertilisation ($N_2O$ from organic fertilizers distribution); iii) indirect emissions related to fertilizer production; iv) indirect emissions related to pesticides production; v) emissions related to products transport (fertilizers and pesticides); vi) emission related to disposal and transport of waste deriving from vineyard management. Emission factors and references are reported in Table 1. Emissions related to fossil fuel consumption has been estimated trough the conversion of hours dedicated to each mechanical operation (Supplementary material - Table 1) to litres of fuel consumed. The conversion has been carried out applying fuel consumption data determined by Sata Studio Agronomico, and based on primary data collected from winegrowers: i) organic fertilisation (8 L $h^{-1}$); ii) pest control (14 L $h^{-1}$); iii) grass mowing (5 L $h^{-1}$); iv) under-row blade (4.5 L $h^{-1}$); v) heavy inter rows tillage (7.3 L $h^{-1}$); vi) light inter rows tillage (5.1 L $h^{-1}$); v) topping (5.9 L $h^{-1}$); vi) pre-pruning (4.5 L $h^{-1}$). In the case of trimming and trunk renewal of vines a coefficient equal to light inter rows, tillage was used, while for mechanical de-suckering the same conversion of topping was adopted. Once the data expressed in litres of fuel were obtained, the emission factor shown in Table 1 was applied to compute $CO_2$-eq emissions. Emissions related to products transport were calculated considering the distances from distributors to company premises. Conversion into $CO_2$-eq was made according to IPCC (2021) considering the following GWPs: i) 1 for $CO_2$; ii) 273 for $N_2O$. Regarding the treated GHG species, methane fluxes are usually originated in soils with high water content and low permeability (IPCC, 2006). Thus, $CH_4$ is usually not produced in well drained soils of vineyards (Carlisle et al., 2009), and it was excluded from CF calculations, with the exception of direct emissions deriving from fuels where a value of 82,5 $CO_2$-eq was used (IPCC, 2021). Where no specific EFs were available, and specifically in case of *Bacillus amyloquiefacens* and *Bacillus thuringiensis*, an EF equal to 6.130 kg$CO_2$-eq was used. This value was derived from the average value of EFs of all fungicides included in Ita.Ca®.

*Table 1 Emission factors used for each item considered and relative data source reference*

| Item | | Emission Factor | UoM | Reference |
|---|---|---|---|---|
| | Fuel consumption | 2.513 | kgCO$_2$-eq L$^{-1}$ | DEFRA (2024) |
| Fertilizers | Manure production (0,8% N) | 0.003 | kgCO$_2$-eq kg$^{-1}$ | Bilan Carbon |
| | Organic pellet fertilizer production (10,5% N) | 5.030 | kgCO$_2$-eq kg$^{-1}$ | ADEME (2014) |
| | N$_2$O from fertilizers | 0.022 | kgN$_2$0 kgN$_{\text{fertilizer}}$$^{-1}$ | Product Category Rules (PCR) (2020) |
| Insecticides | Isonet | 2.425 | kgCO$_2$-eq kg$^{-1}$ | Ecoinvent 3.8 |
| | Mineral Oil | 6.202 | kgCO$_2$-eq kg$^{-1}$ | Bilan Carbon (2011) |
| | Piretrine | 16.608 | kgCO$_2$-eq kg$^{-1}$ | Ecoinvent 3.8 |
| Pesticides | Copper hydroxide | 4.600 | kgCO$_2$-eq kg$^{-1}$ | LCA Manica[1] |
| | Copper oxychloride | 1.300 | kgCO$_2$-eq kg$^{-1}$ | LCA Manica[1] |
| | Sulfur | 0.195 | kgCO$_2$-eq kg$^{-1}$ | Ecoinvent 3.8 |
| Waste disposal | Special waste | 0.354 | kgCO$_2$-eq kg$^{-1}$ | DEFRA (2024) |
| | Paper and cardboard | 0.031 | kgCO$_2$-eq kg$^{-1}$ | DEFRA (2024) |
| Products and waste transport | | 0.00038 | kgCO$_2$-eq kg$^{-1}$ km$^{-1}$ | DEFRA (2024) |

[1]Data communicated directly by production company calculated by LCA

## 2.4 Global net GHG budget of vineyard

The carbon budget of an ecosystem is the balance between sources (+) and sinks (-) of CO$_2$ present in the system. In the same way, the comprehensive carbon budget of the field phase of wine making (GHG$_{\text{NET}}$) was computed as the algebraic sum of the amount of GHG emitted (+) or absorbed (-) by its components:

$$GHG_{net} = NEE + F_{CF} + F_{yield}$$

where F$_{\text{CF}}$ represents the anthropogenic CO$_2$-eq emissions computed with the CF while F$_{\text{YIELD}}$ represents the amount of carbon leaving the systems due to grape harvest. Indeed, the carbon fixed in grapes was measured by EC, but the yield was moved out of the system boundaries and oxidized in subsequent steps. Thus, it must be considered as a loss of carbon. Differently, pruning debris and manure were left in the field, and the fluxes of $CO_2$ produced by their decomposition were measured with EC and, actually, embedded in NEE amount. The grape yield was converted into kgC considering 10% of carbon content in cluster fresh weight, as suggested by Morandé et al. (2017). Subsequently it was converted into kg of $CO_2$.

Anthropogenic emissions, biogenic fluxes and final net C balance were determinate considering three distinct functional unit (FU): the unit of land (1 hectare - $FU_{ha}$), the unit of yield (1 kg - $FU_{yield}$) and the unit of production (0.75L bottle – $FU_{bottle}$). Besides an easier comparison with literature, calculation of $F_{CF}$ per $FU_{ha}$ allows a clear comparison with the data from EC measurements, which are computed for units of land. On the other hand, as several authors demonstrated, the use of different kind of FUs could be necessary and appropriate to assess the environmental impact of agricultural productions (Charles et al., 2006; Chiriacò et al., 2019; Ferng, 2011; Harada et al., 2007; Meier et al., 2015; Notarnicola et al., 2012). In fact, the mostly used unit of product (yield or bottle) may not be the most suitable for the evaluation of CF in production systems that involve ecosystems management, where a land-based FU could be more comprehensive and effective. Indeed, the greater GHG emissions per hectare due to higher agricultural input and management intensity in intensive production systems could be hidden by the higher productivity in respect to more sustainable managed systems (Chiriacò et al., 2019; Renzulli et al., 2015; Steenwerth et al., 2015). Still, results of CF studies in wine sector, both for field and cellar phase, are often presented considering as FU the classical 0.75L bottle of wine, but this value cannot always clearly be converted in a value per hectare or quantity of grapes harvested, due to lack in presented data. In order to allow for the widest possible comparison with data in literature, the three types of FU were calculated. The harvest of this vineyard is used in the production of different types of wine in conjunction with grapes from other vineyards according to the production specification. Thus, it was not possible to have the actual number of bottles produced or make a correct allocation. Therefore, the conversion to $FU_{bottle}$ was made considering 1.15 $kg_{grape}$ $bottle^{-1}$, the maximum allowed by the production specification. This value is very similar to the one used by Marras et al. (2015).

# 3 Results and discussion

## 3.1 Weather framework

The study period was characterized by almost always higher average (T_avg), minimum (T_min) and maximum (T_max) monthly temperatures compared to the 20-years mean (Figure 1). Average temperature anomalies were constantly positive in all months, being higher during summer (Figure 1a). For the whole time series, the average (ΔT_avg), minimum (ΔT_min) and maximum (ΔT_max) temperatures anomalies were respectively +2.07°C, +2.96°C and +2.54°C. In this overall warmed picture, there were still minimum temperatures below freezing point during the winter (Figure 2b). Others specific freezing spot events were identified, such as the late frosts of Apr 19th 2017 (-0.5 ° C), a cold wave from Feb 28th to Mar 2th 2018 with temperatures during the day always below 0 (minimum of -7.6 ° C) and Mar 23th 2018 (-1 ° C). Beside this, summer average temperatures are also higher than climatic averages and the higher temperatures were reached during heat waves on Aug 3th 2017 (36.5°C) and Jun 27th 2019 (36.3 °C) (Figure 2b). Over the entire study period the average increase in total annual rainfall was minimal (8%), reaching a maximum of 1245 mm (+37%) in 2017/2018 period and a minimum of 758 mm (-17%) in 2016/2017. Strong positive and negative anomalies in monthly precipitations (ΔP) were recorded (Figure 1b), with a tendency of rainfall increase in spring and summer months in respect to 20 years mean, with the higher average precipitations anomaly in May (+86% on average) (Figure 1b).

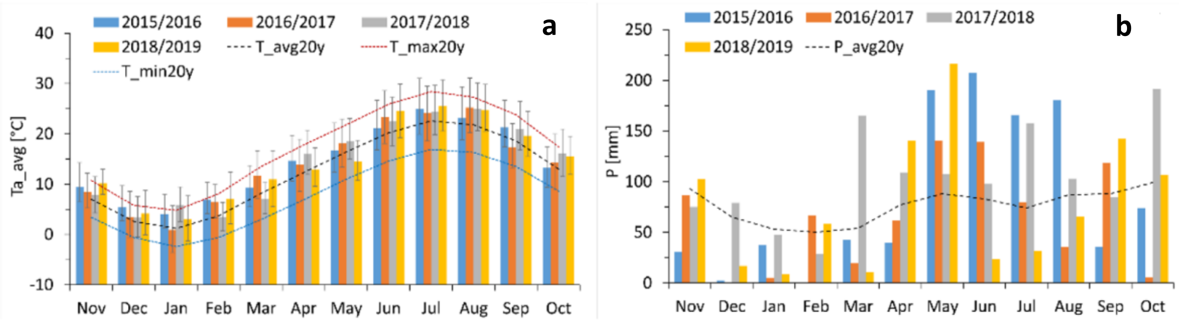

*Figure 1 - Monthly average temperatures (a) and precipitation (b) over the entire study period. Whiskers indicates averages maximum and minimum temperatures, while dashed lines indicates 20 years average of mean, minimum and maximum temperatures;*

Conversely, there was a general decrease in precipitation during the autumn and winter months with a minimum in the average anomaly in December (-62%), with some months with no precipitations (Feb and Dec 2016, Figure 1b). Precipitations were often concentrated in short periods, and the higher daily precipitation was on Oct 29th 2018 (113 mm) (Figure 2b). The result of this general picture was that, especially in spring, temperatures values and rainfall amount above the average could have favored the onset of fungal diseases, while summers are characterized by unstable weather with above-average temperature peaks, interrupted by storm disturbances that, in some cases, could lead to vines damage (for example hailstorm on July 20th 2018).

### 3.2 Vineyard $CO_2$ fluxes measurements

Vineyard demonstrated to be a sink of carbon in all the four years of the study, reaching a net C budget at the end of the period of -2.5 $kgCO_2\ m^{-2}$ (or -694 $gC\ m^{-2}$), that correspond to an average intake of 6.4±1.8 $MgCO_2\ ha^{-1}\ year^{-1}$. The annual carbon balance was variable in the four years of measurements, being -838.1, -616.5, -362.4 and -726.8 $gC\ m^{-2}$ from 2016 to 2019, respectively. As expected, there was a clear seasonal pattern in the biogenic $CO_2$ fluxes (Figure 2a) of the vineyard, with positive daily integrals (corresponding to emissions of $CO_2$ in the atmosphere) in autumn and winter, that eventually turns slightly negative during early spring and reaching a peak of absorption with full canopy development in early summer. In late summer, the magnitude of the absorption becomes lower due to heat stress and drought, with an eventual moderate increase at the end of the season, thanks to lower temperatures and rainy days. As NEE depends primarily on meteorological and environmental conditions (Vendrame et al., 2020), during spring and summer daily fluxes were less negative or even positive as a consequence of cloudy days or in periods of water/heat stress. This is clearly visible

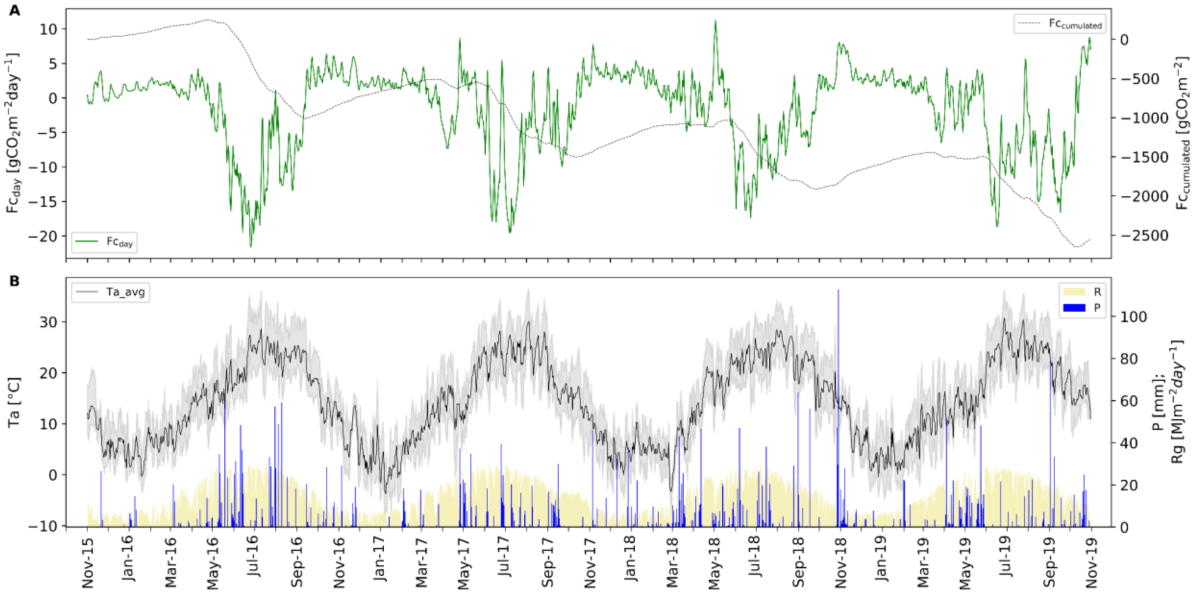

*Figure 2 - (a) Daily integrals ($Fc_{day}$, green line) and cumulative course of NEE ($Fc_{cumulated}$, dotted line) over the 4 years of the study; (b) Daily total precipitation (P, bars), daily total global radiation (Rg, yellow area) and daily average air temperature (Ta_avg, solid line). Grey areas correspond to daily amplitudes of air temperature, i.e. difference between max and min daily temperatures;*

in Figure 2, when relative minimum peak of absorption in spring and summer correspond to lower solar radiation and rainy days.

The described pattern of the absorption trends results distinctly in the monthly C budget of the vineyard (Figure 3): monthly NEE was positive from October to February and it turned negative from June to September. Therefore, summer was the most significant season for the annual carbon absorption, while spring tended to be very variable. In fact, from March to May, monthly NEE sign was unstable, depending on the specific meteorological conditions of the year that influence ground cover activity and vines bud break timing (Figure 3). Indeed, in years in which the absorptions started earlier and the monthly integral in April (or March) was already negative, May occurred with a positive integral. This could be because the sooner the vegetation starts to growth (and therefore the absorptions), the more it will be subject to bad weather (and eventually frost) in the first part of the development and, eventually, it would suffer a slowdown in photosynthetic activity. In addition, in case of early development, in May there will be more biomass to maintain and, therefore, more respiration in periods of unfavorable weather with low photosynthesis rate. For example, in 2017 daily NEE starts to be negative quite early (about mid-March) and continues until half April when, on 19th, a late frost damaged most of the vines shoots and decreased grass activity. This event is clearly visible on daily integrals ($Fc_{day}$) and on the cumulative ($Fc_{cumulated}$) amount of C (Figure 2), where the curve presents a "hump" after an initial early and sharp decreasing. It is interesting to note that another effect of this late frost was the forward shift of the absorption peak of this year compared to the others, being on July 7th instead around June 20th (Figure 2). In spring 2018 NEE was slightly negative in March and April but, in May, daily net absorption of $CO_2$ tends to be constant and effective only at the end of the month, due to frequent cloudy and rainy days. In 2019, absorptions started in early March due to sunny days and previous precipitations during February, but soon had a stagnation period in April and May due to frequent precipitations (Figure 2).

It is well known that the C budget of the vineyard results from the combination of both vines and grass activity, where grass can reach about one third of the annual $CO_2$ absorption (Gianelle et al., 2015; Tezza et al., 2019). The importance of grass cover became clearer in early spring and late summer, when $CO_2$ absorption started before vines bud break and continued after vines seasonal decay and dormancy onset. During summer, the ground cover became less relevant, eventually turning into a source of C in case of drought (Tezza et al., 2019). Usually, vines growers prevent natural herbaceous vegetation growth just under the vines. However, in some cases and in specific periods of vegetation development, they prefer to avoid grass growth also in the alley, performing soil management during the season. Mechanical hoeing, intended to disrupt the soil herbaceous cover, was performed in April 2016, while in the other years it was performed in May. The schedule of this extensive soil management may have been another reason for the positive monthly NEE of May 2017, 2018 and 2019 and negative in May 2016 (and reverse for April). This activity is of remarkable relevance in orchard soil management because may had led to a decrease in absorption and an increase of emissions of $CO_2$ (Marras et al., 2015; Tezza et al., 2019), acquiring a considerable importance in the vineyard C balance.

After the spring, the slope of the accumulation curve becomes very steep (Figure 2) indicating that carbon absorption increases very rapidly, thanks to the rapid and vigorous growth of vines. In effect, the most important period for $CO_2$ absorption was June and July, with an average net uptake of respectively -348±68 and -292±79 $gCO_2\ m^{-2}\ month^{-1}$ (Figure 3). The average absorption peak in the four years was -19 $gC\ m^{-2}\ day^{-1}$. In mid and late summer uptake rates were lower than before due to heat and water stress, but still consistent and effective on total C budget, with average monthly NEE in August and September of -183±73and -144±109 $gCO_2$ $m^{-2}$. On mid-September fluxes tended to be less negative, becoming positive in late September early October. The average annual C absorption found in this study (173±48 gC $m^{-2}$ $year^{-1}$) was in the lower range of the average values founded in literature, from 69 to over 850 gC $m^{-2}$ $year^{-1}$ (Callesen et al., 2023; Chiriacò et al., 2019; Gianelle et al., 2015; Guo et al., 2014; Marras et al., 2015; Meggio and Pitacco, 2016; Vendrame et al., 2019; Xue et al., 2024). The C intake of this vineyard was slightly higher than the average C budget of a three-year study over a non-irrigated vineyard also in northern Italy (Vendrame et al., 2019). Our founds were closed to the study conducted by Marras et al. (2015) over an irrigated vineyard in Sardinia, and another by Chiriacò et al. (2019) over a rainfed vineyard in central Italy.

To our knowledge, just a few long-term studies allow to determine the inter-annual variability of vineyard C budget. Our research presents a standard deviation of annual C budget slightly lower than existing previous studies, being 66 gC $m^{-2}$ $years^{-1}$ in Guo et al. (2014) and 57 gC $m^{-2}$ $years^{-1}$ in Vendrame et al. (2019). These results confirm the high potential of vineyards, and in general of orchards, as $CO_2$ sink. This, thanks to their capacity to fix the absorbed carbon in a permanent woody structure, build an extensive root system and being a complex and structured ecosystem, where the arboreal culture is associated with the herbaceous vegetation, both complementary to the final goal of C sequestration. The results also reflect the dependence of the vineyard C budget to annual climate conditions and the kind of vineyard

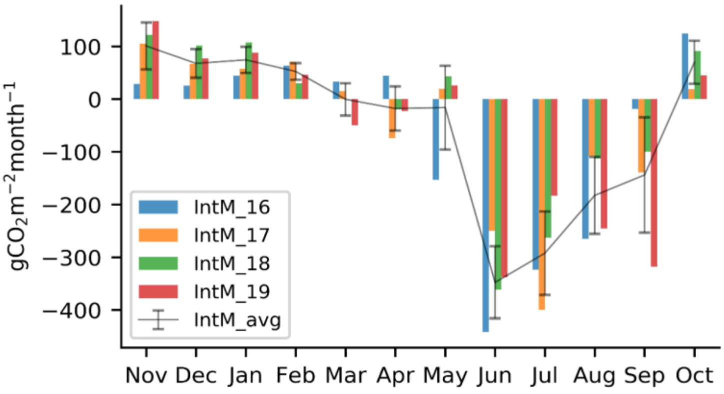

*Figure 3 - Monthly integrals of net vineyard CO2 exchange for each year: 2016 (intM_16, blu), 2017 (intM_17, orange), 2018 (intM_18, green), 2019 (intM_19, red). Grey line represents the average monthly NEE (intM_avg) with related standard deviation;*

management. Indeed, sustainable and conservative soil management practices are fundamental to improve agricultural $CO_2$ sink capacity, to increase agro-ecosystem services and to decrease $CO_2$ emissions caused by agriculture.

### 3.3 Anthropogenic emissions from vineyard management

Emissions from agricultural practices were quantified through CF calculations and reported in Table 2. The average yearly anthropogenic emission of $CO_2$ was 2.56±0.91 $MgCO_2$-eq $ha^{-1}$, equivalent to 0.40±0.24 $kgCO_2$-eq per kg of yield or 0.46±0.28 $kgCO_2$-eq per bottle. These values result slightly higher but in line with previous studies that found a total GHG balance of 1.86 $MgCO_2$-eq $ha^{-1}$ (Adoir et al., 2019). Other authors showed values substantially lower and equal to 0.24 $MgCO_2$-eq $ha^{-1}$ (Chiriacò et al., 2019) but they did not use chemicals for pests or weeds control, they used compost from grape residues as fertilization and recorded a lower value of fuel consumption and fungicides. Differences among studies depend mainly on management and climatic conditions, that affect the number of pesticide treatments and soil management strategies and, when reported to units, of course from yield. Other influencing factors could be related to different cut-off criteria or categories of contributing factors considered by different studies, exclusion rules, system boundaries and allocation rules (Adewale et al., 2018; Adoir et al., 2019; Arzoumanidis et al., 2014b). Considering emissions referred to yield, our results agree with those obtained by Marras et al. (2015) that recorded a value of 0.39 $kgCO_2$-eq $kg^{-1}$ yield deriving from grape production phase. However, these authors excluded pesticide emissions, considered negligible since a few amount was applied per year, and included emissions derived from human labor and crop residues (although very low), excluded in our study because they are short-cycle emissions. Chiriacò et al. (2019) reported a value of 0.10 $kgCO_2$-eq $kg^{-1}$ yield in central Italy, probably in relation to consideration reported above. Other authors confirm the wide range of variability. For example, (Tomaz et al., 2024) found a range of 0.097-0.111 $kgCO_2$-eq $kg^{-1}{}_{yield}$ in three different vineyards in Portugal but (Steenwerth et al., 2015) found a range of 0.087-0.584 $kgCO_2$-eq $kg_{yield}{}^{-1}$ over ninety vineyards and two hundred forty management regimes.

*Table 2 - Anthropogenic emission estimated from CF calculations, differentiated per source and per reference period;*

| **item** | **source of GHG considered** | | **2015 - 2016** | **2016 - 2017** | **2017 - 2018** | **2018 - 2019** | **Avg** | **Dev. std** |
|---|---|---|---|---|---|---|---|---|
| plant protection | fuel | Mg $CO_2$-eq | 7.36 | 6.77 | 5.52 | 6.26 | 6.48 | 0.78 |
| | fungicides (production) | Mg $CO_2$-eq | 1.27 | 0,78 | 0.82 | 0.42 | 0.82 | 0.35 |
| | insecticides (production) | Mg $CO_2$-eq | 0.17 | 0.24 | 0.01 | 0.01 | 0.11 | 0.12 |
| fertilization | fuel | Mg $CO_2$-eq | 0.71 | 0.03 | - | 0.43 | 0.39 | 0.34 |
| | fertilizer (production) | Mg $CO_2$-eq | 8.47 | 0.96 | - | 1.66 | 3.69 | 4.15 |
| | $N_2O$ | Mg $CO_2$-eq | 24.44 | 11.86 | - | 24.09 | 20.81 | 5.99 |
| soil management | fuel | Mg $CO_2$-eq | 10.26 | 9.20 | 17.99 | 14.86 | 13.08 | 4.09 |
| canopy management | fuel | Mg $CO_2$-eq | 1.18 | 1.98 | 1.18 | 0.81 | 1.29 | 0.49 |
| product transport | fuel | Mg $CO_2$-eq | 3.87 | 3.54 | 0.09 | 5.81 | 3.33 | 2.38 |
| waste | fuel/disposal | Mg $CO_2$-eq | 0.86 | 0.19 | 0.26 | 0.17 | 0.37 | 0.33 |
| $F_{CF\ ha}$ | | Mg $CO_2$-eq $ha^{-1}$ | 3.44 | 2.08 | 1.52 | 3.20 | 2.56 | 0.91 |
| $F_{CF\ yield}$ | | kg $CO_2$-eq $kg^{-1}$ yield | 0.41 | 0.72 | 0.14 | 0.32 | 0.40 | 0.24 |
| $F_{CF\ bott}$ | | kg $CO_2$-eq $bott^{-1}$ | 0.48 | 0.82 | 0.16 | 0.37 | 0.46 | 0.28 |

Moving to bottle as functional unit, Rugani et al. (2013) considered the results from 29 papers and obtained an average field phase CF of 0.38 kg$CO_2$-eq bottle $^{-1}$, which was very similar to our results (0.47 kg$CO_2$-eq bottle $^{-1}$). Other authors reported values included from 0.07 to 2 kg$CO_2$-eq bottle $^{-1}$ (Bosco et al., 2013, 2011; Fusi et al., 2014; Gazulla et al., 2010; Gierling and Blanke, 2021; Neto et al., 2013), confirming the wide range of results obtainable based on the starting premises. As reported from several authors (Bosco et al., 2011; Chiriacò et al., 2019; Marras et al., 2015; Tomaz et al., 2024), the main sources of GHG emissions over the total CF were represented by diesel consumption and N from fertilizer application (Figure 4). Diesel consumption covered 61% of the GWP impact of the production phase on average, of which 44% and 31% were used during soil management and plant protection. The impact of N lost in atmosphere ($N_2O$) from fertilizer application over the total CF were estimated to be about 31%, on average. It is important to underline that, in CF calculation, the EF associated to $N_2O$ emissions from fertilizers was 0.022 $KgN_2O\ kgN_{fertilizer}^{-1}$, in agreement with Product category rules (PCR) for arable crops (2020). This EF takes into account the global emissions resulting from the fertilizer supply, without excluding background emissions (i.e. emissions that are naturally generated by the soil even in the absence of fertilization). This EF and approach are similar to that proposed by IPCC (2019b) which EFs were confirmed in a study conducted in northern Italy (Minardi et al., 2022).

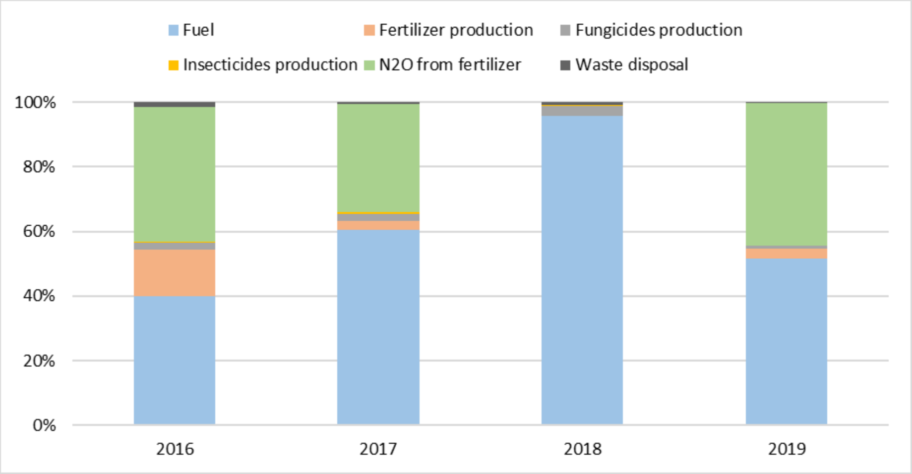

*Figure 4 - Relative global warming potential impact of the items considered in the yearly CF of field phase of wine making, presented in Table 3 (diesel consumption from different items has been joined into a single voice).*

However, these authors found that the EF related to $N_2O$ emissions only from fertilizers addition, excluding the background emissions, was about 38% lower (Minardi et al., 2022), therefore suggesting that the accounted $N_2O$ emissions due to fertilization could be overestimated in the CFs. Indeed, using the fertilizer-related EF suggested by these authors, $N_2O$ accounting would have been 45% lower and the average CF would have been 2.13±0.56 $MgCO_2$-eq $ha^{-1}$. The production of fertilizers was a low relevant source of GHG, that covers about 5% of the total CF, as the majority of used fertilizer was manure that presents low production EF. Considering emission due to plant protection products (fungicides+insecticides production) the average percentage is equal to 2% corresponding to an average value of 0.05 Mg ha$^{-1}$. This value is similar to the value reported in Chiriacò et al. (2019), that found a value of 0.01 Mg ha$^{-1}$ related to plant protection and Marras et al. (2015), that considered negligible this value. Differences in CF among years were mostly related to plant protection, fertilisation, soil and canopy management. Variation in pest control strategy was related to meteorological differences among years. Highest emissions related to plant protection were recorded during the period 2015-2016 (Table 2). This was related to the meteorological trend of May, June and July 2016 (Figure 1) where high level of rainfall was recorded. This has led to an intensification of fungicide treatments that results in higher emissions from both fuel consumption and quantity of products used. Differences in fertilization were determined by the strategy adopted by the winery; organic fertilization was managed in relation to the needs detected directly on the vines year by year. During 2015-2016 organic pelleted fertilizer was used in addition to manure. This has determined an increase of emissions mostly deriving from fertilizer production, due to highest emission associated to production of organic pellet fertilizer compared to manure (Table 1). In 2016-2017 only manure has been applied leading to a significant reduction of emissions compared to the previous period. In 2017-2018 no organic fertilization was carried out while in the following period a high quantity of manure was distributed, leading to high emissions values both from fertilizer production and distribution. Emissions derived from soil management resulted almost the same during 2015-2016 and 2016-2017 while this value significantly increases in 2017-2018 and 2018-2019. This is related to the high number of heavy inter-rows tillage carried out during the last two years of the study. The late frost occurred in April 2017 was responsible for the deterioration of large portions of vine trunks, for about 50% of the vines in the vineyard. This caused the highest value in emissions from canopy management in 2016-2017, due to an intense and unusual vine trunk renewal during the pruning phase. The loss of production resulting from late frost led to an increase of CF per kg of yield and per bottle even though CF per hectare was lower than 2015-2016 and 2018-2019.

In our calculations, we considered the amount of $CO_2$ and $N_2O$ released due to the application of manure during the four years. The first was embedded in NEE measurements, the second was calculated in the CF through the adoption of the EF proposed in PCR 2020:07

(Supplementary material - Table 1). Nevertheless, it is well known that the application of manure, compost or, in general, organic fertilizers results in an increase in soil C stock in the long term (20 or more years), with non-linear dynamics during time (IPCC, 2006; Maillard and Angers, 2014; Morlat and Chaussod, 2008; Tommaso et al., 2018; Triberti et al., 2008). But carbon content in soil matrix has high spatial variability and is well known that, often, this spatial variability could be higher than time-variability. Therefore, the direct measurement of changes in soil C stock over a few-year period could be poorly representative. For this reason, despite the fundamental importance of soil management practices on sustainability of agronomic systems, SOC variation is often not accounted into a CF, and no common methodology for its inclusion currently exist (Bosco et al., 2013; Brandão et al., 2013; Brandão and Levasseur, 2011; Goglio et al., 2015; Rugani et al., 2013). Some authors tried to include SOC changes due to fertilizers application in vineyards, using different approaches. For example, Chiriacò et al. (2019) derived an average annual rate of SOC increase due to compost application from literature, while Bosco et al. (2013) used a modelling approach. The first calculated an average net annual sink of C of 0.99 MgCO$_2$-eq ha$^{-1}$, while the second found that SOC accounting in farms with manure distribution, grassed alleys and incorporation of pruning debris, could turn the CF of the field phase of wine production from a GHG source to a modest sink (from 0.120 to -0.011 kgCO$_2$-eq bottle$^{-1}$). If we considered the increase in SOC thanks to the application of manure in our context, considering a prudent C-retention coefficient of 12% calculated by Maillard and Angers (2014) over a meta-analysis of 42 studies across the world, the cumulated organic carbon in the soil at the end of the study period would be 3 MgCO$_2$-eq ha$^{-1}$. This corresponds to an average of 1 MgCO$_2$-eq ha$^{-1}$ per year of manure application, very close to the value used in Chiriacò et al. (2019) for compost and in the range of 0.66-3.48 MgCO$_2$-eq ha$^{-1}$ founded in literature for manure (Freibauer et al., 2004; Guo et al., 2019; Triberti et al., 2008). If considered into the CF, this value would lower the average CF by 30% per unit of land and 35% per unit of yield (1.81±0.48 MgCO$_2$-eq ha$^{-1}$ or 0.26±0.08 kgCO$_2$-eq kg$_{yield}$$^{-1}$), confirming that the use of organic fertilization is a high-value operation for the purpose of retaining C in soil. These values are in line with those founded by Litskas et al. (2017) where, over ninety vineyards in Cypro Island, found a CF range of 0.283-0.846 kgCO$_2$-eq kg$_{yield}$$^{-1}$ that dropped by 40-67% considering the manure contribution, reaching a range of 0.096-0.507

$kgCO_2$-eq $kg_{yield}^{-1}$. All these considerations highlight that it is necessary to pay close attention when comparing results from different production areas and viticulture systems, which may have specific and peculiar features that lead to substantial differences in CF, possibly also calculated with different methods and EFs. Furthermore, results underline the importance of considering a multi-year approach to reach reliable and solid estimates because, as shown, climate conditions and agronomic choices have great influences on management strategies and, thus, on CF results.

## 3.4 Comprehensive vineyard GHG budget

Table 3 shows the overall net GHG budget ($GHG_{NET}$) of the field phase of wine production during the time span of this study, calculated summing up its components. These latter were the anthropogenic emissions caused by agricultural activity ($F_{CF}$), the amount of C leaving the system due to grape harvest ($F_{YIELD}$) and the net ecosystem $CO_2$ exchange (NEE). As explained in section 2.4, grape yield must be accounted as emission because it was completely removed from the system and oxidized later, while pruning debris were left in the field and the $CO_2$ produced by their decomposition were detected in NEE measurements. The overall net GHG budget at the end of the study period was -3.39 $MgCO_2$-eq $ha^{-1}$, resulting in an effective net sink of carbon, although instable during the years due to high variability in its components (Table 3). The yield was quite variable in the years and was not correlated with yearly NEE, as reported in other studies (Vendrame et al., 2019; Zanotelli et al., 2015). In fact, 2017 was the year with the lowest harvest due to April frost. In this year, also NEE was lower than in 2016 and 2019, but the lowest NEE value was during 2018, the year with higher yield (Table 3). The higher value of $F_{yield}$ than NEE in 2018 should not be misled the goodness of the data. In fact, this does not mean that the harvest includes more C than the vines have absorbed. It is worth remembering that NEE is not just the net $CO_2$ flux from vines but from the whole ecosystem, therefore including ground fluxes (i.e. produced by soil respiration, green pruning and mowed grass decomposition) and its value depends also on the weight of ecosystem respiration over gross primary production (GPP).

*Table 3 - Comprehensive field phase GHG budget (per vintages and total study period);*

| | $F_{CF}$ MgCO$_2$-eq ha$^{-1}$ | $F_{yield}$ MgCO$_2$-eq ha$^{-1}$ | NEE MgCO$_2$-eq ha$^{-1}$ | $GHG_{net}$ [$FU_{ha}$] MgCO$_2$-eq ha$^{-1}$ | $GHG_{net}$ [$FU_{yield}$] kgCO$_2$-eq kg$^{-1}$ yield | $GHG_{net}$ [$FU_{bottle}$] kgCO$_2$-eq bottle$^{-1}$ |
|---|---|---|---|---|---|---|
| 2015-2016 | 3.44 | 2.99 | -8.38 | -1.95 | -0.24 | -0.28 |
| 2016-2017 | 2.08 | 1.07 | -6.16 | -3.02 | -1.04 | -1.20 |
| 2017-2018 | 1.52 | 4.02 | -3.62 | 1.92 | 0.17 | 0.20 |
| 2018-2019 | 3.20 | 3.61 | -7.27 | -0.45 | -0.05 | -0.05 |
| sum | 10.27 | 11.69 | -25.44 | -3.51 | -1.15 | -1.32 |

The resulting averaged $F_{CF}$, $F_{Yield}$ and NEE were 2.56±0.79, 2.92±1.13 and -6.36±1.76 MgCO$_2$-eq ha$^{-1}$ respectively (Figure 5**Error! Reference source not found.**). These averages reveal that $F_{CF}$ was about 40% of NEE on average, while the preponderant part of the C lost was due to the harvest (about 46% on average). These results are quite close to that founds by Marras et al. (2015), while Chiriacò et al. (2019) found that harvest account for about 22% of the NEE, but they pointed out that in the year of the study the harvest was particularly lower compared to the standard yield due to unusual weather and organic management. In addition, in Chiriacò et al. (2019) pruning debris were not accounted in NEE, as they were removed from the field and used to produce compost, which then returned to the field. In that study, emissions in the field phase were only about 7% of NEE but rose to 41% considering C from pruning management. In most of the years $GHG_{net}$ was negative, and the resulting average was -0.88±1.85 MgCO$_2$-eq ha$^{-1}$, equivalent to -0.29±0.46 kgCO$_2$-eq kg$^{-1}$ yield or -0.33±0.53 kgCO$_2$-eq bottle$^{-1}$ (Figure 5), indicating that the field phase of the wine production could be a potentially capable net sink of carbon. The higher net GHG net sequestration was in 2017 (-3.02 MgCO$_2$-eq ha$^{-1}$), the year with lowest yield, while the only year with positive $GHG_{net}$ (2018, +1.92 MgCO$_2$-eq ha$^{-1}$) was a consequence of the combination of lower NEE, probably due to particularly unfavorable

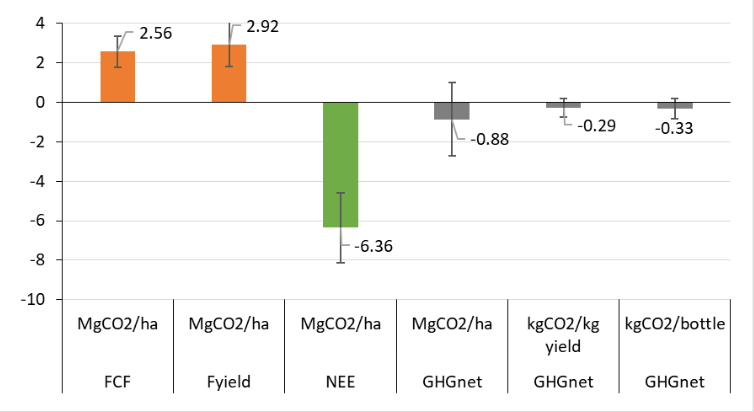

*Figure 5 - Comprehensive field phase GHG budget indicating average and std. dev. of emissions (FCF and Fyeld, orange), absorptions (NEE, green) and net budget considered with different functional units (GHGnet, grey) over the entire study period;*

weather conditions, and high yield. Singularly, it was also the year with the lowest FC, resulting from the non-distribution of fertilizer, which could also be a further reason for the low NEE. These results are more encouraging compared to those found by other authors. Indeed, Marras et al. (2015) had found that the overall net GHG balance resulted in a $CO_2$ source of about 0.4 $MgCO_2$-eq $ha^{-1}$, while Chiriacò et al. (2019) found potential carbon neutrality of the field phase. In a study without NEE measurements but modelled SOM changes accounted in CF, Bosco et al. (2013) found that the average CF for the field phase (over 9 wineries) results in a slight absorption of 0.011 $kgCO_2$-eq $bottle^{-1}$, with a maximum of 0.037 $kgCO_2$-eq $bottle^{-1}$, also lower than our results. Differences from literature could depend on several causes. Some could be linked to the peculiar combination of agronomic management, inputs and pedo-climatic conditions of the analyzed vineyards. As discussed earlier, and reported by other authors (Chiriacò et al., 2019; Marras et al., 2015), the weather of the season acquires a crucial impact on $GHG_{net}$, both directly (NEE and yield) and indirectly (vineyard management). Others could be introduced during CF calculation, due to the lack in the use of a common standard methodology that leads to different choices about boundaries of the analyzed system, phases included in the analysis or the emission factors, to cite some (Adewale et al., 2018; Arzoumanidis et al., 2014b; Vázquez-Rowe et al., 2013). As an example, considering the increasing soil C stock due to manure addition, the average comprehensive C budget would almost double reaching an average value of -1.63±2.24 $MgCO_2$-eq $ha^{-1}$. Furthermore, we underline that it will be useful to further clarify with more studies the EFs relating to $N_2O$ emissions, which could overestimate the emissions due to manuring including also those in the background (Minardi et al., 2022) and, therefore, overestimate the CF. Indeed, considering an $N_2O$ EF only for fertilizer addition from Minardi et al. (2022) the average comprehensive C budget would be -1.31±2.00 $MgCO_2$-eq $ha^{-1}$. These results indicate that sustainable management of the vineyard (organic, in this case) can have a positive impact on GHG emissions. Considering agriculture ecosystem services, can be important for structuring agricultural policies that focus not only on productivity, but also on environmental issues and, particularly, on the mitigation of anthropic GHG emissions and enhancing of SOM.

## 4 Conclusions

This was the first multi-annual study on comprehensive C balance in vineyards. Results over 4 years indicated a real sink capacity of vineyard, able to yield a net positive C balance of viticultural phase in organic conduction. We found high temporal variability in yearly NEE and CF even for the same site, highlighting the need for greater clarity and standardization of the processes for calculating emissions and thus, their interpretation. In this perspective, we have emphasized the importance of accounting C introduced through fertilization and on increasing the reliability and specificity of EFs used for calculating $N_2O$ emissions from fertilizer. The high temporal variability also underlines the importance of multi-annual analysis for both absorption and emissions and further studies are needed to clarify the trend, magnitudes and variability of total C balance, also in different contexts.

As already reported by other authors (Tezza et al., 2019; Xue et al., 2024), we have confirmed that soil management (i.e. green cover and organic fertilization) is crucial for increasing absorption and stock of C. On current C exhausted agricultural lands, mitigation and adaptation interaction can be mutually reenforcing, particularly for improving resilience to increased climate variability under climate change (Rosenzweig and Tubiello, 2007), leading to a double-gain play. In fact, for example, a management that increases absorptions and carefully handles soil C stock increasing SOM, will improve soil healthy and fertility in the long term, to support agriculture and associated environmental services for future generations.

The net sink amount reported in this study, considerable for an agricultural ecosystem, can represent an important base to quantify the role of viticulture in the perspective of carbon farming initiatives. Even if it can be objected that this sink may be only temporary and the built-up can be substantially disrupted at the end of the vineyard life cycle, these results show that there is a concrete possibility of storing carbon in agricultural soils. In this sense, vineyards, and orchards in general, seem to be good candidates for carbon farming. Proper practices can be defined to preserve this storage at best, greatly contributing to the global carbon budget and boost the role of agriculture in climate change mitigation initiatives.

The calculation of the complete C footprint in the agricultural sector can be very useful for directing the management of tree crops with a perspective of climate neutrality. By giving a new image to agriculture, recognizing its active role in mitigating GHG emissions, when possible, we can enhance the work of farmers and make them involved and aware of their potential. Managing tree crops is not the same as industrial production, is dealing with a likelihood virtuous role. We need agricultural models that can limit emissions and optimize absorption through careful and conscious agronomic choices, directing agronomic strategies increasingly towards low carbon agriculture. This type of study is crucial to determine these strategies, driving national adaptation and emissions mitigation plans and pointing out vineyards potential role in the context of AFOLU emission accounting

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## Declaration of competing interest

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

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# Supplementary material

*Supplementary material - Table 1 - Carbon Footprint inventory of agronomic practices carried out during the field phase (Hours)*

| Reference period | Agronomic practice | | Hours |
|---|---|---|---|
| November 1st 2015 - October 31st 2016 | Soil management | Organic Fertilization | 69 |
| | | row blade | 181 |
| | | Heavy inter-rows tillage | 383 |
| | | Light inter-rows tillage | 55 |
| | | Grass mowing | 36 |
| | Pest management | Treatments | 209 |
| | Canopy management | Topping | 64 |
| | | Pre-pruning | 27 |
| November 1st 2016 - October 31st 2017 | Soil management | Organic Fertilization | 38 |
| | | row blade | 202 |
| | | Heavy inter-rows tillage | 370 |
| | | Light inter-rows tillage | 138 |
| | | Grass mowing | 143 |
| | Pest management | Treatments | 196 |
| | Canopy management | Topping | 64 |
| | | Pre-pruning | - |
| | | Trimming | 6 |
| | | Vines trunk renewal | 81 |
| November 1st 2017 - October 31st 2018 | Soil management | Organic Fertilization | - |
| | | row blade | 183 |
| | | Heavy inter-rows tillage | 759 |
| | | Light inter-rows tillage | 30 |
| | | Grass mowing | 129 |
| | Pest management | Treatments | 157 |
| | Canopy management | Topping | 76 |
| | | Pre-pruning | - |
| | | Mechanical de-sukering | 7 |
| | | Brush cutting | 11 |

|  |  |  |  |
|---|---|---|---|
| November 1st 2018 - October 31st 2019 | Soil management | Organic Fertilisation | 39 |
|  |  | row blade | 118 |
|  |  | Heavy inter-rows tillage | 617 |
|  |  | Light inter-rows tillage | 103 |
|  |  | Grass mowing | 70 |
|  | Pest management | Treatments | 178 |
|  | Canopy management | Topping | 59 |
|  |  | Pre-pruning | - |
|  |  | Trimming | 1 |

# GENERAL CONCLUSIONS

This thesis followed the duality in the relationship between viticulture and climate, whereby one has influence over the other. In this way, a more integrated understanding of viticultural sustainability was achieved, with useful insights for planning future tailored adaptation and mitigation strategies that can take into consideration both product quality and carbon efficiency.

Through the longer Eddy Covariance time-series in vineyards, with presented data from 2015 to 2023, we assess the average water requirements for a representative vineyard in the DOC Prosecco. Water consumption has been significantly different in non-irrigated and irrigated years, but very stable inside these two periods. Standard FAO Kc has been confirmed from data filtered for comfort conditions (no water stress), once the application periods have been adapted for the local season timing.

The zoning of Prosecco DOC has elucidated significant regional differences in heat and water stress risk levels, production quality and yield. It was able to discriminate: an homogeneous area with higher productivity and intermediate quality levels (the plains); a high risk zone with lower acidity levels (Berici and Euganei hills), where medium- and long-term adaptation strategies will probably be needed in future; a low risk zone with higher acidity levels (pre-alps), to date little dedicated to viticulture; peculiar features for Trieste area, with higher tartaric acid levels that drives titratable acidity in contrast to the others zones where malic acid is the driver for total acidity. We found the maximum and minimum concentrations of malic acid better related to precipitation rather than thermal levels, raising some hypotheses and aspects to be explored in future about malic acid course and drivers. The results confirmed the crucial role of irrigation in preserving titratable acidity and malic acid during warmer months after veraison in Glera variety, but until a certain level: over-irrigation did not bring improvements compared to the company standard.

We presented the first nitrous oxide high resolution infield monitoring that brings more information about fine dynamics of $N_2O$ fluxes. This study allowed to calculate emissions for different soil and fertilizer management showing the standard emission factor as comprehensive of overall emissions (including background emissions) while, considering fluxes due only to organic fertilization, it would be about a half. We confirmed the linear relationship between $N_2O$ emission and soil TOC content. These findings underline the importance of clarifying the computation of EF and their use in literature.

Also, we presented the first multi-annual study on comprehensive carbon balance in vineyards that shows net negative carbon balance of viticultural phase for organic conduction in four years, with high temporal variability in its components (carbon footprint, net ecosystem exchange and yield) even for the same site. We have highlighted some issues and critical aspects about carbon footprint calculations in agriculture and confirmed that soil management and organic fertilization are crucial for increasing both carbon uptake and soil stock in orchards. Results demonstrate that sustainable practices, together with vineyard carbon fluxes accounting, can lead the field phase to be climate neutral or even positive in tree crops.

This integrated approach can enable to understand specific strengths and weaknesses of local viticulture, allowing for designing more tailored and effective strategies regarding cultivation practices, grape selection, and resource management for each context. These kind of study and results are crucial to drive management of tree crops through future climate changes, addressing agricultural policies and strategies towards higher environmental sustainability, with a perspective of climate neutrality thanks to low carbon agricultural models.