UNIVERSITÀ
DEGLI STUDI
DI BRESCIA

DOTTORATO DI RICERCA IN INGEGNERIA CIVILE, AMBIENTALE, DELLA COOPERAZIONE INTERNAZIONALE E DI MATEMATICA

settore scientifico disciplinare AGR/03 ARBORICOLTURA GENERALE E COLTIVAZIONI ARBOREE

CICLO
XXXV

Advanced methodologies for sustainable viticulture

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Ringraziamenti

Ringrazio il Prof. Gianni Gilioli per la sua disponibilità, per i preziosi insegnamenti e per il costante appoggio durante l'intero periodo di Dottorato.

Ringrazio il Dott. Valenti per le conoscenze che mi ha trasferito e per il suo costante supporto in qualsiasi momento del mio percorso formativo e professionale.

Ringrazio la Dott.ssa Simonetto per il prezioso contributo durante il percorso di ricerca intrapreso e per avermi fatta sentire parte di un gruppo fin dal primo giorno di lavoro insieme.

Ringrazio Sata Studio Agronomico per le opportunità e gli insegnamenti che mi hanno consentito di adottare un approccio applicato alla ricerca. Ringrazio in particolare il Dott. Pierluigi Donna e il Dott. Tonni che hanno contribuito allo sviluppo di alcuni dei vari lavori pubblicati e in preparazione.

Ringrazio tutti i colleghi del gruppo di ricerca Agrofood Research Hub con i quali ho avuto il piacere di condividere i successi raggiunti fino ad ora.

Ringrazio tutti gli altri co-autori che hanno contribuito allo sviluppo dei prodotti della ricerca.

Un ringraziamento speciale alla Prof.ssa Oriana Silvestroni e al Prof. Giovambattista Mattii per il loro lavoro di revisione e valutazione della Tesi.

Ringrazio i miei genitori che mi hanno sempre appoggiato in ogni mia scelta e aiutato in ogni situazione di difficoltà.

Ringrazio mio marito Luigi, maestro e compagno di vita, risorsa per me fondamentale sia nella vita privata che nell'ambito professionale.

Ringrazio i miei figli Leonardo e Matilde che mi danno sempre la gioia necessaria a superare fatiche e difficoltà.

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ABSTRACT
The European Commission adopted several policy initiatives aimed at boosting the efficient use of resources by moving to a clean, circular economy, stop climate change, reduce biodiversity loss and pollution. These initiatives are included in Strategies provided by the European Green Deal and could have a substantial impact on European agriculture and food. Specifically, the climatic component of the Green Deal as well as the Farm to Fork strategy and the Biodiversity 2030 strategy included among their objectives: i) ensure food security facing climate change and biodiversity loss; ii) reduce the environmental and climate footprint of the EU food system; iii) strengthen the EU food system's resilience; iv) lead a global transition towards competitive sustainability from farm to fork.

To achieve these objectives a set of coordinated actions needs to be implemented.

The Common Agricultural Policy (CAP) after 2020 represents a strategic plan structured to combine a wide range of targeted interventions to deliver tangible results in relation to Green Deal objectives. Among the ten Specific Objectives (SO) of the CAP, three are mainly related to environmental sustainability in agriculture: i) SO-4 Contribute to climate change mitigation and adaptation, as well as sustainable energy; ii) SO-5 Foster sustainable development and efficient management of natural resources such as water, soil and air; iii) SO-6 Contribution to the protection of biodiversity, enhance ecosystem services and preserve habitats and landscapes.

In the Introduction of this Thesis (Chapter 1) research activities developed during my PhD are introduced. The final aim to integrate methodologies and models in a framework supporting sustainability analysis in viticulture is described. The framework is conceived to cope with the SO-4, SO-5 and SO-6 of CAP after 2020. Chapter 2 explores methodologies related to SO-4. In relation to climate change mitigation an example about the evaluation of carbon footprint deriving from vineyard management is considered. The effect of leaf removal and shading on vine production, grape and must and wine quality is reported as an example of agronomic strategies to reduce the impact of climate change on grapevine production. Chapter 3 explores methodologies related to SO-5. The quantification of nitrous oxide emissions from vineyard soil are regarded as assessment procedures supporting actions improving air quality and reducing nutrient leakage. Chapter 4 explores methodologies related to SO-6. Actions enhancing biodiversity protection in vineyard ecosystems (with particular emphasis to soil arthropods) are evaluated with respect to environmental conditions and agronomic practices. Actions aiming at enhancing provision ecosystem services in vineyard agroecosystem are evaluated in the Franciacorta winegrowing area. Conclusions (Chapter 5) are dedicated to summarize results obtained in Chapters 2, 3 and 4 showing the advantages of integrating methodologies and disciplines in a framework supporting sustainability analysis in viticulture.

Keywords: environmental sustainability, winegrowing sector, vineyard, climate change, biodiversity.

RIASSUNTO
La Commissione europea ha adottato diverse iniziative volte a promuovere l'uso efficiente delle risorse attraverso la transizione verso un'economia pulita e circolare, fermare il cambiamento climatico, ridurre la perdita di biodiversità e l'inquinamento. Queste iniziative sono incluse nelle strategie fornite dal Green Deal e potrebbero avere un impatto sostanziale sull'agricoltura e sull'alimentazione europea. In particolare, la componente climatica del Green Deal, nonché la strategia Farm to Fork e la strategia Biodiversità 2030 includono tra i loro obiettivi: i) garantire la sicurezza alimentare di fronte ai cambiamenti climatici e alla perdita di biodiversità; ii) ridurre l'impronta ambientale e climatica del sistema alimentare dell'UE; iii) rafforzare la resilienza del sistema alimentare dell'UE; iv) condurre una transizione globale verso una sostenibilità competitiva dall'azienda alla tavola del consumatore. Per raggiungere questi obiettivi è necessario attuare una serie di azioni coordinate. La politica agricola comune (PAC) dopo il 2020 rappresenta un piano strategico strutturato per combinare un'ampia gamma di interventi mirati a fornire risultati tangibili in relazione agli obiettivi del Green Deal. Tra i dieci Obiettivi Specifici (Specific Objectives SO) della PAC, tre sono principalmente legati alla sostenibilità ambientale in agricoltura: i) SO-4 Contribuire alla mitigazione e all'adattamento ai cambiamenti climatici, nonché all'energia sostenibile; ii) SO-5 Favorire lo sviluppo sostenibile e la gestione efficiente delle risorse naturali quali acqua, suolo e aria; iii) SO-6 Contribuire alla protezione della biodiversità, al miglioramento dei servizi ecosistemici e alla conservazione di habitat e paesaggi. Nell'Introduzione di questa tesi (Capitolo 1) vengono enunciate le attività di ricerca sviluppate durante il Dottorato. Viene inoltre descritto l'obiettivo finale di inserire metodologie e modelli in una struttura integrata a supporto dell'analisi della sostenibilità in viticoltura. Questa struttura è stata impostata per far fronte agli SO-4, SO-5 e SO-6 della PAC dopo il 2020. Il Capitolo 2 esplora le metodologie relative a SO-4. In relazione alla mitigazione del cambiamento climatico viene considerato un esempio di valutazione dell'impronta carbonica derivante dalla gestione del vigneto. L'effetto della sfogliatura e dell'ombreggiamento sulla produzione della vite e sulla qualità di uva e mosto viene riportato come esempio di strategie agronomiche per ridurre l'impatto dei cambiamenti climatici. Il Capitolo 3 esplora le metodologie relative a SO-5. La valutazione dell'impronta carbonica e la quantificazione delle emissioni di protossido di azoto dal suolo vitato sono considerate quali procedure di valutazione a supporto di azioni volte a migliorare la qualità dell'aria e a ridurre la dispersione di nutrienti. Il Capitolo 4 esplora le metodologie relative a SO-6. Le azioni che migliorano la protezione della biodiversità negli ecosistemi viticoli (con particolare attenzione agli artropodi del suolo) sono valutate rispetto alle condizioni ambientali e alle pratiche agronomiche. Nell'area vitivinicola della Franciacorta vengono valutate azioni volte a migliorare l'erogazione di servizi ecosistemici nell'agroecosistema vigneto. Le Conclusioni (Capitolo 5) sono dedicate a riassumere i risultati ottenuti nei Capitoli 2, 3 e 4 mostrando i vantaggi dell'inserimento di metodologie e discipline in una struttura integrata a supporto della sostenibilità in viticoltura.
Parole chiave: sostenibilità ambientale, settore vitivinicolo, vigneto, cambiamento climatico, biodiversità

CHAPTER 1: Introduction

1.1 Sustainability in agriculture: a general overview

The concept of sustainable agriculture is fundamental for the present and future condition of environments, economies, and societies (Smit and Smithers, 1993). A great variety of different views, paradigms and assessment schemes of sustainability in agriculture have been proposed and numerous attempts to gain a shared definition of sustainable agriculture have been made (Velten et al., 2015).
In 1988, FAO defined sustainable agricultural development as "*the management and conservation of the natural resource base, and the orientation of technological change in such a manner as to ensure the attainment of continued satisfaction of human needs for present and future generations. Sustainable agriculture conserves land, water, and plant and animal genetic resources, and is environmentally non-degrading, technically appropriate, economically viable and socially acceptable*" (FAO, 1989). This definition introduces the three dimensions of sustainability: environmental sustainability, economic sustainability, social sustainability (Yunlong and Smit, 1994). The fourth dimension of governance must be integrated as governance sustainability represents a fundamental element to guarantee any realistic prospects of an orderly transition to sustainability (Adger and Jordan, 2009).
Among the proposed sustainability assessment schemes focusing on agri-food and rural systems, the FAO-SAFA (Sustainability Assessment of Food and Agriculture Systems, FAO, 2014) represents one of the most complete four-dimensions assessment methodology. SAFA is hierarchically organized in a series of themes and sub-themes, as summarized in the following figure (**Figure *1***):

GOOD GOVERNANCE

CORPORATE ETHICS	Mission Statement	Due Diligence			
ACCOUNTABILITY	Holistic Audits	Responsibility	Transparency		
PARTICIPATION	Stakeholder Dialogue	Grievance Procedures	Conflict Resolution		
RULE OF LAW	Legitimacy	Remedy, Restoration & Prevention	Civic Responsibility	Resource Appropriation	
HOLISTIC MANAGEMENT	Sustainability Management Plan	Full-Cost Accounting			

ENVIRONMENTAL INTEGRITY

ATMOSPHERE	Greenhouse Gases	Air Quality			
WATER	Water Withdrawal	Water Quality			
LAND	Soil Quality	Land Degradation			
BIODIVERSITY	Ecosystem Diversity	Species Diversity	Genetic Diversity		
MATERIALS & ENERGY	Material Use	Energy Use	Waste Reduction & Disposal		
ANIMAL WELFARE	Animal Health	Freedom from Stress			

ECONOMIC RESILIENCE

INVESTMENT	Internal Investment	Community Investment	Long-Ranging Investment	Profitability	
VULNERABILITY	Stability of Production	Stability of Supply	Stability of Market	Liquidity	Risk Management
PRODUCT QUALITY & INFORMATION	Food Safety	Food Quality	Product Information		
LOCAL ECONOMY	Value Creation	Local Procurement			

SOCIAL WELL-BEING

DECENT LIVELIHOOD	Quality of Life	Capacity Development	Fair Access to Means of Production		
FAIR TRADING PRACTICES	Responsible Buyers	Rights of Suppliers			
LABOUR RIGHTS	Employment Relations	Forced Labour	Child Labour	Freedom of Association & Right to Bargaining	
EQUITY	Non Discrimination	Gender Equality	Support to Vulnerable People		
HUMAN SAFETY & HEALTH	Workplace Safety and Health Provisions	Public Health			
CULTURAL DIVERSITY	Indigenous Knowledge	Food Sovereignty			

Figure 1 Dimensions, themes, and subthemes of the SAFA - - Sustainability Assessment of Food and Agriculture Systems - Guidelines (FAO, 2014).

Among the four dimensions, environmental sustainability is one of most relevant issues in agriculture as far as environmental degradation is concerned (Shyamoli and Dasgupta, 2022). Environmental dimension of sustainability, considering the physical inputs of productions, emphasizes environmental life-support systems without which neither production nor humanity could exist

(Goodland, 1995). Therefore, environmental sustainability is a necessary prerequisite to global sustainability (Baumgärtner et al., 2010). Following the importance given to environmental sustainability, in the present Thesis emphasis is given to environmental aspect with the belief that reducing environmental impact of agricultural practices, benefits in terms of economic and social sustainability can be achieved.

1.2 Policies promoting sustainable agriculture: the role of new European Common Agricultural Policy (CAP) 2021-2017

In 2015 the 193 United Nations Members States signed a 15-year global framework, the 2030 Agenda, introducing 17 Sustainable Development Goals (SDGs) to approach global challenges, including poverty, inequality, climate change, environmental degradation, peace and justice (UN, 2015).

The European Commission, to address the issues of 2030 Agenda, adopted several policy initiatives aimed at boosting the efficient use of resources by moving to a clean, circular economy and stop climate change, revert biodiversity loss and cut pollution. These initiatives are included in Strategies provided by the European Green Deal (European Commission, 2019) and could have a substantial impact on European agriculture and food system. Specifically, the climatic component of the Green Deal as well as the Farm to Fork strategy (European Commission, 2020) and the Biodiversity 2030 (European Commission, 2021) strategy included among their objectives:

- ensure food security facing climate change and biodiversity loss;
- reduce the environmental and climate footprint of the EU food system;
- strengthen the EU food system's resilience;
- lead a global transition towards competitive sustainability from Farm to Fork.

To achieve these objectives a set of coordinated actions need to be implemented. The new Common Agricultural Policy (CAP) 2021-2027, represents a strategic plan structured to combine a wide range of targeted interventions to deliver tangible results helping the achievement of the ambitions of the Farm to Fork and Biodiversity strategies. This new version of CAP was formally adopted on 2nd of December 2021, and it is due to be implemented by January 2023. The CAP 2021-2027 has been conceived with a strong emphasis on results and performances, defining a set of monitoring frameworks based on indices focusing on 10 Specific Objectives (SOs) (European Commission, 2018) as listed below:

- SO-1: Support viable farm income and resilience across the Union to enhance food security;
- SO-2: Enhance market orientation and increase competitiveness;
- SO-3: Improve the farmers' position in the value chain;

- SO-4: Contribute to climate change mitigation and adaptation, as well as sustainable energy;
- SO-5: Foster sustainable development and efficient management of natural resources such as water, soil and air;
- SO-6: Contribution to the protection of biodiversity, enhance ecosystem services and preserve habitats and landscapes;
- SO-7: Attract young farmers and facilitate business development in rural areas;
- SO-8: Promote employment, growth, social inclusion and local development in rural areas, including bio-economy and sustainable forestry;
- SO-9: Improve the response of EU agriculture to societal demands on food and health, including safe, nutritious and sustainable food, as well as animal welfare;
- SO-10: Fostering knowledge & innovation.

Among these SOs, SO-4, SO-5 and SO-6 are mainly related to environment and climate representing a core part of the new CAP 2021-2027 and providing three clear environmental goals, each of them are echoed in the European Green Deal, Farm to Fork and Biodiversity 2030 strategies. These three SOs have been conceived focusing on a set of Specific Actions (SAs), namely:

- SO-4 (tackling climate change): SA-4.1) Contributing to climate change mitigation; SA-4.2) Enhancing carbon sequestration; SA-4.3) Increasing sustainable energy in agriculture;
- SO-5 (protecting natural resources): SA-5.1) Reducing soil erosion; SA-5.2) Improving air quality; SA-5.3) Improving water quality; SA-5.4) Reducing nutrient leakage; SA-5.5) Reducing pressure on water resource;
- SO-6 (enhancing biodiversity): SA-6.1) Increasing farmland bird population; SA-6.2) Enhancing biodiversity protection; SA-6.3) Enhancing provision of ecosystem.

1.3 The research topic of PhD

During my PhD I had the opportunity to explore various areas of sustainability in viticulture. The interest for this agricultural sector is related to my background that included training, professional and research activities in winegrowing sector. Viticulture is one of the most relevant agricultural compartments, covering approximately 7.3 million hectares worldwide, with approximately 3.3 million located in Europe (OIV, 2019). Sustainability within this sector is becoming a major issue. A variety of systems, methodologies and tools have been implemented to assess sustainability in winegrowing compartment (Corbo et al., 2014), and the scientific community is putting a lot of efforts in this direction. Baiano (2021) reported 334 and 262 papers founded, respectively in Scopus and WoS (Web of Science), considering “sustainable viticulture” as searching keyword.

With the aim to promote an international standard for winegrowing sector the International Organisation of Vine and Wine (OIV) has introduced the 2020-2024 Strategic Plan to deal with the various challenges the international wine-growing sector is facing (OIV, 2020). The OIV Strategic Plan is structured around six strategic axes with the aim to integrate in the OIV work the 2030 Agenda perspectives of the Sustainable Development Goals (SDGs):

- AXIS I - Promote environmentally-friendly vitiviniculture: climate change, environmental performance, natural resources related SDGs;
- Axis II - Promote economic activity according to principles of sustainable development and of market growth and globalization: value chain, statistical analysis related SDGs;
- Axis III - Contribute to social development through vitiviniculture: social development, health and wellbeing related SDGs;
- Axis IV - Pursue the development of a harmonised regulatory environment:mproduct definition, oenological practices, analysis methods related SDGs;
- Axis V - Facilitate the digital transition of the sector: adaptation, transition, harmonization related SDGs;
- Axis VI - Consolidate the role of the OIV as a global scientific, technical and cultural reference organization: international cooperation, specialisation centre, communication related SDGs.

To achieve these multiple goals and in view of the complexity of the winegrowing sector, approaches and tools that allow to deal with sustainability through a systemic conception proposing integrated solutions are needed (Costa et al., 2022). In line with the indications emerging from the European Commission and OIV documents, during my PhD program I identify the need of exploring seven main directions on which actions can be taken to improve environmental sustainability in viticulture:

- Technological innovation;
- Biodiversity and ecosystem services;
- Biotechnologies;
- Genetic composition;
- Crop models;
- Environmental footprints;
- Integrated management strategies.

Among the seven directions, I had the possibility to deepen three of them on which my scientific effort has been focused:

- Biodiversity and ecosystem services through research on

    - vineyard soil biodiversity in particular studying soil arthropod communities through soil biological quality evaluation (QBS-ar Index) and metabarcoding approaches;
    - vineyard agroecosystem biodiversity.
- Environmental footprints through the assessment of
    - Carbon footprint;
    - Water footprint.
- Integrated management strategies aiming at
    - Reducing nitrous oxide emissions form vineyard soil;
    - Conserving and improving biodiversity;
    - Mitigating and adapting to climate change.

Research activities developed during my PhD were informed by the need of integrating methodologies and models in a framework supporting sustainability analysis in viticulture. In the present Thesis the elements to build this methodological framework are described.

1.4 Aims of the Thesis

The main path followed by the studies conducted in this Thesis is organized around the new CAP 2021-2027 considering its relevance in delivering tangible results to improve sustainability in viticultural sector. As mentioned above, I focused on SOs that are mainly related to environment and climate issues: SO-4, SO-5 and SO-6.

A selection of SAs main representative of the research developed were identified:

- SA-4.1 - Contributing to climate change mitigation;
- SA-5.2 - Improving air quality, and SA-5.4 Reducing nutrient leakage;
- SA-6.2 - Enhancing biodiversity protection, and SA-6.3 - Enhancing provision of ecosystem.

The main research activities carried out during the PhD program, reported in papers already published or submitted, can be associated to the selected SAs following the scheme reported in **Figure *2***.

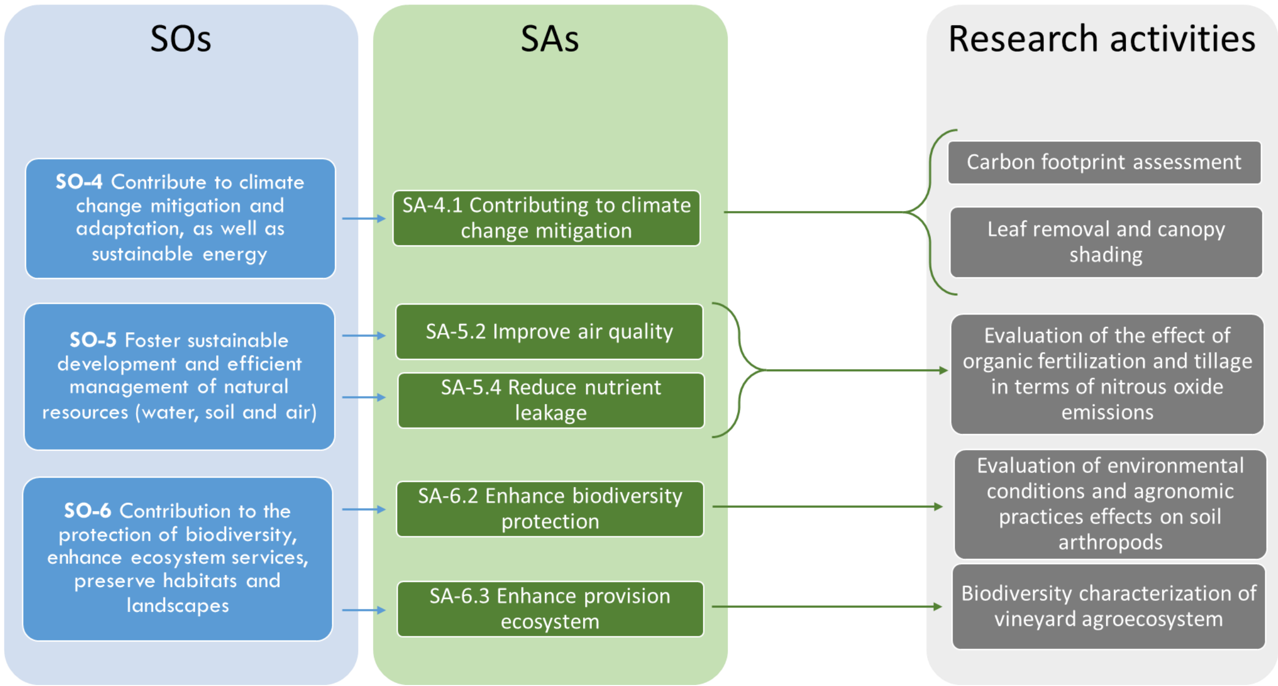

Figure 2 Research activities carried out during PhD and their association to selected SOs and SAs

In detail, to deal with SO-4 (SA-4.1), Chapter 2 describes research activities aimed at contributing to climate change mitigation and adaptation:

- in Ghiglieno et al. (submitted to Journal of the Science of Food and Agriculture) a carbon footprint assessment in viticultural sector is described considering 25 Italian wineries. The paper reported the comparison between carbon footprint conventional and organic vineyards in northern Italy. Results obtained from this research underlined that no significant differences are revealed between organic and conventional in terms of total emissions, while differences can be revealed considering each source of emissions separately;
- in Ghiglieno et al. (2019) the effect of canopy management to reduce the impact of climate change on grapevine production is explored. Leaf removal and shading effects on vine production and must quality are described in sparkling wine production. The practice of artificial shading emerges as an interesting agronomic strategy to support winegrowers to contrast climate change effects.

Chapter 3 explores research activities related to SO-5 (SA-5.2 and SA-5.4):

- in Minardi et al. (2022) the effect of organic fertilization and tillage in terms of nitrous oxide emissions from vineyard soil is considered. In the paper the variation of direct nitrous oxide emissions changing soil and organic fertilization management is investigated. The effect of fertilizer incorporation in increasing nitrous oxide emissions is underlined.

In Chapter 4 research activities carried out in the context of SO-6 are addressed (SA-6.2 and SA-6.3).

- in Ghiglieno et al. (2021) actions aimed at enhancing biodiversity protection are evaluated in vineyard agroecosystem with particular emphasis to soil arthropods. A paper describing the impact of environmental conditions and management on soil biological quality referred to arthropod communities (QBS-ar Index) is reported. Results obtained by this research identified soil temperature and soil texture as the abiotic factors exerting the most significant effect on the QBS-ar values. Organic vineyards exhibited higher QBS-ar values compared to those conventionally managed vineyards, and subsoiling negatively influenced the soil biological quality;

- in Ghiglieno et al. (2022b), actions aiming at enhancing provision ecosystem services in vineyard agroecosystem are evaluated in the Franciacorta winegrowing area. The research proposed introduces a methodology aimed at developing a unitary framework able to describe vineyard ecosystems biodiversity considering both local (morphological characteristics, internal ecological infrastructure, and management) and landscape (land-use) components. This integrated approach to the assessment of vineyard biodiversity allows managing the complexity of the vineyard landscape providing a useful instrument to increase knowledge about vineyard system biodiversity.

The Chapter 5 is dedicated to some concluding remarks. An attempt to integrate the results obtained by the research activities in my PhD program is proposed defining a methodological scheme supporting sustainability analysis in viticulture.

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CHAPTER 2 – Specific objective 4 – Specific Action 4.1 - Contribute to climate change mitigation and adaptation, as well as sustainable energy

2.1 Comparing the carbon footprint in northern Italy's conventional vs organic vineyards

Chapter 2.1 has been submitted to Sustainability.

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Abstract

The carbon footprint is an index used to assess the impact of an activity in terms of greenhouse gas emissions. Viticulture contributes to greenhouse gas emissions due to the use of fuels, fertilizers, pesticides, soil erosion and degradation. In this regard, in recent decades, a sustainability-based approach has been increasing in vineyard management systems to ensure efficiency in terms of energy and water consumption, limit the use of environmentally harmful products and contain the release of pollutants. Organic viticulture differs from conventional one mainly because of the absence of synthetic products, the use of soil processing practices and the level of organic carbon in soil. The purpose of the study was to determine the actual differences between conventional and organic vineyard management in terms of greenhouse gas emissions, comparing multiannual data from 25 wineries in northern Italy. No statistically significant differences were found between the overall mean values of conventional and organic management. In both systems, the main component of GHG emissions is fuel consumption and fertilizing. In organically farmed vineyards, a higher incidence of fuel consumption was observed, while in conventionally-farmed vineyards higher emissions were observed due to the use of such products as pesticides and fertilizers. No differences were found between the two management systems in terms of emissions resulting from direct fertilizing, although further assessment of potential sequestration of organic fertilizing would be necessary.

Keywords: Viticulture, Organic vs. traditional management, Carbon Footprint, Greenhouse gas (GHG), Sustainable viticulture

1. Introduction

The Carbon Footprint (CF) is an indicator of global warming (IPCC, Geneva, Switzerland, 2022). The CF expresses the amount of greenhouse gas emissions (GHGs) generated during the production or consumption of goods and converted into CO_2 equivalent (CO_2-eq) according to their global warming potential (Röös et al., 2013). It can be considered a simplified LCA focusing on global warming as the only impact category (Pattara et al., 2012). CF can be assessed either at the corporate level, according to the ISO 14064 standard (2018) and the GHG Protocol for organizations (2004 and 2011), or at the product level, according to the ISO 14067 standard (2018) and the GHG Protocol for products (2011). The corporate CF method consists of calculating direct and indirect GHG emissions that a company generates over one year while performing its activities. Direct emissions are generated from sources controlled by a company, while indirect emissions are a consequence of the activities of a company (Borsato et al., 2020). All company products are included in the assessment of the corporate CF, only one company product is assessed in the product CF (Navarro et al., 2017a). Corporate CF is therefore a method used to assess the sustainability of a company according to the impact of its production activities on global warming.

Viticulture contributes to GHG emissions due to the use of fertilizers, pesticides, water and fuels, soil erosion and degradation, not to mention the production of a significant amount of organic waste (Bandinelli et al., 2020). Even though viticulture leads to carbon sequestration by the vines and all cover crops, intensive vineyard cultivation needs to be correctly managed to reduce GHG emissions (Chiriacò et al., 2019). In this perspective, many wineries are now gearing towards sustainable grape-growing practices (Tsalidis et al., 2022). The key sustainable measures contemplate innovation and improvement in terms of energy and water consumption, the restricted use of environmentally harmful products, such as pesticides and fertilizers, and the limitation of other pollutants potentially released in the ecosystem (Volanti et al., 2022). Among the certifications considering sustainable viticultural practices, the certification of organic wine is considered the main widespread (Letamendi et al., 2022). In the European Union, this certification can be obtained if the vine grower complies with specific organic farming rules set forth by the European Parliament and Council Regulation 2018/848/EU. As compared to conventional management, organic viticulture management does not use synthetic products, such as fertilizers and pesticides, applies different soil tillage practices and leads to a different level of organic carbon sequestered in croplands (Ahrens et al., 2022). The wineries certified to sustainable organic viticulture tend to be perceived as realities with a generally beneficial impact on the environment compared to those adopting conventional viticulture (Baiano, 2021). The FAO has highlighted that both conventional and organic agriculture are key models to address global warming (FAO, 2007). The question arises as to whether conventional and organic viticulture has an impact in terms of GHG emissions. Some authors have highlighted that organic practices may not necessarily lead to a reduction in CF values compared to conventional ones. The lower yields, the possible increase in the consumption of fuels necessary for the greater number of phytosanitary treatments, the need of adopting mechanical weeding, the high number of soil management interventions (Litskas et al., 2020) and the transport of a large amount of manure and organic fertilizers could generate higher GHG emissions than conventional farming (Venkat, 2012).

Furthermore, a number of studies have shown that conventional systems maximizing productivity have a reduced environmental impact according to several indicators, including the CF (Korsaeth, 2008).

The current literature shows little knowledge of the real differences in the amount of greenhouse gas emissions in the organic versus conventional management of vineyards. It emerges the need for an environmental assessment that takes into account the distinctive features of the two systems, based on a large sample of wineries with different characteristics over several years. This paper aims to obtain a comparative overview of organic and conventional vineyard management, focusing on the main categories of carbon dioxide emissions. The approach used is the corporate carbon footprint, involving 25 wineries in northern Italy over nine different vintages.

2. Materials and Methods

2.1 Geographical location and years of study

The study involved 25 wineries, located in four administrative regions of northern Italy. Six wineries adopting organic management practices (ORG) were certified according to EU Regulation 2018/848, while 19 wineries were conventionally managed, without any specific certification (CONV). The data collected refer to the 2009-2017 years. A case study represents the overall data collected in one year in one farm. Table 1 shows the administrative regions, the years and the total vineyard surface area managed by each winery available for CONV and ORG for the number of case studies.

Table 1 Number of case studies considered, years of data collection and vineyard surface area according to administrative region and management system.

Administrative Region	Management	Years of data collection	Number of case studies	Vineyard surface area (ha)	
				Mean	**[min; max]**
Friuli-Venezia Giulia	CONV	2012, 2013, 2015, 2017	4	69.31	[38.85; 83.65]
Lombardy	ORG	2009, 2010, 2011, 2014, 2015, 2016, 2017	9	49.24	[3.41; 84.65]
	CONV	2009, 2010, 2011, 2012, 2013, 2014, 2016	34	57.37	[9.22; 180.63]
Piedmont	CONV	2010	1	93.01	[93.01; 93.01]
Veneto	ORG	2012, 2013	2	17.19	[17.18; 17.19]
Overall			50	55.96	[3.41; 180.63]

2.2 Description of the system, boundaries and exclusions

Fig. 1 describes the processes associated with organic and conventional vineyard management. According to specific European Parliament and Council rules on organic agriculture (Regulation 2018/848/EU), ORG differs from CONV in a number of agronomic aspects: i) control of weeds and pests is allowed only with the application of mechanical and physical methods, ii) the exclusive use of natural or natural-derived substances, such as organic or mineral low-solubility fertilizers, iii) the exclusive use of natural or mineral products for pest control, such as sulfur and copper for fungal treatments and plant-extracted pyrethrins. for insecticide treatments with specific restrictions (EC, 2021; EP, EC, 2018).

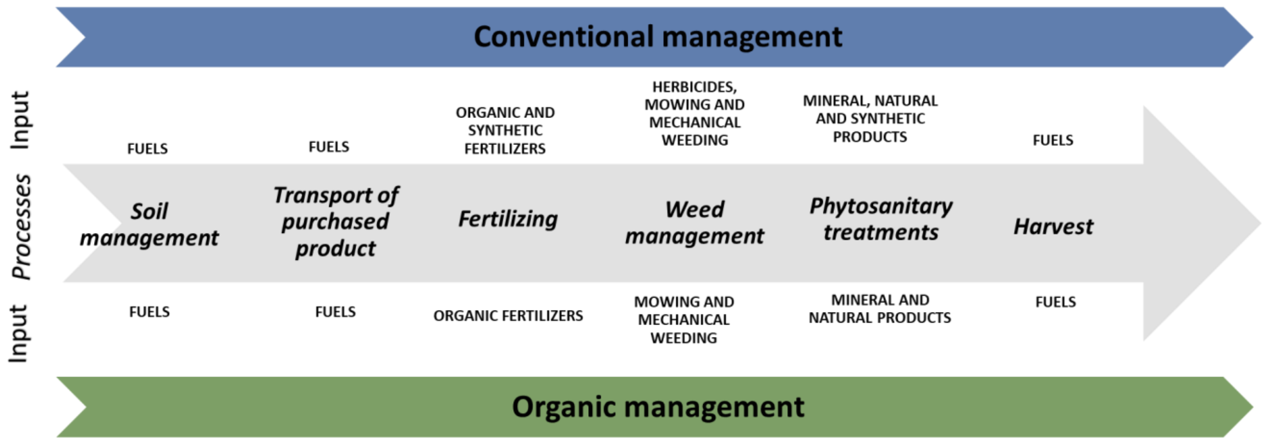


Fig. 1. Description of the grape production system, processes and inputs in conventional and organic management

In this study, a cradle-to-gate approach is adopted, therefore the system boundaries include all the main sources of GHG emissions during the production processes (in accordance with ISO 14064, 2018) from post-harvest operations through to the delivery of the following year's grapes to the winery, regardless of the post-agricultural life cycle stages. According to other studies (Villanueva-Rey et al., 2014), co-products from grape production (e.g. pruning waste) do not fall within the production system boundaries. Indirect GHG emissions from machinery, infrastructure (including vineyard planting) and vehicles were excluded because they were considered negligible compared to the overall impact (D'Ammaro et al., 2021). Emissions generated from waste management were also considered negligible as, unlike wine-making or bottling operations, they are not relevant during grape production (ANPA, 2001). No complete data was available on irrigation water and energy required by the pumps for vineyard irrigation, therefore these sources of emissions were neglected. The vine nursery phase was also excluded from the study considering that the average number of vines replaced on an annual basis is low (Villanueva-Rey et al., 2014). As envisaged in the latest version of the ISO 14064 standard, the transport of the purchased products was calculated by determining the weight of the products and the mileage covered upstream of the supply chain. Workers' commuting trips were not considered due to lack of data.

2.3 Primary data acquisition

A survey was prepared and submitted to wineries in order to obtain the primary data necessary for CF calculation: type of management (conventional/organic), total vineyard surface area managed (ha), type and quantity of fertilizers consumed, type and quantity of pesticides consumed, amount of water used, transport of purchased products, fuel consumption of vehicles owned by the winery, rented or owned by agricultural contractors. Data on fuel consumptions related to the use of farming machinery owned by the winery or agricultural contractors were collected directly as the amount of fuel consumed (e.g., kilos of diesel and petrol); whereas fuel consumptions related to the use of vehicles for other activities, such as off-road vehicles, were collected as mileage traveled. The amount of specific active principle was collected for the mineral or synthetic fertilizers, pesticides and herbicides. The concentration stated on the label was used to determine the amount in kg of nitrogen contained in synthetic fertilizers. In the case of organic fertilizers (e.g. manure, compost, solid fraction of digestate), the nitrogen content was determined using data on organic matrices collected in several Italian geographical areas during the LIFE VITISOM Project (Valenti et al., 2019).

2.4 Corporate carbon footprint method

Primary data were aggregated and classified into three categories and five subcategories according to ISO 14064:2018. A total of twelve entries were defined based on data collected from questionnaires submitted to wineries (Table 2). Starting from the wineries' primary data, the corporate CF was determined adopting the Italian wine carbon Calculator (Ita.Ca.®), developed according to the ISO 14064 standard (Donna et al., 2012; ISO 14064, 2018). The carbon footprint can be quantified using the following equation:

$$CF = \Sigma\ (Pd_i\ \cdot\ EF_i)$$

where Pd_i is the *primary datum* quantifying the *i-th* process and EF_i is the *emission factor* of the *i-th* process. Ita.Ca.® provides the use of specific Emission Factors (EFs –

Table **2**) derived from the main LCA databases, including the UK Department for Environment, Food and Rural Affairs (DEFRA, 2022), French ADEME, Agence de l'environnement et de la maîtrise de l'énergie, Bilan Carbone database (ADEME, 2020), and Ecoinvent database, as well as publications of the Joint Research Centre (JRC), the European Commission's science and knowledge service and the Intergovernmental Panel on Climate Change (IPCC). The adoption of different databases allows to increase accuracy and specificity of emission factors (Rugani et al., 2013). In Table 2, EFs used for direct emissions from the use of vehicle fuels include indirect emissions related to the production and transportation of fuels. A specific EF has been used for each type of fuel or vehicle (e.g., diesel, LPG, petrol), derived by DEFRA (2022). In case of plant protection products, specific EFs found in the Ecoinvent and Bilan Carbone databases were used, when available, otherwise an average EF value was applied. For herbicides, the glyphosate emission factor was used (Ponstein et al., 2019). In the case of urea, it was considered that during fertilizing, CO_2 is also released into the atmosphere in addition to N_2O emissions (IPCC, 2006). The allocation of the environmental impact of organic fertilizers can be a controversial issue in agricultural systems (Villanueva-Rey et al., 2014; Vázquez-Rowe et al., 2012; Luo et al., 2009). As to manure, digestate and the waste from other production systems, the approach proposed by several author was adopted, which only includes the impact directly related to viticultural practices, such as the transport and direct fertilizing concerning the release into the atmosphere of GHGs, such as nitrous oxide, into the atmosphere (D'Ammaro et al., 2021; GHG Protocol for products., 2011; Navarro et al., 2017b). As indicated by Zampori and Pant (2019), N_2O direct and indirect emissions must be estimated taking into account 0.022 kg of N_2O emitted into the atmosphere for each kg of synthetic N fertilizer and organic fertilizer applied.

Table 2 Breakdown of primary data and sources of emission factors used (EFs).

Categories according to ISO 14064	Subcategories according to ISO 14064	Entries	Emission factor sources
Direct GHG emissions	Direct emissions from mobile combustion	Fuels for field operations, Fuels for other vehicles	DEFRA - Department for Environment, Food Rural Affairs, 2021
	Direct fugitive emissions arising from the release of GHGs in anthropogenic systems	Synthetic fertilizing Organic fertilizing (manure, compost, digestate)	For nitrous oxide emissions from fertilizing: Joint Research Centre (JRC), the European Commission's science and knowledge service provides scientific evidence throughout the whole policy cycle. (Zampori and Pant, 2019) For carbon dioxide emissions from urea use: Intergovernmental Panel on climate Change (IPCC, 2019, 2006)
Indirect GHG emissions from transportation	Indirect emissions from upstream transport for goods	Transport of purchased goods	DEFRA - Department for Environment, Food Rural Affairs, 2021
Indirect GHG emissions from products and services used by organization	Indirect emissions from purchased goods, which are associated with product manufacturing activities	Synthetic fertilizers Organic fertilizers, Fungicides, Herbicides, Insecticides	ADEME's Bilan Carbone database, ADEME 2020 The ecoinvent database (Frischknecht and Rebitzer, 2005)
	Indirect emissions from mobile combustion	Fuels for rental or non-owned vehicles Fuels for agricultural contractors.	DEFRA - Department of the Environment, Food Rural Affairs, 2021

We decided to express the functional unit as mass per unit of surface area, i.e., kg of CO_2 equivalent per hectare ($kg\ CO_2 - eq \cdot ha^{-1}$) (IPCC, Geneva, Switzerland, 2022). A comparison among wineries of different dimensions and different limits of yield defined by wine origin regulations, as those considered in the present study, could not be performed using kg CO_2-eq per kg of grape as the functional unit (Chiriacò et al., 2017; Renzulli et al., 2015).

2.5 Data analysis

An Analysis of Variance (ANOVA) was conducted to assess whether the total impact per hectare differed significantly between the two types of management, CONV and ORG. The relevance of the crop years was also tested given that different agronomic practices may have been adopted due to climatic conditions and pest pressure. Therefore, the effects of management and winery, management and year (two-way ANOVA) and the combination of management, winery and year (three-way ANOVA) were tested (function aov and TukeyHSD, R software). The second stage of analysis focused on the difference between the CF of direct and indirect emission subcategories according to management type. The non-parametric Wilcoxon rank sum test for two independent groups of samples was used (function Wilcox. Test, R software).

3. Results

3.1 Inventory data for vineyard inputs

Table 3 shows the mean, standard deviation, minimum and maximum values obtained for the key inputs in vineyard according to the management systems (ORG or CONV).

Table 3 Mean, standard deviation (SD), minimum and maximum values of vineyard inputs by surface area (ha) in organic and conventional vineyards. (a.s.): active substance

Vineyard inputs	**Organic**		**Conventional**	
	Mean (± SD)	[min; max]	Mean (± SD)	[min; max]
Fuels for field work (kg ha^{-1})	324.31 (± 97.44)	[216.44; 475.32]	237.1 (± 125.12)	[0; 500.81]
Fuel for 8others vehicles (km ha^{-1})	169.22 (± 197.45)	[0; 459.28]	139.14 (± 221.24)	[0; 693.86]
Manure (q ha^{-1})	3659.09 (± 6115.74)	[0; 16738.65]	2098.3 (± 3456.63)	[0; 13731.48]
Compost (q ha^{-1})	562.33 (± 1735.34)	[0; 5781.81]	78.62 (± 361.03)	[0; 2029.78]
Nitrous-based fertilizers (kg N ha^{-1})	-	-	8.07 (± 15.15)	[0; 72.59]
Urea-based synthetic fertilizers (kg ha^{-1})	-	-	5.99 (± 10)	[0; 45.08]
Phospho-potassium fertilizers and others (kg a.s. ha^{-1})	-	-	20.43 (± 23.81)	[0; 167.72]

Sulphur-based fungicides (kg a.s. ha^{-1})	125.07 (± 65.92)	[38.16; 225.39]	47.63 (± 48.94)	[0; 210.06]
Copper-based fungicides (kg a.s. ha^{-1})	4.66 (± 0.86)	[3.68; 5.76]	3.43 (± 2.75)	[0; 10.44]
Unspecific fungicides (kg a.s. ha^{-1})	-	-	9.84 (± 20.05)	[0; 116.31]
Herbicides (kg a.s. ha^{-1})	-	-	0.73 (± 0.88)	[0; 3.38]
Insecticides (kg a.s. ha^{-1})	-	-	0.46 (± 0.46)	[0; 2.05]
Natural insecticides (kg a.s. ha^{-1})	0.59 (± 1.17)	[0; 3.92]	0.25 (± 0.62)	[0; 2.43]
Fuels for rental or non-owned vehicles (km ha^{-1})	21.32 (± 28.38)	[0; 73.99]	2.37 (± 7.72)	[0; 37.02]
Fuels for agricultural contractors (kg ha $^{-1}$)	238.91 (± 534.52)	[0; 1440.00]	1213.02 (± 2596.32)	[0; 9489.15]

Inputs for fertilizers, fungicides, insecticides and herbicides are reported as active substances. Some inputs considered in categories of fertilizers, fungicides, herbicides and insecticides are not considered in the case of ORG system (-) because they cannot be used in organic agriculture on the basis of the specific rules and regulations set forth by the European Parliament and Council Regulation 2018/848/EU. The minimum value recorded in case of fuels (direct and indirect emissions) is zero because some wineries carry out farming operations using owned vehicles only (direct emissions) or, conversely, using only rented or farm contractor vehicles only (indirect emissions).

3.2 Overall organic and conventional viticulture carbon footprint results

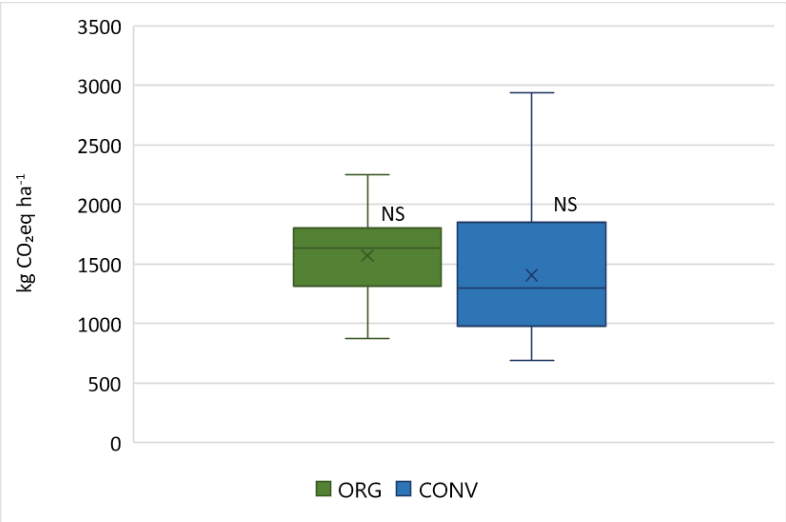


Fig. 2. Box plots showing variability of CFs in ORG and CONV systems and the results obtained by ANOVA analysis: NS not significant differences.

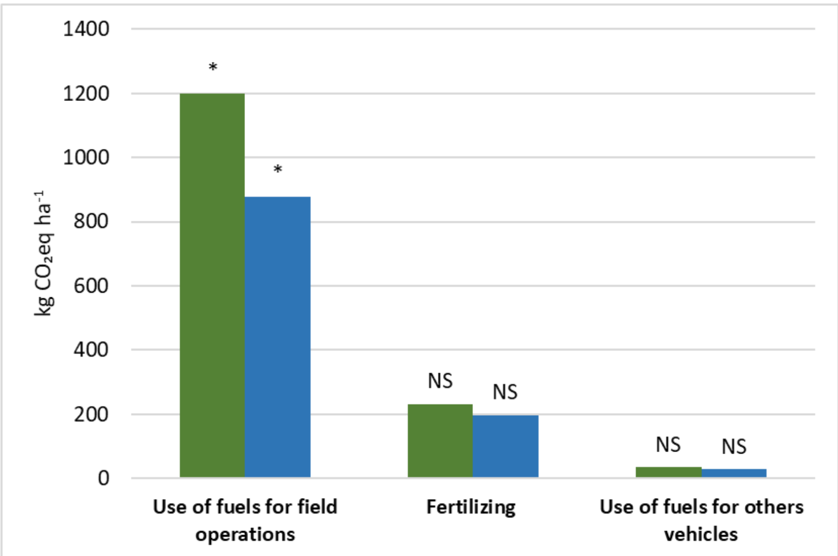
The overall emissions from the wineries considered range between 690.39 and 2937.03 kg CO_2-eq ha^{-1}. The median carbon footprint is 1408.34 and 1568.77 kg CO_2-eq ha^{-1} for CONV and ORG wineries, respectively. Data analysis (Fig. 2) reveals a high variability of data in the ORG system, even higher in the CONV system. The mean values of the overall impacts in the CONV and ORG systems show no significant differences.

Table 4 Mean, standard deviation (SD), minimum and maximum estimates of overall GHG emissions (kg CO_2-eq ha^{-1}) in ORG and CONV wineries

Management	**GHG emissions Mean (± SD)**	**GHG emissions [min/max]**
ORG	1568.77 (± 396.80)	[876.99; 2253.34]
CONV	1408.34 (± 535.27)	[690.39; 2937.03]
ALL	1443.63 (± 508.76)	[690.39; 2937.03]

3.3 Direct emissions in organic and conventional management

As shown in Fig. 3, emissions from "Use of fuels for field operations" show a high range of variability. This category represents the main contribution to direct emissions for both ORG and CONV systems, with a mean value of 1199.48 kg of CO_2-eq ha^{-1} in the case of organic management and 878.72 kg of CO_2-eq ha^{-1} in the case of conventional management. Direct nitrogen emissions generated by fertilizer distribution (Fertilizing) show an overall average value of 205.42 kg of CO_2-eq ha^{-1}; these emissions together with the consumption of fuels represent the greatest contribution to the value of direct emissions.



	Mean (± SD)	[min/max]	Mean (± SD)	[min/max]	Mean (± SD)	[min/max]
ORG	**1199.48** (± 361.55)	[798.61; 1755.37]	**232.27** (± 39.74)	[0; 877.78]	**34.22** (± 318.82)	[0; 91.17]
CONV	**878.72** (± 463.09)	[0; 1867.9]	**197.84** (± 203.24)	[0; 859.5]	**28.28** (± 45.27)	[0; 142.34]

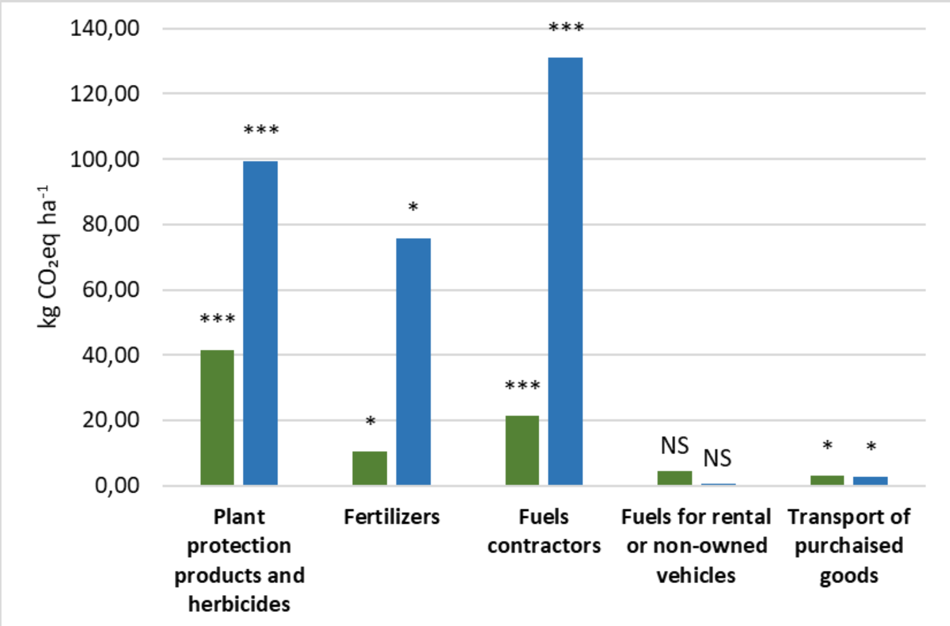
Fig. 3. The figure shows the ORG vs CONV results in terms of direct emissions. The significance of the differences was measured using the Wilcoxon test: * p-value < 0.05 and NS not significant.

The category "Use of fuels for field operations" refers to emissions from the use of own agricultural machinery; while the category "Use of fuels for other vehicles" refers to cars and vehicles, other than own agricultural machinery.

The emissions due to "Use of fuels for field operations" resulted statistically significant (p-value <0.01) between ORG and CONV, while no statistically significance is detected for Fertilizing and Use of fuels for other vehicles (Fig. 3).

3.4 Indirect emissions in organic and conventional management

Under the categories “Fertilizers” and “Plant protection products and herbicides” indirect emissions (Fig. 4) in CONV where higher than those in ORG. The lower value in the “Fertilizers” category for ORG vs CONV is due to the exclusion of indirect emissions for manure and digestate, whose production emissions are excluded as waste from other production systems. Emissions from the transport of goods purchased account for less than 1% of overall indirect emissions, both in CONV and ORG systems.



	Mean (± SD)	[min/ max]	Mean (± SD)	[min/ max]	Mean (± SD)	[min/ max]	Mean (± SD)	[min/ max]	Mean (± SD)	[min/ max]
ORG	**41.64** (± 14.5)	[22.74 ; 61.94]	**10.46** (± 21.98)	[0 ; 69.15]	**21.33** (± 47.72)	[0 ; 128.56]	**4.46** (± 5.85)	[0 ; 15.18]	**3.07**(± 1.74)	[0.62 ; 5.74]
CONV	**99.42** (± 121.14)	[16.25 ; 737.12]	**75.78** (± 81.94)	[0 ; 293.94]	**130.95** (± 265.33)	[0 ; 897.59]	0.53 (± 1.74)	[0 ; 8.63]	2.53 (± 3.6)	[0.01 ; 18.58]

Fig. 4. The figure shows the results comparing ORG and CONV systems in terms of indirect emissions. The significance of the differences was measured using the Wilcoxon test: *** p-value < 0.001; * p-value < 0.05 and NS not significant.

Wilcoxon test (Fig. 4) confirmed the existence of statistically significant differences between CONV and ORG. The indirect emissions due to Fertilizers, Plant protection products and herbicides, and Fuels for contractors in CONV are higher than those associated with ORG (p-values < 0.01, < 0.001, < 0.001, respectively). Indirect emissions from transport of goods purchased are higher for the ORG system (p-value < 0.001). No significant differences are found for "Fuels for rental or not-owned vehicles”.

4. Discussion

Overall GHG emissions. In this study, the overall mean value of corporate CF is 1443.63 kg of CO_2-eq ha^{-1}. For ORG the mean value is 1568.77 kg of CO_2-eq ha^{-1}, while for CONV the mean value is 1408.34 of CO_2-eq ha^{-1}. High variability was observed with corporate results ranging from 690.39 to 2937.03 kg CO_2-eq ha^{-1}. No statistically significant difference was found probably also due to the high heterogeneity of company CF estimated within each management system.

In a winery in the North-East of Italy, Borsato et al. (2020) a study involving an organically-managed vineyard and a conventionally-managed one shows a greater emission of greenhouse gases for the conventional vineyard, about 2534 kg CO_2-eq ha^{-1} versus 1827 kg CO_2-eq ha^{-1} in organic vineyard. Different values were found by Volanti et al. (2022), who, in a study involving three Spanish wineries using different management systems, estimated a CF ranging 57.4 to 289.3 kg CO_2 -eq ha^{-1} for ORG and 438.3 to 481.0 kg CO_2-eq ha^{-1} for CONV wineries. Average values similar to those presented here, have been found by Renaud-Gentié et al. (2020) who, in a multi-year study conducted on 12 plots using different management systems, located in three different French wine-growing regions, found average CF values of about 1300 kg CO_2-eq ha^{-1}.

According to Tuomisto et al. (2012), ORG and CONV should be understood not as one viticulture system but rather as a set of different practices. Therefore, the level of greenhouse gas emissions depends more on the choice of winery management rather than the management system. Some ORG wineries may find low CF values as a result of minimal use of inputs, fertilizing based on the addition of organic soil improvers and the use of non-synthetic products with a low emission impact, as argued by Reganold and Wachter (2016). Similarly, some CONV wineries may find lower CF values to optimize energy inputs and fertilize not only synthetic products but also organic soil improvers.

Direct GHG emissions. The contribution of the category "Use of fuels for field operations", with an average value of 1199.48 kg of CO_2-eq ha^{-1} accounts for 79.69% of the main emissions in the case of ORG systems, while it accounts for 61.99% in CONV systems, with an average value of 878.72 kg of CO_2-eq ha^{-1}. (Fig. 5).

As noted by Rouault et al. (2016) in a study comparing the organic and conventional system in a Chenin Blanc vineyard in the Loire Valley, the major carbon footprint impact in both management systems is due to fuel consumption, especially for plant protection treatments and soil management operations. The high contribution of “Fuels for field operations” to corporate CF is confirmed also by other studies. In a research involving 14 grape producers from four German administrative regions, Ponstein et al. (2019) observed the higher incidence of this category, with an average value of 565.59 kg of CO_2-eq ha^{-1}, compared to the overall emissions . Litskas et al., (2020) analyzed three different vineyards in Cyprus, with high or low conventional input and organic management, and observed a higher incidence of emissions from fuel consumption in ORG systems than from other factors, which is in line with what has emerged in this paper.

Increased fuel consumption in ORG systems vs CONV systems can be expected in relation to the high number of tractor transits in ORG systems (Litskas et al., 2020; Probst et al., 2008). In fact, non-synthetic copper-based fungicides are largely lost in foliar wash-off from vine leaves treated due to the action of rainfalls (Pérez-Rodríguez et al., 2015), with the consequent need for numerous interventions in rainy periods and hence greater diesel consumption. Similarly, the non-use of herbicides entails the need for a greater number of tillage operations, such as hoeing and mowing, for mechanical weed control (Rouault et al., 2016).

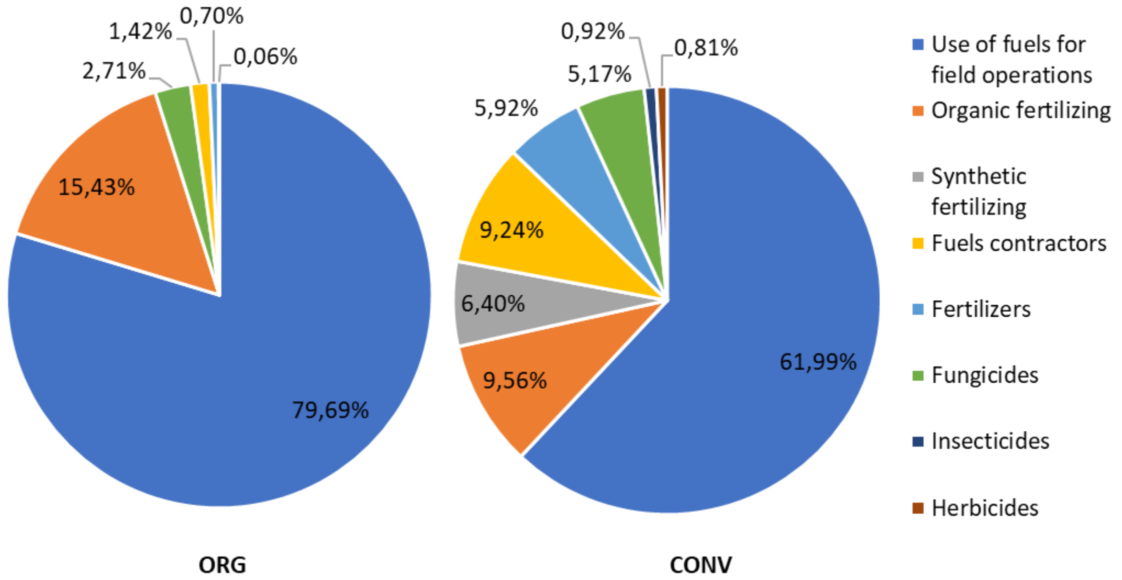


Fig. 5. Contributions of the emission categories in ORG and CONV management.

Direct emissions of nitrous oxide and carbon dioxide into the atmosphere during "Fertilizing" did not revealed any significant differences between the CONV and ORG systems. For both operations, some companies recorded zero emissions for lack of fertilizing in the study year.

In accordance with the results of Venkat (2012), which compared 12 agricultural products, including wine grapes grown in California with ORG and CONV management systems, direct emissions for fertilizing are similar for both systems, where CONV wineries limit the use of synthetic fertilizers.

As required by the legislation on organic farming, emissions related to synthetic fertilizing only concern CONV wineries when using synthetic fertilizers such as urea. For this reason, in ORG systems, the contribution of organic carbon is generally higher than that of CONV systems. Although the direct emissions from organic fertilizing are significant, it would be interesting to deepen the environmental benefits deriving from the use of these matrices, such as the improvement of the chemical-physical structure of the soil, the stimulation of the soil microbiota (Fregoni, 1999, p. 629), and the natural sequestration and maintenance of soil carbon stock (Brunori et al., 2016; Patinha et al., 2018). Furthermore, it is to be considered that unlike synthetic fertilizers produced specifically for agricultural fertilizing, organic fertilizers, which are often waste products from other activities, would still have an environmental impact.,.

Indirect GHG emissions. In CONV wineries the amount of indirect emissions due to "Fertilizers" and "Plant protection products and herbicides" is higher than that in ORG wineries (Figs. 5). A total of 99.42 kg of CO_2-eq ha^{-1} indirect emissions from "Pesticides and herbicides" were estimated on average in the CONV compared to 41.64 kg of CO_2-eq ha^{-1} in the ORG; while the average estimated emissions due to "Fertilizers" in CONV is 75.78 kg of CO_2-eq ha^{-1}, which more than 7 times the value measured in the ORG (10.46 kg of CO_2-eq ha^{-1}).

Our estimates are consistent with the results measured by Chiriacò et al. (2019). The authors assessing the GHG balance in an organic winery in the Lazio administrative region (Central Italy) found that organic wineries can state emissions for the category "Plant protection products" lower than 10 kg of CO_2-eq ha^{-1}, due to the non-use of synthetic products. In the Global Warming category, also Volanti et al. (2022), found a significant impact of conventional farming as the result of the use of fertilizers and synthetic products as herbicides.

Synthetic fertilizers, pesticides and herbicides are key inputs in CONV systems; by contrast, ORG systems are based on the use of natural mineral or organic substances, generating less indirect emissions (Briar et al., 2007).

Regarding the differences highlighted in the "Fuels for contractors" category, where high values were found in CONV cellars, it is important to note that, although they are divided, these emissions are similar to fuel consumption for field operations. In fact, if the winery did not employ contractors, it would be required to directly manage the operations in the field. This category depends very much on the strategic choices and the winery's specific characteristics; indeed, equipment cost and size as well as the rapid pace of technological innovation all affect the choice of whether to use contractors, especially in small and medium-sized farms (Nye, 2020).

5. Conclusions

In conclusion, the study of 25 wineries, including 19 CONV and 6 ORG, in several years of harvesting, did not reveal statistically significant differences between the overall CF of ORG and CONV wineries. The extent of the dataset made it possible to highlight the differences in impact of each category within the two systems.

In relation to the total amount of GHG emissions, beyond the management system (ORG or CONV), a specific management approach is to be considered depending on each winery's characteristics and production strategies. A management approach encompassing the agricultural technical specifications according to the characteristics of the winery is more effective in mitigating corporate emissions. In both management systems, the greatest impact is due to fuel consumption for field operations, which suggests precise monitoring of fuel consumption in order to optimize management strategies. This is particularly relevant in ORG systems due to a high number of plant protection treatments and mechanical weed control.

Nitrogen fertilizing has an important impact on CF, both in ORG and CONV systems, mainly in relation to the release of nitrous oxide into the atmosphere. Organic fertilizing, which is more common in ORG vineyards, should also be considered as a mitigation action contributing to the increase of naturally fixed carbon stock into the soil. The integration of organic carbon sequestration in the CF analysis can consequently lead to a more complete comparison of ORG and CONV systems. Considering indirect emissions, synthetic products have a greater impact in terms of greenhouse gas emissions, although these emissions have a low share overall.

From what has been observed with respect to the main GHG emissions, it is possible to adopt some mitigation strategies for both management systems: i) innovation in technology introducing agricultural machineries with a greater efficiency in fuel consumption, ii) reduce the number of interventions in the field, reducing the depth of soil tillage (Navarro et al., 2017b), iii) optimize the number of treatments based on weather conditions and previous fungal infections (Mian et al., 2021).

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.

Acknowledgment

The authors thank all the wineries that provided the primary data for this study.

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2.1 The effects of leaf removal and artificial shading on the composition of Chardonnay and Pinot noir grapes

Chapter 2.1 has been published in OENO One 2020, 54, 4, 761-777 DOI:10.20870/oeno-one.2020.54.4.2556

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Abstract
Aims: The aim of this study was to assess the effects of leaf removal and bunch shading on the analytical composition of Pinot noir and Chardonnay (*Vitis vinifera* L.) grapes suitable for making premium sparkling wine.

Method and results: Total bunch defoliation (TD) and different treatments using shading nets (TD1L, TD2L and ND1L) were evaluated in comparison with a test with no defoliation and shading (ND) over three seasons in the southern part of Franciacorta, one of the most famous Italian sparkling wine regions. Micrometeorological variables, yield components, musts and grapes chemical composition were evaluated. Shading practices lead to a delay in ripening and they improve the acidic content of must, thus resulting in a potential improvement in the quality of juice suitable for producing sparkling wines. Furthermore, this particular type of vine canopy management leads to changes in the phenolic content of grapes.

Conclusions: From the results obtained it was possible to underline the positive effect - delaying ripening, preserving acid concentration and reducing flavonol content - of shading on the composition of Pinot noir and Chardonnay grapes suitable for making premium sparkling wine.

Significance of the study: This study shows the importance of shading, because it delays grape ripening, and thereby preserves the acidic content of musts, and, specifically, deals with the problem of early ripening related to the climate change now underway.

Keywords: vine, shading, grape, must, leaf removal.

Introduction

The average increases in temperature and different rainfall distributions have led to major repercussions in the agricultural sector; in the case of vines in particular, the different stages of development generally take place earlier and the time between veraison and ripening is shorter

(Schultz, 2000; Jones *et al*., 2005). This can affect grape and wine quality by increasing alcohol content and reducing aroma and acidity (Webb *et al*., 2007; Hall and Jones, 2009). Acidity is an important determinant of sparkling wine quality and longevity (Ribéreau-Gayon *et al*., 2000).

The effect of defoliation and shading on vine cultivation and grape quality has been widely studied in the past (Crippen and Morrison, 1986; Jackson and Lombard, 1993; Downey *et al*., 2006). Moreover, several studies have focused on the relationship between canopy management and the variation in temperature of grapes and berries. Berries shaded by canopies show thermal behaviour very similar to that of air (Reshef *et al*., 2017); however, different studies have demonstrated a steady increase in the temperature of exposed bunches (Spayd *et al*., 2002), which can reach values between 7 °C and 12 °C higher than the air temperature (Kliewer and Lider, 1968; Smart and Sinclair, 1976; Bergqvist *et al*, 2001). Bunches exposed to direct solar radiation can reach temperatures higher than 37 °C (Crippen and Morrison, 1986), thus exceeding the optimum temperature range for berry development, which has been identified as between 25 °C and 35 °C (Hale and Buttrose, 1974). This influences berry ripening and metabolism, particularly in terms of reducing titratable acidity and increasing malic acid degradation (Lakso and Kliewer, 1978; Conde *et al*., 2007; de Oliveira *et al*., 2019). The majority of studies to date have characterised the combined influence of solar irradiance and its accompanying climatic component, temperature - both of which are known to influence several metabolic processes - on berry composition. Berry temperature is determined by the energy balance of the fruit and is strongly affected by direct exposure to solar radiation (Cola *et al*., 2009). At a practical level, solar irradiance is the most easily and readily controlled climatic factor (Reshef *et al*., 2017).

For some time, studies have indeed demonstrated the effect of shading on delaying ripening (Rojas-Lara and Morrison, 1989; Percival *et al*., 1994; Filippetti *et al*., 2014; Martin *et al*., 2016) and preserving acidity, both in terms of titratable acidity (Reynolds *et al*., 1986; Smart *et al*., 2017) and malate concentration (Dokoozlian and Kliewer, 1996; Martin *et al*., 2016). Some studies have associated artificial shading with an increase in pH and potassium (Smart *et al*., 1985; Scafidi *et al*., 2013; Martinez and Balda, 2014), although more recent studies have reported that this treatment does not significantly affect **this** parameter (Filippetti *et al*., 2014).The exposure of bunches to sunlight can also modify the content of anthocyanins (Bergqvist *et al*., 2001; Dokoozlian and Kliewer, 1996; Downey *et al*., 2003; Haselgrove *et al*., 2000; Mori *et al*., 2005; Spayd *et al*., 2002) and other polyphenols in berries. Above a certain temperature range, both anthocyanin and polyphenol synthesis are inhibited, as reported in various previous studies (Kliewer and Torres, 1972; Price *et al*., 1995; Pastor del Rio and Kennedy, 2006; Fernandes de Oliveira and Nieddu, 2015). Nevertheless, some authors have reported that a decrease in the exposure of Pinot noir grapes to sunlight can cause

a reduction in total anthocyanin concentration (Dokoozlian and Kliewer, 1996), changing the pattern and leading to lower percentages of delphinidin-glucoside, cyanidin-glucoside, petunidin-glucoside and malvidin-glucoside, with an increase in peonidin-glucoside (Cortell and Kennedy, 2006).
In general, the response of grapes to different levels of exposure, in terms of accumulation of anthocyanins and phenolic substances, also seems to be related to the cultivar's sensitivity to temperature (Fernandes de Oliveira *et al*., 2015). Recently, the complex influence of the spatial pattern of incoming irradiance and fruit temperature on the metabolic profile within grape clusters of Cabernet Sauvignon was described in a vineyard in the Negev desert, Israel, where excess solar irradiance and midday temperatures are known to reduce grape quality. The higher irradiance increased the concentration of several amino acids and polyamines (proline, valine, leucine, GABA, putrescine and ethanolamine) and of tartaric acid in the pulp, while decreasing malic acid. Irradiance increased the concentration of phenylalanine, flavonols, naringenin-chalcone-4-O-glucoside and cyanidin-3-glucoside in the skins, while decreasing malvidin-3-glucoside, hydroxycinnamic acids and monomeric and dimeric flavanols (Reshef *et al*., 2017).
This paper aims to compare different canopy shading levels for *Vitis vinifera* L. cv. Chardonnay and Pinot noir suitable for producing sparkling wine. The effects of different levels of bunch exposure on the vine, must and berry composition, and the relationship between treatments and micro-meterorological variables are described.

Materials and methods

1 Experimental trial

This research was conducted in three consecutive years (2013, 2014 and 2015) in a vineyard belonging to Azienda Agricola Castello Bonomi Tenute in Franciacorta, located in the southern part of the Franciacorta viticultural area (Lombardy Region). This geographical context is characterised by temperatures about 3 °C higher than the average for other vineyards in this winegrowing area. The vineyard was planted in 2004, cordon-trained, oriented from north to south and grass-covered.
To further confirm the results obtained, treatments were applied to two international *Vitis vinifera* L. cultivars (Chardonnay clone ENTAV-INRA® 96 and Pinot noir clone 292, both grafted onto Kober 5BB rootstock), which are both traditionally cultivated in this area.
Five different treatments were compared in all the years considered: a comparative test without defoliation and shading (ND), a test with total defoliation (east and west side) (TD), and three different systems adopting shading nets applied along the bunch zone; two of the shaded treatments were defoliated as for TD and covered with one layer of shading net (TD1L) or two layers of shading net (TD2L), while a third treatment was covered by only one layer of shading net, but not defoliated (ND1L).

For both cultivars, the treatments were organised into three randomised blocks, each consisting of 25 vines. The treatments were maintained in the same blocks during the whole trial period. Leaf removal and shading net application took place at about 20 % veraison and was carried out along the bunch zone (about six basal leaves removed equal to about 35 % of total leaf area), while a polyethylene UV stabilised net of approximately 95 g/m^2 was used for shading (shading net OF50N provided by Retes srl). Preliminary tests were carried out in order to evaluate the percentage of global solar radiation passing through the nets. The transmittance of global solar radiation of the single layer and double layer nets was reduced by 50 % and 70 % respectively.

2 Meteorological data

Two types of measurement were carried out in all three years of the study with the aim of better understanding the effects of shading on canopy and berry temperatures. Specifically, for the cultivar Chardonnay alone, five field weather stations were installed to monitor temperature and humidity during the period between the beginning of veraison (when defoliation and shading were implemented) and harvesting. Each weather station consisted of an Onset Hobo datalogger endowed with a silicon pyranometer and an air temperature/relative humidity sensor placed in a solar shield. One station monitored atmospheric variables outside the canopy and the sensors were placed outside the vineyard at standard heights, following the recommendations of the World Meteorological Organization (WMO, 2009). In the case of the other four stations, the sensors were placed at the height of the bunches (first wire level) in order to monitor the variables under the canopy for each of the four main treatments: ND, TD, TD1L and ND1L. We decided to focus on these four treatments, because they best represent the different conditions of the canopy (presence-absence of leaves; absence of artificial cover). Monitoring took place at a 5-minute time step. Subsequently, the data were aggregated to provide hourly and daily time steps.

Internal berry temperature was measured withan Onset Hobo Copper–Constantan thermocouple inserted into the berry. Data were collected with a specific datalogger. The measurements were carried out with a reduced time step of 1 min during the period, ranging from post-veraison to harvest (see Table 5): from 5 to 27 August in 2013, from 23 July to 17 August in 2014 and from 16 July to 7 August in 2015.

Inner berry temperature monitoring followed the protocol adopted in Cola *et al*. (2009): the thermocouple tip was inserted into the berry, previously pierced with a spike; the thermocouple tip was placed on a berry in the external-middle part of the cluster; the thermocouple was relocated to a new berry every week in order to maintain optimal conditions of the living organs, so that withering could not influence the measurements. Each thermocouple was installed in a single randomised block for each treatment. No replicates of the measurements were taken.

In order to evaluate the thermal conditions of berries, the following indices were calculated for each treatment from berry temperature measurements for the period between fruit set and physiological maturity:

- GDD - Growing Degree Days, calculated from average daily berry temperature using 10 °C as a base (as with the Winkler Index (Amerine and Winkler, 1944), cumulated from fruit set to physiological maturity;
- NHH - Normal Heat Hour Index, which represents the accumulation of hourly thermal resources useful for berry maturation (Cola *et al*., 2020), cumulated from fruit set to physiological maturity;
- HHH - High Heat Hour Index, which represents the accumulation of hourly thermal excess (Cola *et al*., 2020), cumulated from fruit set to physiological maturity.

The main limitation of the GDD approach is the overestimation of high temperatures: a very hot summer day will show a high mean daily temperature. This translates into high GDD, meaning optimal conditions for plant growth. However, since temperature can be detrimental to biological processes, the NHH and HHH approaches (Mariani *et al*. 2012, Cola *et al*. 2017) measure hourly temperature (Th) based on four cardinal temperatures: LC - low cardinal (6 °C), LOC - low optimal cardinal (24 °C), UOC - upper optimal cardinal (26 °C) and UC - upper cardinal (33 °C). LC and UC limit the cardinal range within which phenological development occurs, while LOC and UOC define the optimum for phenological development. The response function (Figure 1) translates hourly temperature into thermal effective hour: Th gives 0 NHH, if outside the cardinal range, and 1 NHH, if within the optimal range. As Th moves from LC to LOC, NHH linearly increases from 0 to 1 and, similarly, NHH linearly decreases from 1 to 0 as Th moves from UOC to UC (Cola *et al*. 2016). The values of the four parameters LC, LOC, UOC and UC proved to perform well for all the studied cultivars (Cabernet Sauvignon, Chardonnay, Barbera and the Georgian cultivars Mtsvane Kakhuri, Rkatsiteli, Ojaleshi and Saperavi) (Mariani *et al*. 2013, Cola *et al*. 2014, Cola *et al*. 2016).

It is well known that shaded berries show a thermal regime very close to air (Cola *et al*., 2009, Berquivst *et al*., 2001), while the temperature of sun-exposed black berries exceeds air temperature by up to 10 °C.

Several authors have discussed the relationship between environmental temperature and ripening processes (Abeysinghe *et al*., 2019, Kuhn *et al*. , 2014, Mori *et al*., 2005, Downey *et al*., 2004, Downey *et al*., 2003, Spayd *et al*., 2002, Haselgrove *et al*., 2000), while few have tried to consider berry temperature (Wu *et al*., 2019, Lecourieux *et al*., 2017, Greer and Weedon, 2014, Bergqvist *et al*., 2001); it is therefore hard to understand how the temperature of air affects the temperature of clusters and then the ripening.

The NHH response curve was parameterised in order to describe the phenological development of grapevine and is strictly related to the net photosynthesis response to temperature (Greer, 2017 and Greer and Weedon, 2012). The use of the same response curve to calculate thermal resources of berries can be seen as an attempt to summarise the bulk of different physiological processes of ripening, each of which is characterised by a specific response function.

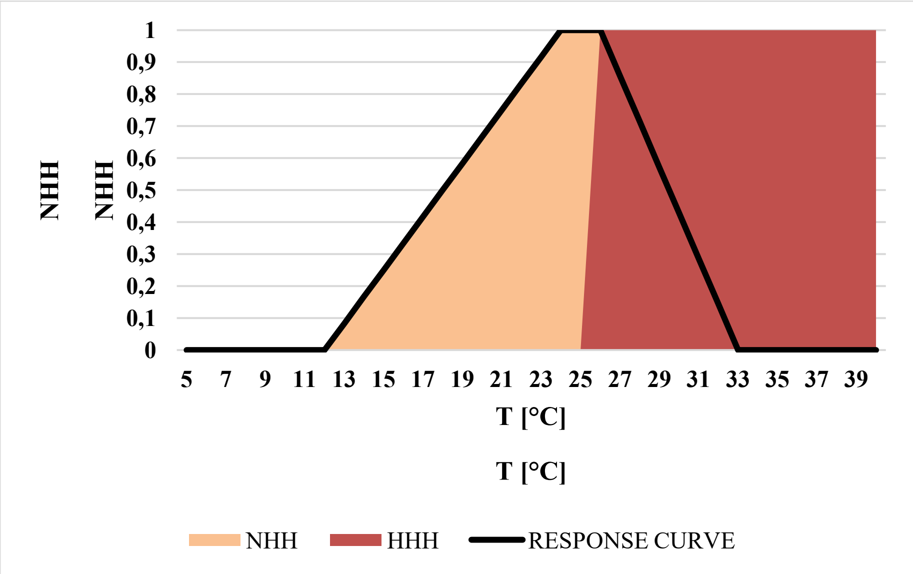

Figure 1. NHH and HHH Response curves relating thermal resources and temperature.

Furthermore, the three seasons were characterised according to the reference period 1988-2015, representative of the current warm phase that started in Europe at the end of the 1980s (Mariani *et al.*, 2012). The analysis was carried out with data from the weather station of Rovato, provided by the Agrometeorological Network of the Province of Brescia, located 2 km from the experimental field.

3 Yield components and the composition of grape juice

The evolution of ripening for both Chardonnay and Pinot noir was monitored by periodically sampling each of the five treatments. Seventy berries were collected from each randomised block (35 berries collected from both the eastern and the western sides and then grouped together in a single sample). The first sample was collected on the same day the shading net was installed and when defoliation took place, to obtain evidence regarding the condition of the parcels before setting up the trial. From that moment on, weekly samples were taken until harvesting time approached, when the frequency of sampling was intensified.

For both cultivars and all treatments, the harvesting time was established at about 10.5 % of potential alcohol. In this case the tests were called "fixed alcohol" and indicated with -FA at the end of the treatment code (i.e., ND-FA, TD-FA, TD1L-FA, TD2L-FA, ND1L-FA). With the aim of having a more complete view of the ripening trend, for the Chardonnay cv alone, another harvest was carried out on the same date, when the earliest treatment reached 10.5 % potential alcohol. In this case the tests were called "fixed date" and indicated with -FD at the end of the treatment code (i.e., ND-FD, TD-FD, TD1L-FD, TD2L-FD, ND1L-FD). Each harvest date is reported in detail in Table 5.

Table 5 Harvest dates specified by year and treatment. Dates of treatment application and harvesting are reported both for Chardonnay and for Pinot noir. In case of Chardonnay harvesting dates are divided by -FD (fixed date) and -FA (fixed alcohol).

Year	**Cultivar**	**Dates of treatment application**	**Dates of FD harvesting**	**Dates of FA harvesting**
2013	**Chardonnay**	29/07	All treatments 28/08	ND 28/08; TD 28/08; TD1L 28/08; TD2L 2/09; ND1L 2/09
	Pinot noir		/	ND 22/08; TD 20/08; TD1L 23/08; TD2L 22/08; ND1L 24/08
2014	**Chardonnay**	16/07	All treatments 17/08	ND 17/08; TD 17/08; TD1L 17/08; TD2L 17/08; ND1L 21/08
	Pinot noir		/	ND 11/08; TD 11/08; TD1L 11/08; TD2L 13/08; ND1L 13/08
2015	**Chardonnay**	15/07	All treatments 10/08	ND 11/08; TD 10/08; TD1L 10/08; TD2L 12/08; ND1L 12/08
	Pinot noir		/	ND 5/08; TD 4/08; TD1L 4/08; TD2L 5/08; ND1L 6/08

Experimental harvesting was organised by selecting a total of 18 vines per treatment (ND, TD, TD1L, TD2L and ND1L), corresponding to 6 plants for each of the three randomised blocks. For each vine, total yield (TY), average bunch weight (AWG), yield/pruning weight (Ravaz Index - RI) and bud fertility (BF) were determined. Bud fertility was calculated from the ratio between the total number of bunches and the total number of buds, including shoots and not sprouted buds. A sample of three bunches was collected to check juice quality. These samples were then crushed and the total soluble solids concentration (TSS), pH, titratable acidity (TA) and malic acid (MA) concentration were measured in the grape juice. These measurements were determined respectively using a traditional handheld refractometer for soluble solids concentration, a Crison compact titrator analyser both for pH and TA, and the enzymatic method (Hyperlab wine analyser) to determine malic acid concentration.

4 Grape anthocyanin and polyphenol composition

At harvesting, both at 10.5 potential alcohol and in the case of Chardonnay on the fixed date, 1 kg racemes sample was collected for each randomised block (for a total of three samples for each treatment). The sample was taken by collecting about 500 g from 20 clusters on each side (east and west) and immediately sent to the laboratory at the Edmund Mach Foundation, where it was stored at -80 °C until analysis. The sub-sampling procedure, which aimed to obtain a smaller representative sample, consisted of two steps. After removing the pedicels, a sample of 100 g of berries was randomly selected. From this a further subsample consisting of 30 randomly picked deep-frozen berries was ground under liquid nitrogen using an IKA analytical mill (Staufen, Germany) to obtain a frozen powder. A total of 3 g of the powder from each sample was extracted in sealed glass vials using 10 mL of a water/methanol mixture (30:70). After vortexing for 1 min, the samples were transferred to an orbital shaker for 15 min at room temperature. Samples were centrifuged at 1000 g and 4 °C for 10 min. Extraction was repeated by adding another 5 mL of water/methanol (30:70) and after centrifugation, the two extracts were combined, brought to 20 mL with demineralised water, and filtered through a 0.2 µmPTFE filter prior to analysis.

Chromatographic, separation and detection conditions were the same as those extensively validated for the quantitative analysis of phenols, as described by Vrhovsek *et al*. (2012). Briefly, Ultra Performance Liquid Chromatography separation of phenolic compounds, lasting 17 min, was performed on a Waters Acquity UPLC by means of a Waters Acquity HSS T3 column 1.8 µm, 150 mm × 2.1 mm, kept at 40 °C. Mobile phase A comprised water containing 0.1 % formic acid; mobile phase B comprised acetonitrile containing 0.1 % formic acid. The flow was 0.4 mL/min. This targeted method was developed for the quantification of 60 phenolics, including benzoic acid derivatives, phenylpropanoids, coumarins, stilbenes, flavan-3-ols, flavonols, anthocyanins and thiols.

5 Statistical analysis

The statistical analysis was carried out with SPSS software (Statistical Package for Social Science). In the preliminary data analysis, outliers were deleted; i.e., observations with values greater than 1.5 interquartile ranges (IQRs) above the third quartile, or lower than 1.5*IQRs below the first quantile. To analyse analytical and growth-productivity results, a linear mixed effects model ($p < 0.05$) was performed, including "treatment" and "block" as fixed factors, and "year" as a random factor. The block was not included as a fixed factor in ANOVA, which was carried out on phenolic and thiol variables, because a single observation was made for each block. A post-hoc REGWF (Ryan, Einot, Gabriel, and Welsch F) procedure was implemented to compare the pairs of treatment means while controlling fixed and random factors.

A Principal Components Analysis (PCA) was carried out to visualise the pattern of behaviour for productive and quality variables, together with microclimatic variables (GDD, NHH and HHH). We could only apply this analysis to data obtained from Chardonnay-FD, as the thermocouple tip was inserted into the berry on this cultivar in the period between post-veraison and the fixed harvest date (-FD). We carried out this analysis on the 6 plants for each treatment and each year, in the block where the thermocouple had been installed. We therefore referred to the specific microclimatic data and the specific UPLC data of that randomised block. PCA was performed with the R package, FactoMineR, scaling data to unit variance. We also computed the correlation matrix of the microclimatic variables with the productivity and quality variables.

Results

1 Weather data

Figure S1 and S2 show the temperature and rainfall in the three seasons, compared to the reference period (average for the 1988-2015 period).

In terms of temperature, the 2013 results were, in general, similar to the reference period: maximum monthly temperatures were close to normal values, with a slightly positive anomaly in February, March, April and July. The minimum temperatures were slightly above the normal values throughout the year. Yearly precipitation was slightly higher than in the reference period (1027 vs 971 mm, +5.8 %). Strong positive anomalies characterised March and May, while negative anomalies were recorded in June and September.

2014 was characterised by very high minimum temperatures during the first four months, average values in summer and high values from September to December. Maximum temperatures were close to average values, with the exception of July and August, characterised by low values. The yearly precipitation was high (1298 Vs 971 mm + 34 %), with highly positive anomalies in January, February, July, August and November, while spring was characterised by a negative anomaly.

2015 was a fairly average year in terms of both minimum and maximum temperatures. Above-average temperatures were recorded in June, July and August. Precipitation was very low for the area, with 565 vs 971 mm (-42 %). Negative anomalies characterised the whole year, with the sole exception of October.

1.1 Micro-meteorological data

Based on data measured directly inside the berries, thermal resources during the ripening periods were analysed. An example of berry temperature monitoring is shown in Figure 2.

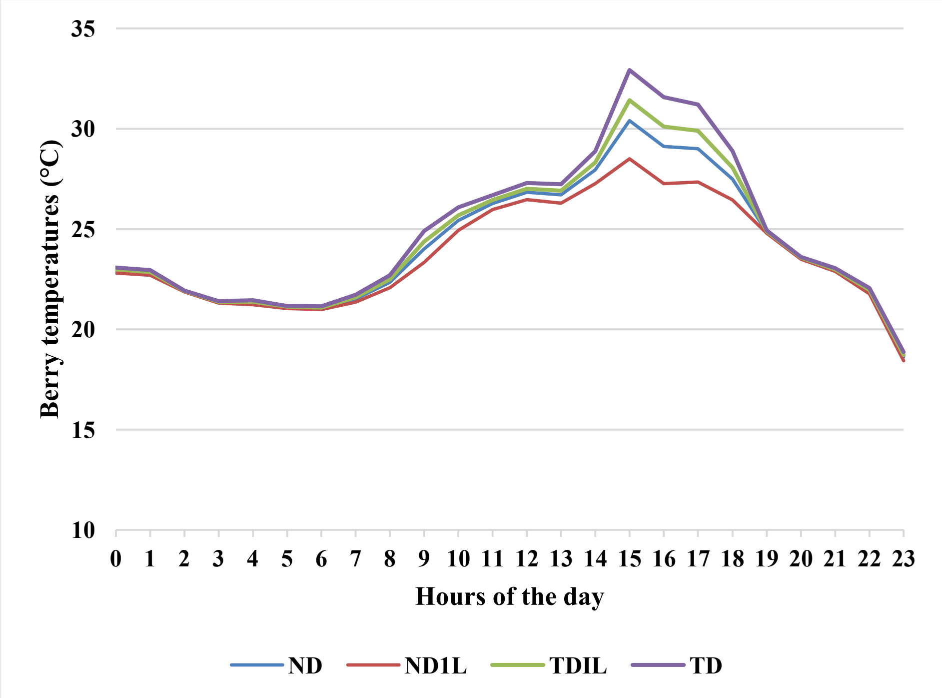

Figure 2. Thermal trend in berries for the four monitored treatments (2 August 2014).

Table 6. GDD, NHH and LHH accumulation during Chardonnay ripening.

Index	**Year**	**ND**	**ND1L**	**TD1L**	**TD**
GDD	**2013**	338.5	314(-7.2 %)	344.2(1.7 %)	361.6(6.8 %)
	2014	394.6	362.2(-8.2 %)	407.9(3.4 %)	430.5(9.1 %)
	2015	439.5	409.9(-6.7 %)	458.4(4.3 %)	474.2(7.9 %)
NHH	**2013**	337.6	346.4(2.6 %)	333.2(-1.3 %)	327.2(-3.1 %)
	2014	454.1	472.8(4.1 %)	442.9(-2.5 %)	428.8(-5.6 %)
	2015	313	324.2(3.6 %)	308.1(-1.6 %)	301.7(-3.6 %)
HHH	**2013**	106.4	97.1(-8.7 %)	111.1(4.4 %)	117.2(10.2 %)
	2014	63.5	36.3(-42.8 %)	78.2(23.3 %)	97.0(52.9 %)
	2015	201.8	189.2(-6.2 %)	207.3(2.7 %)	214.5(6.3 %)

% variation compared to the ND treatment is shown

Table 6 shows GDD, NHH and HHH based on inner berry temperature during ripening with the different treatments. For each season, the variability between treatments was expressed as a percentage increase/decrease compared to the ND treatment.

Regarding accumulation of GDD, variability between treatments was low, the maximum difference in 2014 when the highest level was reached by TD (+9.1 % compared to ND) and the lowest by ND1l (-8.2 %). As regards variability between years, 2013 was the year with the lowest GDD values, while 2015 showed the highest levels for all treatments (on average +31 % in 2015 compared to 2013). The ranking from the lowest to the highest GDD value was NDL1, ND, TDL1 and TD in all the years. Variability in the accumulation of NHH with the treatments was low, and 2014 was again the year with the highest variability: +4.1 % for NDL1 and -5.6 % for TD. As regards the years, 2014 was the year with the highest NHH accumulation, while 2015 had the lowest (on average +33.7 % in 2014 and -7.3 % in 2015 compared to 2013). The ranking from the lowest to the highest NHH value was TD, TDL1, ND and NDL1 in all the years. The ranking was reversed in comparison to GDD. This can be explained by the levels of thermal stress caused by above optimal temperatures, as shown by the HHH Index. The higher variability of the HHH index can be explained by the lower values of the accumulated index. As a consequence, the percentage variation may be higher. 2014 showed the highest percentage variability (from -42-7 % for NDL1 to +52.9 % for TD) and the lowest absolute values. 2015 was the year with the highest HHH accumulation, while 2014 had the lowest (on average +88.5 % in 2014 and -37.4 % in 2015 compared to 2013). The ranking from the lowest to the highest HHH accumulation was NDL1, ND, TDL1 and TD in all the years.

2 Growth-productivity results

The total yield (TY) and average bunch weight (AWG) results averaged over the three years of observations are shown in Table 7. TY and AWG were significantly affected by the cultivar. TD showed a higher production level compared to other treatments for Chardonnay-FA, while for Pinot noir-FA, ND showed the highest TY value. As expected, AWG behaviour was similar to TY, but the differences were only significant when comparing TD and ND1L for Chardonnay-FA. Pinot noir-FA AWG increased for ND and TD2L compared to TD. Chardonnay-FD showed higher TY values for ND1L and TD compared to other treatments, while AWG was significantly different in ND1L and ND, TD1L and TD2L. Considering other growth-productivity parameters, data related to the Ravaz Index (RI) and bud fertility (BF) showed minor differences.

Table 7 Total yield (TY), average bunch weight (AWG), Ravaz Index (RI) and bud fertility (BF) averaged over the three years of observation.

	Chardonnay-FA					**Chardonnay-FD**					**Pinot noir-FA**				
	TD	**ND**	**TD1L**	**TD2L**	**ND1L**	**TD**	**ND**	**TD1L**	**TD2L**	**ND1L**	**TD**	**ND**	**TD1L**	**TD2L**	**ND1L**
TY (kg)	$2,7^{a}$	$2,2^{b}$	$2,4^{b}$	$2,1^{b}$	$2,3^{b}$	$2,7^{a}$	$2,2^{b}$	$2,4^{b}$	$2,2^{b}$	$2,8^{a}$	$2,5^{b}$	$3,0^{a}$	$2,6^{b}$	$2,6^{b}$	$2,6^{b}$
AWG (g)	140^{a}	135^{ab}	132^{ab}	126^{ab}	123^{b}	140^{ab}	131^{b}	131^{b}	130^{b}	145^{a}	111^{b}	123^{a}	120^{ab}	122^{a}	119^{ab}
BF	1,1	0,9	0,9	0,9	0,8	1,1	0,9	0,9	1,3	1,0	$1,0^{b}$	$1,1^{a}$	$0,9^{b}$	$0,9^{b}$	$0,9^{b}$
RI	$4,7^{a}$	$4,0^{ab}$	$4,1^{ab}$	$4,2^{ab}$	$3,6^{b}$	4,7	4,0	4,1	4,4	4,3	4,1	4,9	4,6	4,7	3,9

Different letters indicate significant differences in the REGWF test ($P < 0.05$); when no letters are present no significant differences were found.

3 Influence on ripening

As shown in Figure S3 and S4, ND1L showed a slight delay in ripening for all three years of the study for both cultivars, although in some cases there was only a one-day delay compared to other treatments. ND1L matured two days later in 2013 and 2014 and one day later in 2015 compared to ND, when applied to Pinot noir. In the case of Chardonnay ND1L, sugar accumulation was slower, with a delay of five days in 2013, four days in 2014 and one day in 2015 compared to ND. TD2L showed similar behaviour to ND1L, although in this case a delay was not recorded in all the years considered. When applied to Pinot noir, TD2L showed a delay in ripening of two days in 2013 and 2014, and one day in 2015 compared to TD, while as regards Chardonnay, a five-day delay was recorded in 2013 and a two-day delay in 2015. TD1L showed delayed maturation in the first year of the study when applied to Pinot noir. The total defoliation treatment (TD) led to the fastest accumulation of sugar in all three years of the study.

4. Analytical Results

4.1 Technological characterisation of must

4.1.1 Pinot Noir

The analysis of Pinot noir must show the effect of artificial shading without leaf removal on TA and MA concentration. ND1L-FA had the highest value in terms of MA concentration compared to all other treatments (Figure 3), while TA results increased compared to TD-FA and ND-FA. TD-FA had the lowest level of TA, while as regards MA concentration, this treatment gave similar results to TD2L-FA. TD1L-FA had a lower pH level compared to other treatments, such as ND-FA, FD-FA and TD2L-FA, and the results were similar to ND1L-FA.

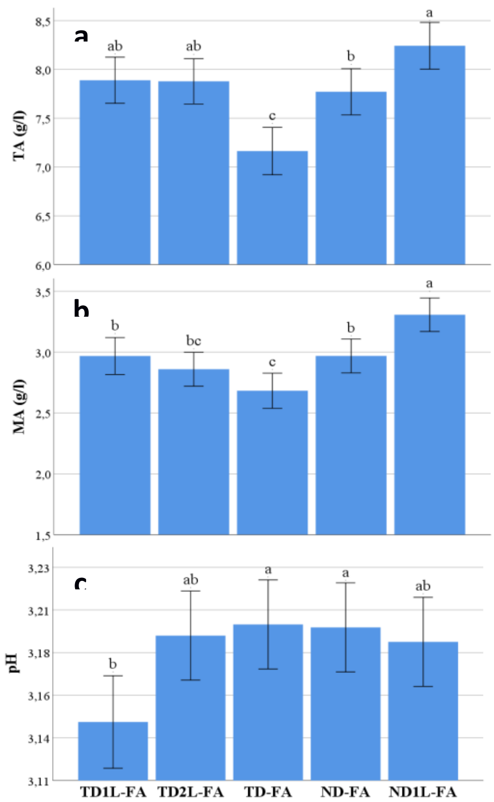

Figure 3. Average titratable acidity (TA) (a), malic acid content (MA) (b) and pH (c) recorded for the 3-year period for Pinot noir-FA.

+/- 2 SE bar error is shown; letters above bars indicate statistical significance in the REGWF test ($P < 0.05$).

4.1.2 Chardonnay

Figure 4 shows the average results obtained for for Chardonnay-FD during the 3-year period. Examining the results obtained for TSS, ND1L showed the lowest value compared to ND, TD1L and TD. In relation to TA and MA, ND1L had the highest value, although the TA results were only significant when compared to the TD-FD treatment. The pH showed similar values for TD1L-FD and ND1L-FD, while a difference was recorded between this treatment and TD1L-FD, TD-FD and ND-FD. The results obtained for Chardonnay-FA are shown in Figure 5. ND1L-FA maintained a higher level of TA and FA, although this treatment was the last to be harvested in all the three years studied (Table 5). TD2L-FA did not preserve the acidic levels observed for the -FD harvest; this is reflected by higher pH values compared to TD-FA, TD1L-FA and ND-FA.

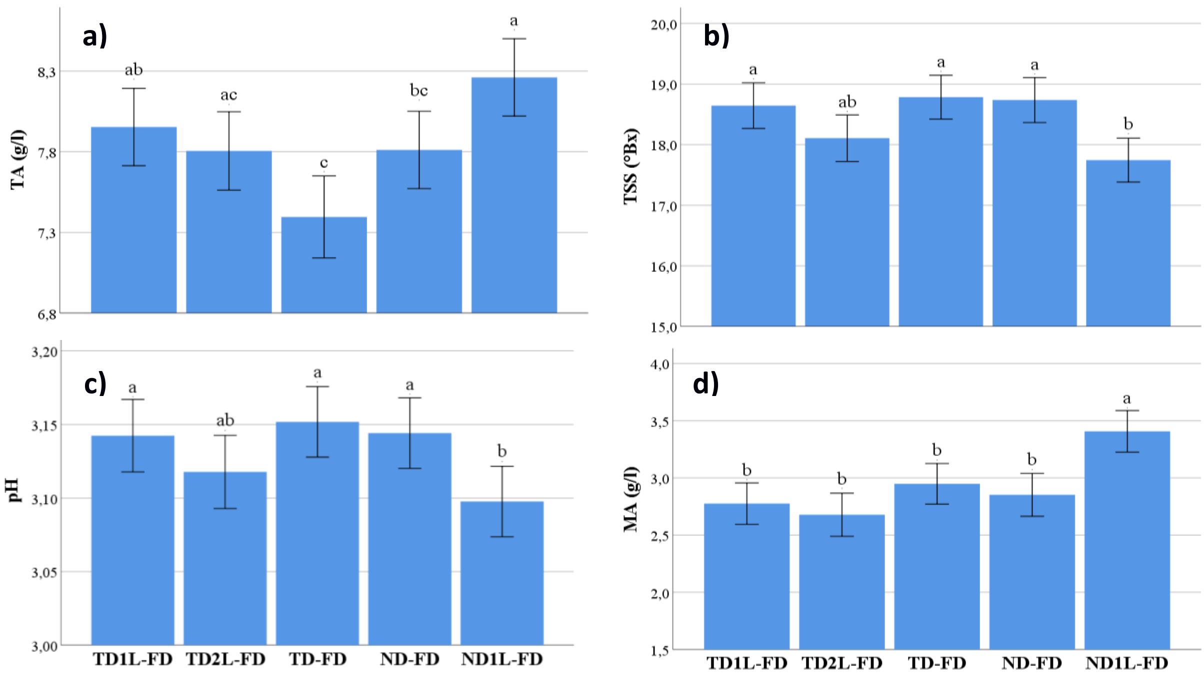

Figure 4. Average titratable acidity (TA) (a), total soluble solids (TSS) (b), pH (c) and malic acid content (MA) (d) for the 3-year period for Chardonnay-FD.
+/- 2 SE bar error is shown; letters above bars indicate statistical significance in the REGWF test ($P < 0.05$).

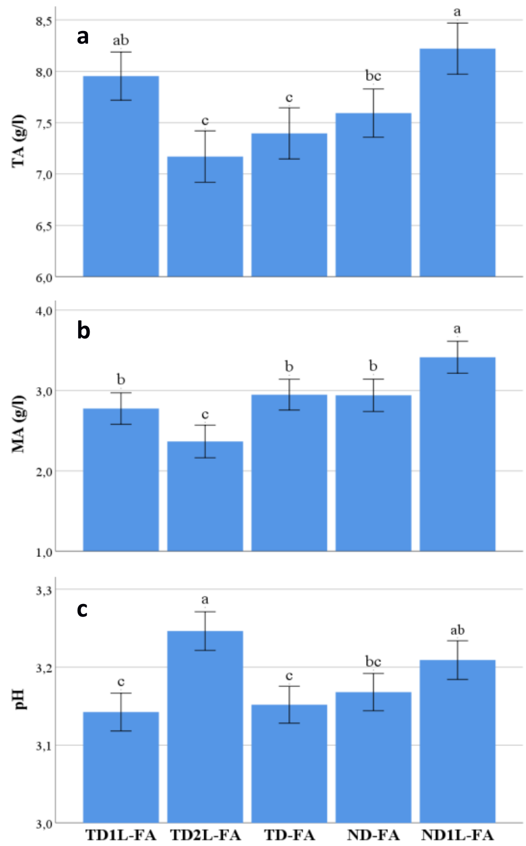

Figure 5. Average titratable acidity (TA) (a), malic acid content (MA) (b) and pH (c) for the 3-year period for Chardonnay-FA.
+/- 2 SE bar error is shown; letters above bars indicate statistical significance in the REGWF test ($P < 0.05$).

4.2 Phenolic and thiol content in grapes

The average results obtained from the 3-year analysis of phenolics and thiols are shown in Table 4. The main results are described below.

4.2.1 Pinot noir

TD showed a higher content of total cinnamic acid and total flavonols compared to other treatments. Total defoliation led to an increase in Caftaric, Fertaric, Gallic and *Trans*-Coutaric acids, Quercetin, Quercetin 3-glucoside, Kaempferol-3-glucuronide, Isorhamnetin-3-glu, Kaempferol-3-glucoside, Syringetin-3-glucoside and Myricetin. Other treatments showed similar results in terms of the total concentration of cinnamic acids and flavonols, while showing differences in the concentration of some specific phenolic compounds. Flavanols did not show significant differences between treatments, while ND had higher values for total stilbenes compared to TD and ND1L. Total anthocyanins did not show any differences between the treatments, but some differences were observed for the pattern: peonidin-3-glucoside (PN-3–Glu) seemed to be enhanced by leaf presence (the highest values were recorded for ND and ND1L), while Delphinidin-3-glucoside (Dp-3-glu) increased with total defoliation. High levels of shading (ND1L) led to an increase in S-glutathionylated precursor of 3-Sulfanylhexan-1-ol (GSH-3MH). This is reflected in the results obtained for total thiols.

4.2.2 Chardonnay

No significant differences were identified for harvesting carried out on "fixed dates" (-FD). Significant results were only shown for Quercetin-3-glucoside, Kaempferol-3-glucuronide, Isorhamnetin-3-glucoside and Kampferolo-3-Glucoside, for which the highest values were recorded for the TD-FD treatment compared to ND1L-FD. The Chardonnay-FA harvest showed significant differences between treatments for total cinnamic and total flavonol concentration. Shaded treatments (TD1L, TD2L and ND1L) led to the highest values in terms of cinnamic acid concentration, while ND1L led to a lower level of flavonols. No significant differences were observed for stilbenes, while a higher level of total thiols was shown for the TD2L treatment compared to ND and TD1L. This was determined by differences identified for S-glutathionylated precursor of 3-Sulfanylhexan-1-ol (GSH-3MH). It is important to underline that, as shown in Table 1, both TD and TD1L were harvested on the same date, reaching the established value of 10.5 % potential alcohol at the same time corresponding to -FD harvest. For this reason, the phenolic and thiol results are the same as for -FD and -FA for these two treatments.

Table 8 . Average phenolic and thiol results obtained for the 3-year period.

Phenolics (mg/kg) and thiols (µg/ kg)	**Chardonnay-FA**					**Chardonnay-FD**					**Pinot noir-FA**				
	TD	**ND**	**TD1L**	**TD2L**	**ND1L**	**TD**	**ND**	**TD1L**	**TD2L**	**ND1L**	**TD**	**ND**	**TD1L**	**TD2L**	**ND1L**
Caftaric acid	96^{b}	102^{b}	119^{a}	124^{a}	118^{a}	96	99	119	114	110	158^{a}	134^{b}	124^{b}	125^{b}	126^{b}
Fertaric acid	1,60^{b}	1,57^{b}	1,70^{b}	2,06^{a}	1,74^{ab}	1,6	1,6	1,7	1,8	1,7	4,6^{a}	3,4^{b}	3,1^{bc}	3,7^{b}	2,6^{c}
Gallic acid	5,9b^{c}	3,2^{c}	3,5^{c}	10,3^{ab}	11,9^{a}	5,9	3,1	3,5	3,0	2,9	10,2^{a}	3,8^{c}	4,5^{bc}	6,2^{b}	3,7^{c}
t- Coutaric acid	16,9^{c}	20,8^{bc}	24,7^{b}	34,2^{a}	31,2^{a}	16,9	21,3	24,7	23,3	23,4	41,0^{a}	25,9^{b}	24,4^{b}	23,4^{b}	25,3^{b}
Ellagic acid	1,01	0,76	0,94	1,03	0,62	1,01	0,86	0,94	1,09	1,09	1,81	1,29	1,49	0,81	1,18
Total Cinnamic acids	**121^{b}**	**128^{b}**	**150^{a}**	**172^{a}**	**164^{a}**	**121**	**126**	**150**	**143**	**139**	**216^{a}**	**169^{b}**	**158^{b}**	**160^{b}**	**159^{b}**
Quercetin-3-glucoronide	10,2	10,9	11,2	13,8	9,1	10,2	10,5	11,2	12,1	11,8	16,9	12,0	15,2	12,6	12,3
dihydrokaempferol	0,47	0,40	0,32	0,43	0,36	0,47	0,40	0,32	0,35	0,19	0,49	0,34	0,38	0,48	0,44
Quercetin	0,035	0,035	0,030	0,042	0,030	0,035	0,035	0,030	0,031	0,026	0,086^{a}	0,051^{ab}	0,034^{b}	0,038^{b}	0,029^{b}
Quercetin-3-glu	20,7^{a}	11,8^{bc}	15,6^{ab}	17,8^{ab}	5,9^{c}	20,7^{a}	11,2^{bc}	15,6^{ab}	14,8^{ac}	7,5^{c}	19,9^{a}	7,2^{c}	11,7^{b}	7,7^{bc}	5,3^{c}
Kaempferol-3-glucuronide	0,41^{a}	0,24^{ab}	0,27^{ab}	0,29^{ab}	0,11^{b}	0,41^{a}	0,21^{ab}	0,27^{ab}	0,27^{ab}	0,16^{b}	0,42^{a}	0,13^{bc}	0,25^{b}	0,15^{bc}	0,11^{c}
Isorhamnetin-3-glu	0,54^{a}	0,32^{ab}	0,35^{ab}	0,37^{a}	0,14^{b}	0,54^{a}	0,33^{ab}	0,35^{ab}	0,50^{a}	0,14^{b}	3,9^{a}	1,8^{c}	2,9^{b}	2,0^{c}	1,4^{c}
Rutin	0,47	0,64	0,67	0,90	0,56	0,47	0,62	0,67	0,82	0,83	1,65	1,48	1,73	1,42	1,52
Kaempferol-3-glucoside	1,6	0,9	0,8	1,1	0,3	1,6^{a}	0,7^{ab}	0,8^{ab}	0,8^{ab}	0,4^{b}	0,5^{a}	0,2^{b}	0,3^{b}	0,1^{b}	0,1^{b}
Syringetin-3-glu	/	/	/	/	/	/	/	/	/	/	0,33^{a}	0,18^{b}	0,26^{ab}	0,18^{b}	0,21^{ab}
Myricetin	/	/	/	/	/	/	/	/	/	/	0,09^{a}	0,02^{b}	0,04^{b}	0,04^{b}	0,03^{b}

Total Flavonols	**34,4^{a}**	**25,3^{ab}**	**29,3^{a}**	**34,8^{a}**	**16,6^{b}**	**34,4**	**24,0**	**29,3**	**29,7**	**21,1**	**44,3^{a}**	**23,3^{b}**	**32,7^{b}**	**24,7^{b}**	**21,5^{b}**
Catechin	46,1	58,5	54,3	58,9	64,7	46,1	60,9	54,3	94,1	47,7	282	259	329	261	320
Epicatechin	54,8	40,9	60,6	39,2	66,5	54,8	50,4	60,6	47,5	42,9	105	127	149	149	167
Gallocatechin	1,9	1,0	1,3	2,1	2,3	1,9	2,2	1,3	2,3	1,9	2,6	2,7	2,0	1,4	4,0
B1	18,2^{b}	14,5^{b}	20,8^{ab}	41,3^{a}	31,5^{ab}	18,2	30,4	20,8	38,0	33,4	40,2	52,4	43,9	34,9	55,2
B2	25,3	19,7	32,0	26,0	33,3	25,3	24,5	32,0	28,6	23,9	99	76	73	93	104
Total Flavanols	146,0	134,6	169,1	167,6	198,4	146,0	168,3	169,1	210,5	149,6	**530**	**518**	**597**	**539**	**650**
trans- Piceide	0,31	0,53	0,42	0,54	0,43	0,31	0,54	0,42	0,52	0,47	1,54^{ab}	1,86^{ab}	1,33^{b}	2,03^{a}	1,51^{b}
cis- Piceide	0,44	0,51	0,49	0,48	0,41	0,44	0,52	0,49	0,76	0,44	2,6^{b}	4,4^{a}	3,3^{ab}	4,0^{ab}	3,0^{b}
Total Stilbenes	**0,75**	**1,05**	**0,91**	**1,02**	**0,83**	**0,75**	**1,06**	**0,91**	**1,29**	**0,91**	**4,09^{b}**	**6,23^{a}**	**4,67^{ab}**	**6,05^{ab}**	**4,55^{b}**
MV-3-glu	/	/	/	/	/	/	/	/	/	/	379	333	339	333	342
CN-3-glu	/	/	/	/	/	/	/	/	/	/	10,2	9,2	7,7	7,9	9,1
PN-3-glu	/	/	/	/	/	/	/	/	/	/	167^{c}	204^{a}	168^{bc}	200^{ab}	224^{a}
DP-3-glu	/	/	/	/	/	/	/	/	/	/	122^{a}	81^{b}	88^{b}	67^{b}	81^{b}
PT-3-glu	/	/	/	/	/	/	/	/	/	/	55,7	41,7	45,1	42,2	45,2
Total Anthocyanins	/	/	/	/	/	/	/	/	/	/	**734**	**670**	**648**	**650**	**701**
CYS-3MH	7,2	4,1	7,8	11,9	12,5	7,2	10,2	7,8	12,1	6,3	27,6	28,2	12,7	25,0	21,5
GSH-3MH	81^{ab}	66^{b}	53^{b}	115^{a}	80^{ab}	81	83	53	66	59	266^{ab}	190^{b}	234^{ab}	216^{b}	304^{a}
Total Thiols	**88^{ab}**	**70^{b}**	**61^{b}**	**126^{a}**	**92^{ab}**	**88**	**93**	**61**	**78**	**65**	**294^{ab}**	**219^{b}**	**247^{ab}**	**241^{b}**	**326^{a}**

Different letters indicate significant differences in the REGWF test ($P < 0.05$); where no letters are present no significant differences were found.

4.3 Relationship between microclimatic data and productivity and quality variables

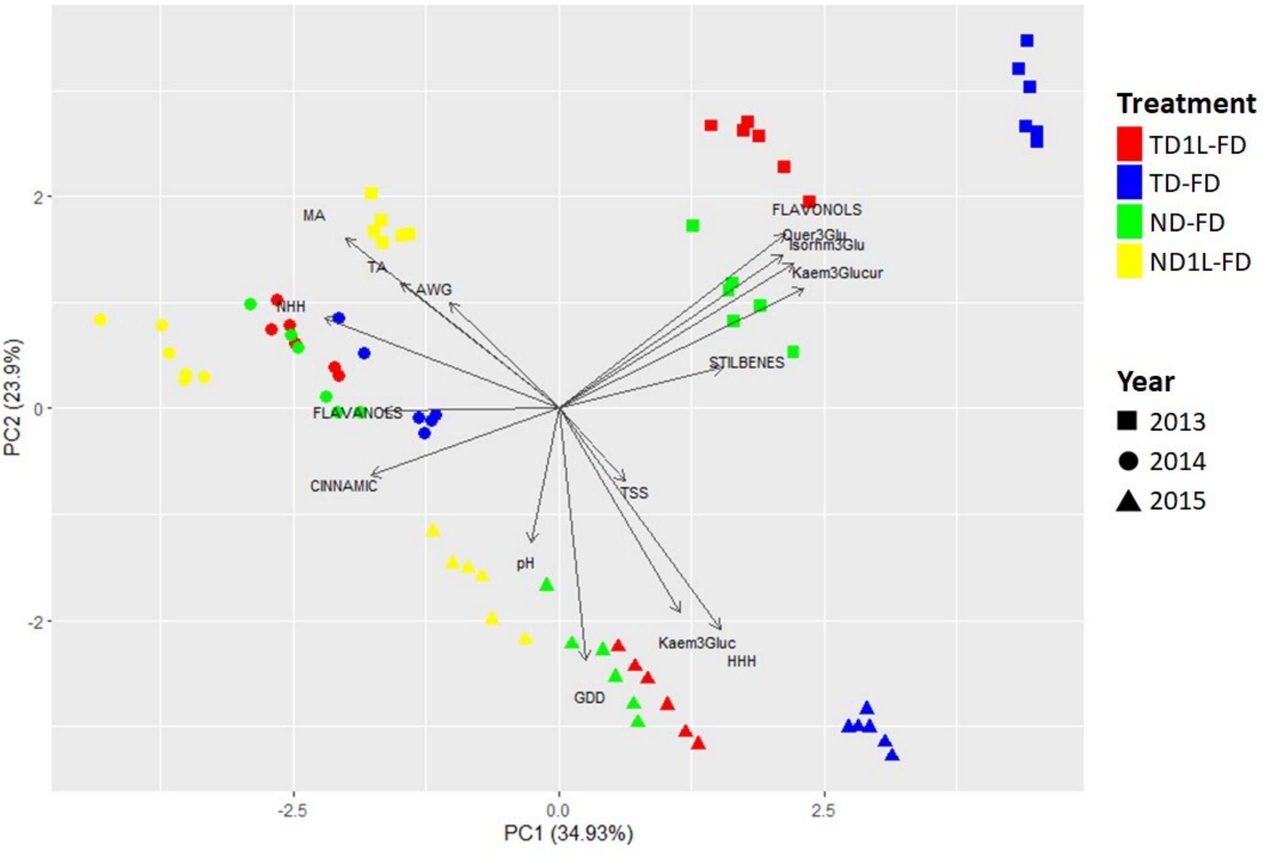

Figure 6. Biplot of principal component analysis of treatment parameters and microclimatic variables for Chardonnay-FD data.
Observations are plotted in a new 2-dimensional space, which is defined by the x-axis that represents the first principal component (PC1) explaining 34.9 % of overall data variance, and the y-axis that is the second principal component (PC2) explaining 24.9 % of overall data variance. The points are the observations, coloured according to the treatment and differentiated by shape according to the year of observation. Arrows represent the direction of the variables, as projected onto the 2-d plane of the biplot.

PCA makes it possible to summarise multidimensional information defined by productivity and quality variables and microclimatic variables (GDD, NHH and HHH) in a lower-dimensional space. We selected 13 productivity and quality variables as quantitative parameters, (AWG, TSS, TA, MA, pH, total cinnamic acids, Quercetin-3-glucoside, Kaempferol-3-glucuronide, Isorhamnetin-3-glucoside, Kaempferol-3-glucoside, total flavonols, total flavanols and total stilbenes) on the basis of the results obtained from ANOVA, calculated for Chardonnay-FD. Considering the eigenvalues (i.e., the percentage of overall variance explained by the principal components - PCSs), we decided to carry out the analysis only with the first two PCs, preserving 68.8 % of overall dataset variability.

The biplot (Figure 6) shows that the score of observations on PC1 are positive in relation to total flavonols (specifically Kaempferol-3-glucuronide, Isorhmnetin-3-glucoside and Quercetin-3-glucoside), total stilbenes and HHH, and negative in relation to TA, total flavanols, cinnamic acids, MA, and NHH. Data scores on PC2 were positively correlated with total flavonols, MA, and Quercetin-3-glucoside and negatively correlated with Kaempferol-3-glucoside, HHH, GDD. In the two-dimensional space, the data are grouped into three sets, which represent the three survey years. Within the same year, the 4 treatments were well discriminated, always maintaining the same order according to the values on the y axis. Namely, within the same year, the observations referred to as treatment TD-FD always had PC1 scores higher than those of treatment TD1L-FD, which in turn had higher values than treatment ND-FD. Finally, the observations of treatment ND1L-FD always had PC1 scores lower than the other treatments. This makes it possible to visually discriminate different treatment behaviour and differences between years.

Table 5 shows the correlation matrix of microclimatic data and productivity and quality variables selected for PCA. GDD and HHH was positively correlated ($\rho = 0.673$), while there was a high negative correlation between NHH and HHH ($\rho = -0.859$. Kaempferol-3-glucoside was positively correlated with GDD ($\rho = 0.680$) and HHH ($\rho = 0.707$). NHH was positively correlated with MA ($\rho = 0.804$) and cinnamic acids ($\rho = 0.684$). MA was highly negatively correlated with HHH ($\rho = -844$.

Table 9. Correlation matrix of microclimatic data and productivity and quality variables selected for PCA (n=72).

	AWG	TSS	pH	TA	MA	CINNAMIC	Quer3Glu	Kaem3Glucur	Isorhm3Glu	Kaem3Gluc	FLAVONOLS	FLAVANOLS	STILBENES	GDD	HHH	NHH
AWG	1.000															
TSS	-0.107	1.000														
pH	-0.120	0.288	1.000													
TA	0.274	-0.201	-0.212	1.000												
MA	0.437	-0.417	-0.058	0.631	1.000											
CINNAMIC	0.354	0.027	0.298	0.233	0.418	1.000										
Quer3Glu	-0.055	0.047	0.073	-0.181	-0.232	-0.309	1.000									
Kaem3Glucor	-0.161	0.031	0.069	-0.367	-0.368	-0.360	0.957	1.000								
Isorhm3Glu	-0.115	0.053	0.057	-0.248	-0.300	-0.375	0.981	0.937	1.000							
Kaem3Gluc	-0.325	0.183	0.213	-0.472	-0.626	0.053	0.078	0.294	0.051	1.000						
FLAVONOLS	-0.029	0.023	-0.069	-0.179	-0.214	-0.357	0.965	0.939	0.952	0.059	1.000					
FLAVANOLS	0.215	-0.278	0.192	0.161	0.494	0.238	-0.374	-0.349	-0.468	-0.155	-0.475	1.000				
STILBENES	-0.326	0.048	-0.032	-0.276	-0.399	-0.655	0.349	0.466	0.371	0.250	0.450	-0.270	1.000			
GDD	-0.315	0.291	0.476	-0.249	-0.497	0.166	-0.159	-0.063	-0.185	0.680	-0.326	0.092	-0.185	1.000		
HHH	-0.456	0.239	0.057	-0.467	-0.844	-0.422	0.025	0.169	0.075	0.707	-0.007	-0.370	0.330	0.673	1.000	
NHH	0.407	-0.148	0.202	0.474	0.804	0.684	-0.292	-0.416	-0.366	-0.516	-0.354	0.596	-0.594	-0.223	-0.859	1.000

Coloured boxes indicate correlation higher than 0.6 (green) and lower than -0.6 (red).

Discussion

The effects of defoliation and shading on grape quality have been demonstrated by different authors, but the relationships between berry temperature and metabolism have not yet been fully understood. The positive effect of shading on acidity preservation is particularly interesting for sparkling wine production, because acidity is one of the most important sensory characteristics (Ribéreau-Gayon *et al.*, 2000). Grape shading can also mitigate the current problem of ripening anticipation caused by increasing temperature related to global warming (Schultz, 2000; Jones *et al.*, 2005). This paper presents results regarding the effects of different levels of defoliation and shading. Micrometeorological characterisation was carried out by monitoring berry temperature variability in different exposure conditions. Differences among years emerged, with 2014 identified as the most balanced season, with high NHH values and low HHH values. The high thermal levels in 2015 (highest GDD values) were translated into high HHH levels and lower NHH levels. 2013 was in an intermediate position. PCA effectively discriminated the differences among years, while the correlation matrix revealed a positive relationship between GDD and HHH, and a negative relationship between NHH and HHH. This is in agreement with the definition of GDD, HHH and NHH (Amerine and Winkler, 1944; Cola *et al.*, 2020), which associates increases in temperatures with an HHH increase at the expense of NHH. The good correlation showed by NHH and HHH regarding the ripening process suggests that the adopted response curve can provide a synthetic representation of the process. Total defoliation (TD) was associated with a higher level of GDD and HHH and a lower level of NHH, while a higher level of shading (ND1L) gave the opposite result. This caused a delay in ripening observed in the ND1L treatment for both analysed cultivars and was further confirmed by the analitycal results obtained from Chardonnay-FD (ND1L low TSS and pH values and high level of TA and MA). The positive effect of shading on delaying ripening is in agreement with conclusions drawn by other authors (Rojas-Lara and Morrison, 1989; Percival *et al*., 1994; Filippetti *et al*., 2014; Martin *et al*., 2016), and is reflected in must and grape quality. In the correlation analysis, NHH was shown to be positively related to malic acid concentration (MA), while this variable was negatively associated with HHH. This is confirmed by the results obtained from treatment comparisons, where MA was higher for the ND1L treatment, both for Pinot noir and Chardonnay. These positive effects of shading on malic acid preservation have already been described in previous studies (Lakso and Kliewer, 1978; Dokoozlian and Kliewer, 1996; Conde *et al*., 2007; Martin *et al*., 2016; de Oliveira *et al*., 2019). Titratable acidity (TA) followed the same behaviour as MA, showing a higher concentration in the ND1L treatment compared to the TD treatment. The better conservation of the acidic component observed for high level of shading is further confirmed by the fact that this treatment was the last to be harvested in all three years of the study and can be supported by further studies (Reynolds *et al*., 1986; Smart *et al*., 2017). The results obtained for pH showed that TD1L-FA had the lowest value both for Chardonnay and Pinot noir, while other treatments showed differing behaviour between cultivars, in agreement with other studies that have reported that shading does not significantly affect this parameter (Filippetti *et al*., 2014). Total yield and bunch weight showed opposite behaviour between Chardonnay-FA and Pinot Noir-FA when comparing TD and ND, which is in agreement with literature that reported contrasting results with regards to the effects of leaf removal on berry size (Lemut *et al*., 2011). In particular, the average bunch weight of Pinot noir was more affected by defoliation without shading, while in the case of Chardonnay, bunch weight seems to have been affected by the timing of the harvest: ND1L recorded the highest value for harvest at a fixed date (-FD), but this value decreased at harvesting at fixed alcohol -FA. This can probably be related to the delay in ripening associated with ND1L, which showed a delay in -FA harvest compare to -FD harvest in all the three years observed. This delay lead to a decreased in bunch weight; this reduction does not seem to indicate cause the phenomena of acid concentration the phenomena of acid concentration, as the average levels of malic acid and titratable acidity remained constant between -FD and -FA harvest for this treatment, and an increase in average pH level was recorded.

The positive effect of temperature increase on flavonol accumulation (Downey *et al*., 2004; Spayd *et al*., 2002) can be determined from both the correlation and PCA, and from the differences recorded in treatments, in which higher flavonol values were obtained in the TD-FA treatment both for Pinot noir and Chardonnay. These results confirm the findings of other previous studies on the relationship between flavonol concentration and sunlight exposure (Spayd *et al*., 2002; Cortell and Kennedy 2006; Reshef *et al*., 2017). The intensity of light may thus have influenced the development of Quercetin-3-glucoside in particular, as demonstrated by another study by Price *et al*. (1995). Cinnamic acids were shown to be positively related to NHH and had the opposite behaviour in Pinot noir (positive effect of defoliation on the concentration of cinnamic acids) compared to Chardonnay (positive effect of shading in cinnamic acid concentration). Chardonnay displayed the opposite effect of irradiation to that observed for flavonols, as already observed by Rehsef *et al.* (2017).

The effects of shading and, consequently, the delaying of harvest time observed for the shaded treatments did not have a major impact on grape stilbene concentration.

The behaviour of thiols was not clearly defined, as it differed between cultivars. ND was the only treatment maintaining lower values both for Chardonnay and Pinot noir.

Conclusions

This study was carried out over the three-year period of 2013-2015. From the results obtained it was possible to underline the effect of shading on the composition of Pinot noir and Chardonnay grapes. Specifically, leaf shading combined with artificial shading (ND1L) had repercussions on ripening, slightly delaying maturation and maintaining a higher level of acidity. These characteristics in must are important in the context of sparkling wine production, because conservation of sparkling wine depends mostly on acidic composition. In terms of polyphenolic composition, total defoliation led to a higher concentration of flavonols and reduced the concentration of hydroxycinnamates in the berries, in accordance with other studies carried out on exposed grapes. ND1L also seemed to reduce flavonol content, confirming the effect of this type of shading on grape characteristics. These latter results suggest that the shading net afforded additional effective protection from irradiation, which was not entirely expected, considering that clusters are protected by several layers of leaves in the canopy, each one capable of absorbing 60–70 % of visible wavelengths (Schultz,1996). These results also seem to be related to berry temperature, which was highest for the TD treatment and lowest for ND1L, both in terms of GDD and HHH. The knowledge gained from this study could be useful for the wine production sector in terms of adapting defoliation and shading interventions to the meteorological conditions of a specific season. In particular, the effects of shading - delaying ripening, preserving acid concentration and reducing flavonol content - is relevant in relation to Franciacorta-specific oenological issues: indeed, sparkling wines need to be made with highly acidic grapes (in terms of high titratable acidity and malic acid content, and low pH), such grapes being particularly affected by problems resulting from the climate change now underway.

Acknowledgements: We would like to thank Castello Bonomi Tenute in Franciacorta, which hosted the experimental tests and the 'Consorzio per la tutela del Franciacorta', which promoted and inspired collaboration between universities and wine sector.

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Supplementary materials are reported at https://oeno-one.eu/article/view/2556

CHAPTER 3 – Specific objective 5 - SA-5.2 - Improving air quality, and SA-5.4 Reducing nutrient leakage

3.1 Evaluation of nitrous oxide emissions from vineyard soil: Effect of organic fertilisation and tillage

Chapter 3.1 has been published in Journal of Cleaner Production 2022, 380, 134557 DOI: 10.1016/j.jclepro.2022.134557

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Abstract

It is well known that the largest source of N2O is the agricultural sector, where fertilization represents the main source of this GHG. Monitoring N2O 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 N2O 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 N2O emissions. Emission factors of uncorrected (EFt) and corrected for no-fertilizer induced emissions (EFf) were calculated.

Results showed a seasonal trend in N2O 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 N2O fluxes. Cumulated annual emissions varies between 0.54 and 1.38 kg N2O-N ha-1 y-1, depending on treatment and level of soil 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 EFf (referred only to direct fertilizer application) were in range of 0.02 – 0.4% of N input. We found EFf of fertilized not tilled treatments from 17 to 79% lower than fertilized tilled, with different magnitude of reduction depending on soil TOC. Our EFt

were in line with disaggregated EFs from IPCC 2019 but EFf were 43% lower on average, confirming the IPCC reference value to be a good estimator of the overall N2O 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.

Keywords: Nitrous Oxide, Agriculture, Vineyard, Organic fertilizer, Emission factor

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 (N2O), that represent the three major Green House Gases (GHGs). AFOLU are responsible for 23% (12.0 ± 2.9 GtCO2eq yr-1) of the global human-generated emissions of these GHGs, while about 50% of this value (6.2 ± 1.4 GtCO2eq yr-1) is agriculture related (IPPC, 2019).
Agriculture, and especially intensive agriculture, is one of the largest contributors to non-CO2 GHG anthropogenic emissions (IPCC, 2014). Livestock production systems are one of the main producer of CH4 emissions (Heiling, 1994), while agricultural soils are the largest source of human related N2O emissions (IPCC, 2019). In viticulture CH4 emissions are essentially insignificant (Carlisle et al., 2010), and the main non-CO2 GHG produced is N2O (Nistor et al., 2018). Beyond being the dominant ozone-depleting substance emitted in the 21st century (Ravishankara et al., 2009), the critical issue regarding N2O lies in its high global warming potential (GWP), that is about 300 times more effective than CO2 in trapping heat in Earth's atmosphere (Solomon et al., 2008).N2O emissions from soils are directly related to practice of fertilization and tillage. N inputs applied in the field as fertilizers is released through nitrification and denitrification processes, or immediate volatilization (Linton et al., 2020), while impact related to soil tillage in terms of N2O 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) trough 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 represent 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 N2O in agriculture from 1990, with the number of papers steadily increasing per year since 2005. Going more specifically on organic fertilizers or 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-CO2 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 N2O emission are commonly measured trough 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 chambers are variable, generally 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 N2O fluxes present high temporal variability at different scale (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 done to identify the best time to do the single daily measurement, Francis Clar and Anex (2019) affirm that N2O 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 N2O emissions (Longbottom and Petrie, 2015). Annual estimation of cumulative N2O emissions based only on growing-season measurements, could also presents important bias (Gregorich et al., 2005).

On the other hand, the quantification of N2O 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 to 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 N2O 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 N2O analyzer directly in the field, with shorter measurement times (3- 10 minutes), better performance and less environment disturbance, rarely have been used for soil N2O fluxes monitoring (Cowan et al., 2014; Volpi et al., 2018). This because the technology associated with this system is currently very expensive and energy demanding, limiting the possibility to gain detailed information about N2O fluxes directly and continuously in the field. This makes it hard to define the amount of annual N2O emissions from specific fertilizers, soils, and crop management type.

For nitrogen footprint (NF) scope, annual N2O emissions are usually estimated using an average emission factor (EF) derived from literature. In case of N from fertilizers, default EF to estimate direct N2O emissions from managed soils has been quantified in an average value of 0.01 kg N2O–N (kg N)-1 (ranging from 0.001 to 0.018 kg N2O–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 N2O–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 decomposition. This default value is further differentiated between dry and wet climates with 0.006 and 0.005 kg N2O–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 take into account differences in soil properties and agronomic management between different cases studies (Garland et al., 2014; Gu et al., 2019). The effect on N2O 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 the literature with respect to measurements technique and type of crops, soil management and climate. For example, in their review Gu et al. (2019) highlight 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 N2O 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 to 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 N2O fluxes from soil with different management, in a temperate vineyard (North-eastern Italy), with the intent to acquire new information regarding the relationship between N2O emissions, organic fertilization and soil tillage.

Very few high time resolution monitoring have been done on N2O 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 measurements in tree crops and, especially, in temperate climate. The aim of the present work is to i) to increase the knowledge about dynamic of N2O 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 N2O emissions 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-CO2 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 its 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 (Fig. 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.

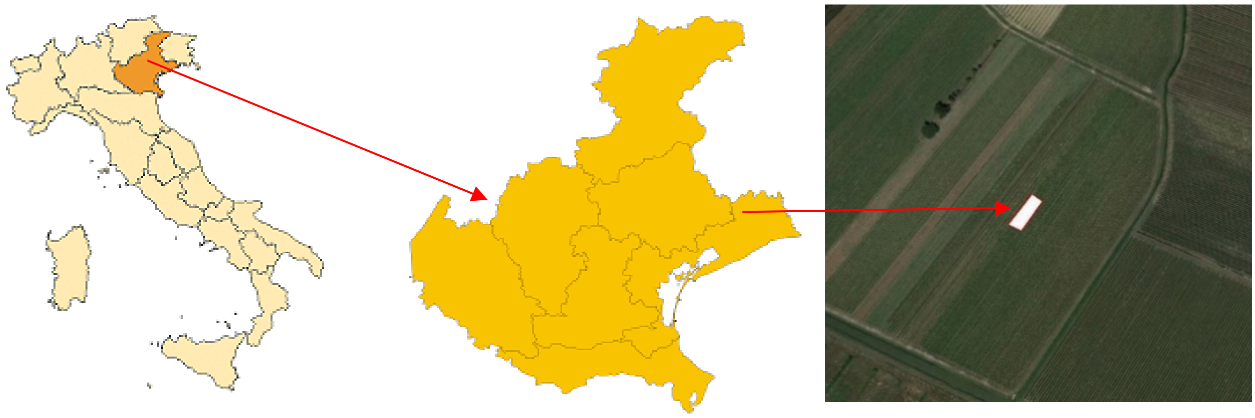

Figure 1 Location of the wine-growing area included in the study

A more detailed description of pedological and climatic characterization of vineyard is reported by Tezza et al. (2019).

2.2 Meteorological characterization

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 N2O 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 were 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 (Fig. 2).

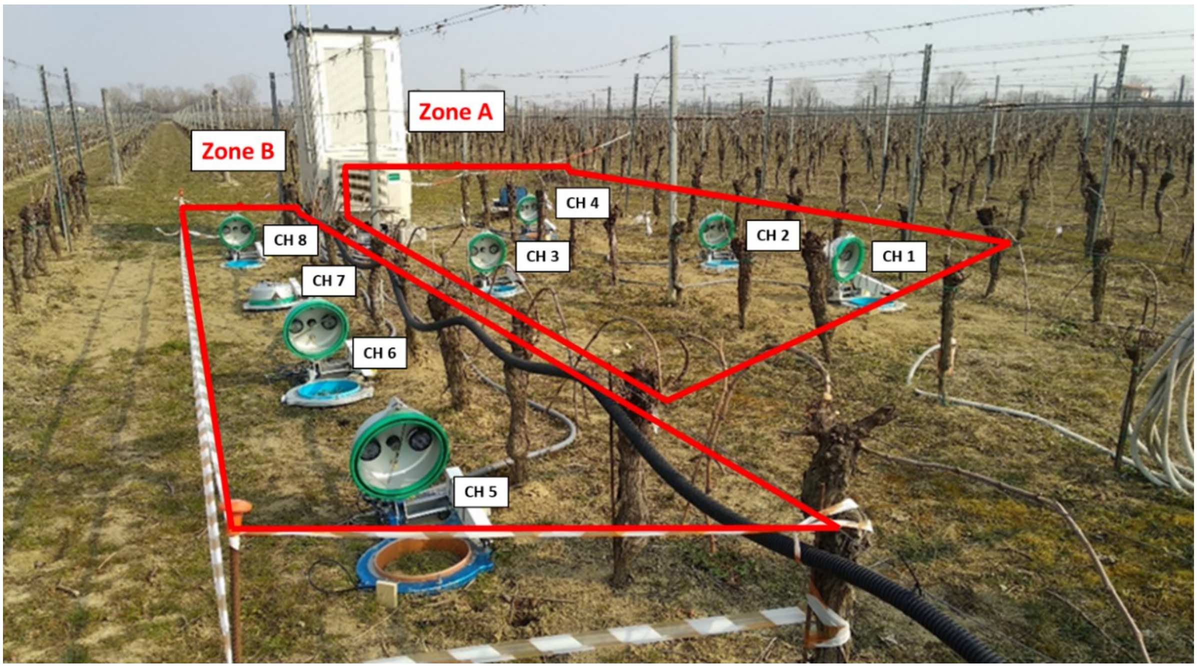

Figure 2 Experimental area where Continuous Monitoring Station of N2O emissions were installed

One accumulation chamber was installed in the central part of each plot for a total of 8 chamber installed. Each chamber was numbered and associated with the specific treatment applied on the plot monitored as reported in Table 1.

Table 1 Experimental plan: treatments associated to 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

2.3.1 Physical and Chemical soil characterization

A soil sample was collected at each plot before the treatment application, to perform a physical and chemical characterization of the soil. 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). Results are reported in Table 2.

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**	**Average values**	**CH5_UNT**	**CH6_TNT**	**CH7_UT**	**CH8_TT**	**Average values**
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
N tot 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

2.3.2 N2O Data collection

The continuous monitoring of soil N2O fluxes was carried out from October 19th2017 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 N2O 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 N2O analyser, 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 µmol 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 N2O 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.

2.3.3 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 N2O 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):

$$Flux = \frac{dC}{dt} \, .ACk$$

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

$$ACk = \frac{P \cdot V}{R \cdot T \cdot A}$$

The calculated N2O 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 main statistical parameters have been calculated (Rstudio software Version 1.4.1103). The confidence interval of N2O 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 N2O emissions from treated and untreated chambers ($P < 0.05$). Total N2O emissions calculated from each plot, expressed in gN2O m-2 d1, were converted in N2O –N, using equation:

$$N_2O - N = N_2O * \frac{28}{44}$$

The obtained data were used to estimate two N2O specific emission factor, one related to the overall emission of N2O (background + fertilization, EFt) and one for fertilizer induced direct N2O emissions (EFf), 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 the literature, which do not always present the results related to emissions from control plots (IPCC, 2006).
The N2O-N total annual emission were divided by the total nitrogen distributed as fertilizer in each plot to obtain EFt, while EFf where calculated subtracting emissions from untreated plots as control plots according to the equation:

$$EF_f = \frac{(Et - Eu)}{N}$$

Where Et is the N2O emission from treated (fertilized) plot, Eu is the N2O 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 N2O time series

The descriptive statistics for N2O daily fluxes are reported in Table 3. Values ranges 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 with value obtained by Lazcano et al. (2022) that reported, in a Mediterranean vineyard, a maximum value of 104 g N2O-N ha^{-1} d^{-1}, equal to 16.343 mg N2O m^{-2} d^{-1}.

Table 3 Minimum, maximum, median and mean value of N2O fluxes from each treatment chamber. 95 Percent confidence interval is defined with t-test (p-value >0.05). Data collected from 1st may 2018 to 30th April 2019

Zone	Plot	Min. N_2O fluxes in mg m^{-2} d^{-1}	Max. N_2O fluxes in mg m^{-2} d^{-1}	Median N_2O fluxes in mg m^{-2} d^{-1}	Mean N_2O fluxes in mg m^{-2} d^{-1} and 95 Percent confidence interval*
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 N2O fluxes measured for each chamber, average air temperature and cumulated precipitation are shown in Figs. 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. Total annual precipitation is not very different from historical mean (-9%) but, as described, 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.

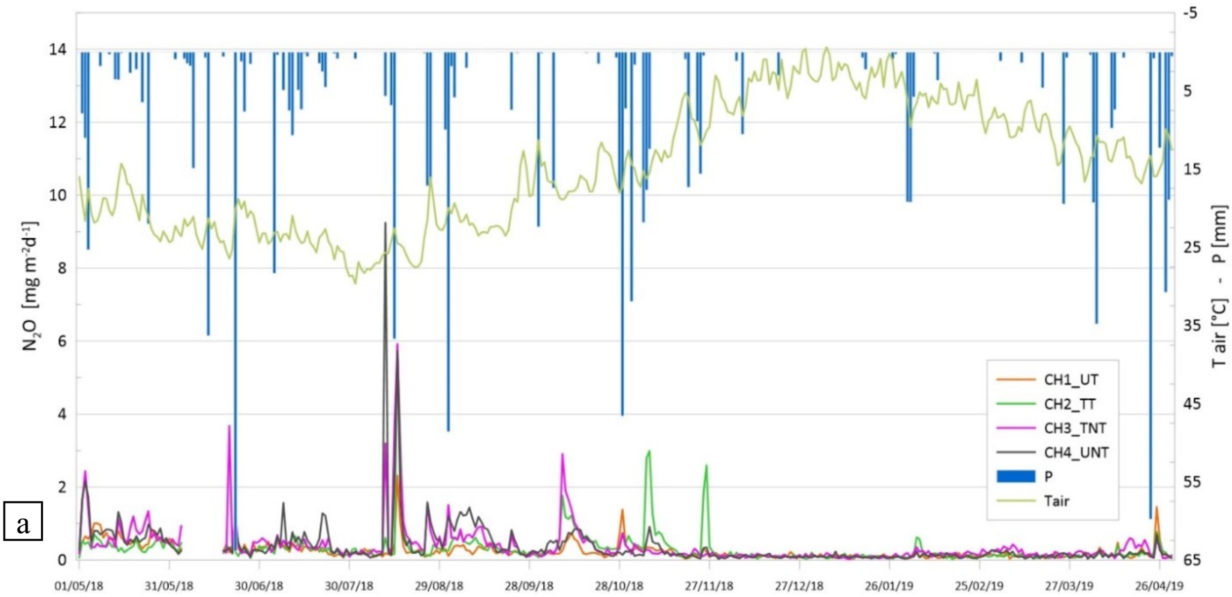

Figure 3 Time series of N2O emissions in mg m-2d-1 measured in each plot of a) zone A and b) of zone B. Detailed chambers fluxes are represented with different colors on left y-axes. Cumulative daily rain and average air temperature data are plotted on the right reverse y-axes.

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 N2O emission, not related with fertilization or rain events, was 0.23±0.10 mg m^{-2} d^{-1} (1.48±0.62 g N2O-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 N2O-N ha^{-1}d^{-1}). These values are higher than reported from other authors for Mediterranean vineyards with averages of about 0.5 g N2O-N ha^{-1}d^{-1} (Garland et al., 2014). From Fig. 3 is visible the lower background fluxes from late November and spring, in particular when air and soil temperature are below 10 °C. The seasonal effect of temperature on the trend of N2O fluxes is highlighted by Table 4 where for each chamber the median value of N2O daily fluxes has been calculated in the warm period (from 1thMay to 8thOctober 2018) and in a colder period (from 1thNovember 2018 to 30thApril 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 O2 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 N2O emissions to climate type (i.e. intertwining of temperature and rainfall).

Table 4 Median value of N2O fluxes expressed in mg m^{-2} d^{-1} for two different period: from 1thmay to 8thOctober 2018 and from 1thNovember 2018 to 30thApril 2019. Period from 8 to 30 October has been exclude because related with the treatment period.

N_2O fluxes [mg $m^{-2}d^{-1}$]	CH1_UT	CH2_TT	CH3_TNT	CH4_UNT	CH5_UNT	CH6_TNT	CH7_ UT	CH8_TT
Median 1/05/2018 to 8/10/2018	0.264	0.266	0.380	0.429	0.275	0.426	0.252	0.473
Median 1/11/2018 to 30/04/2019	0.061	0.087	0.110	0.058	0.120	0.284	0.136	0.206

Apart from this seasonal response, N2O fluxes follow a meteorological pattern, and a strong link appears between N2O emissions peaks and rains. As visible in Fig. 3, in general, there are peaks of N2O emission whenever there was a precipitation. The effect of rains on N2O emissions has been emphasized by other authors, that found an increase of soil N2O 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 N2O fluxes can be related to the effect determined by soil pore space saturation that induces a slower supply of O2 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 N2O 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 (Fig. 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 N2O fluxes can be identified (Fig. 4). 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 (Fig. 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 (Fig. 5). After fertilization, treated plots tend to have higher peaks related to rains than untreated for the rest of the period (Fig. 3 and Table 4).

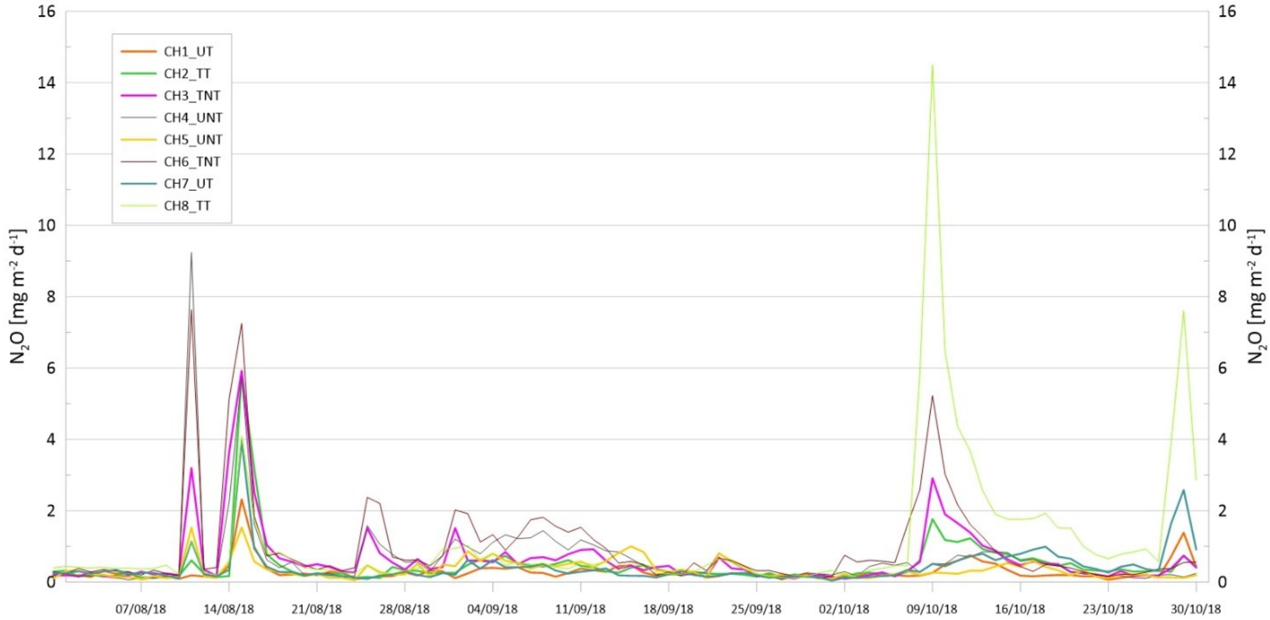

Figure 4 Focus on the three principal peak (August – October 2018) of N2O emissions (mg m^{-2} d^{-1}) detected during the study.

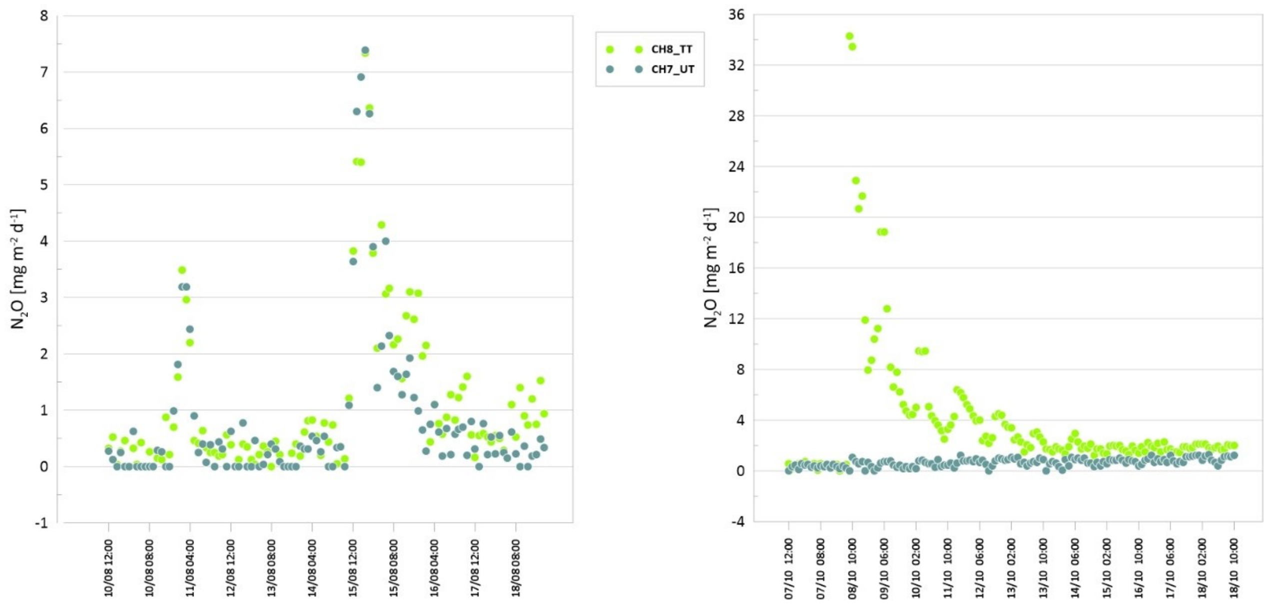

Figure 5 Focus on N2O emissions (data collected every 2 hours) in Chamber 7 (Untreated Tilled) and Chamber 8 (Treated Tilled): from 10 to 18 August the peak after rain (on the left) and from 7 to 18 October the peak after fertilizer application (on the right).

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 N2O – 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 N2O–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 N2O – 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 N2O–N ha^{-1}. Similarly, (Garland et al., 2014) report a maximum peak after fertigation (5 kg N ha^{-1}) of 23 g N2O-N ha^{-1} d^{-1}, in dry climates, but with a maximum peak of 360 g N2O–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 N2O-N ha^{-1} d^{-1} after fall fertilization event, comparable to the major peak of our study. At 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 N2O–N ha^{-1}.

Most of the emissions in fertilized plots occurred during the first 6-7 days after treatment followed by a decrease in N2O 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 N2O 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 N2O 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 peak 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 N2O–N ha^{-1}, similarly to Verhoeven and Six (2014) where total emissions per fertigation event ranging from 54 to 244 g N2O–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 Fig. 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 N2O-N ha^{-1} y^{-1} (Table 5).

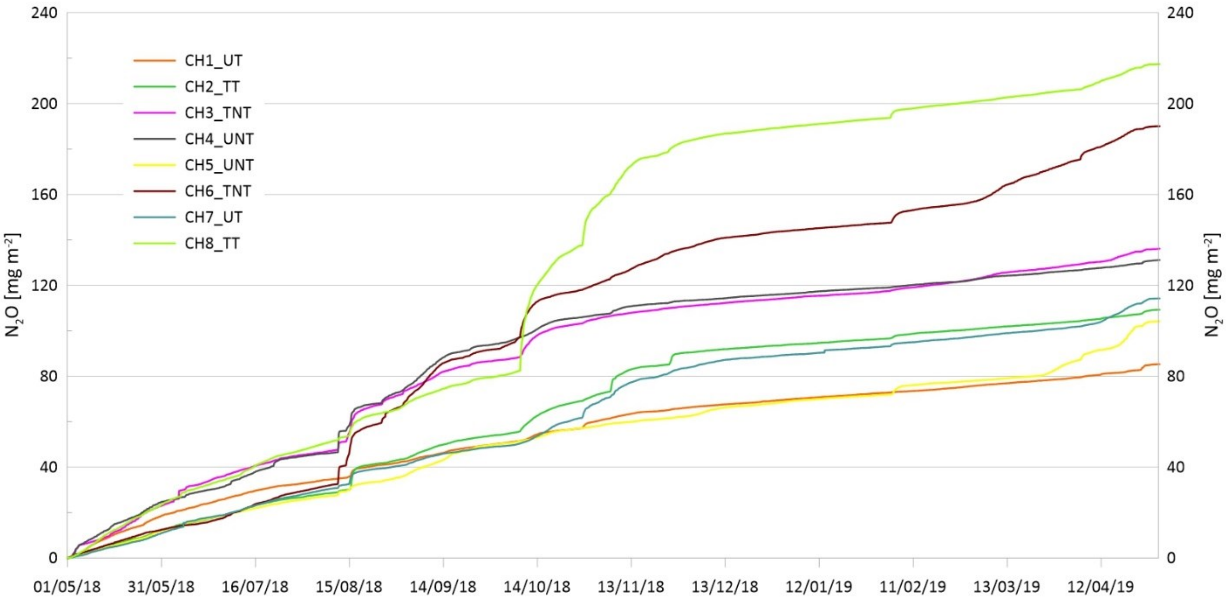

Figure 6 N2O cumulative annual emission expressed in mg m-2calculated for each chamber

Table 5 Total 2 N-N2O emissions for each experimental plot

	ZONE A					ZONE B		
Site	**CH1_UT**	**CH2_TT**	**CH3_TNT**	**CH4_ UNT**	**CH5_ UNT**	**CH6_TNT**	**CH7_UT**	**CH8_TT**
Total N_2O [mg 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 [kg N_2O-N ha^{-1}]	0.54	0.70	0.87	0.83	0.66	1.21	0.73	1.38

*Different lowercase letters indicate significant difference ($P < 0.05$) between untreated and treated treatment chambers (ANOVA analysis).

As described in relation to peaks, a consistent difference in N2O emissions from Zona A and Zone B can be observed for cumulated emissions. This is confirmed by ANOVA analysis that shows significative difference in N2O emissions from Zone A and Zone B for both treatment and untreated chamber, while in Zone B the N2O fluxes in untreated tilled and not tilled chamber are comparable. In Zone B the effect of fertilization and fertilizer incorporation is evident. An average increase of 87% of N2O 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 N2O-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 N2O-N ha^{-1} y^{-1} for perennial crops in Mediterranean climate. Other cumulated emissions from vineyard presented in literature where higher and ranged from 1.6 to 3.92 kg N2O-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 N2O-N ha^{-1} The effect of fertilization is less evident in Zone A, where no relevant differences can be observed between fertilized and not 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 enterprise 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 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 N2O 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 (R2 0.86), where higher N2O fluxes were registered in presence of higher level of organic carbon available for microbial activities. No relationships where 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 EFt and EFf 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 EFt is between 0.004 and 0.008 g N2O-N g N^{-1}, while compost not tilled varied from 0.005 to 0.007 g N2O-N g N^{-1}.

Table 6 Emission Factor for N2O emissions in vineyard: related to overall soil N2O emissions (EFt) and direct related only to fertilizer application (EFf)

	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

Average EFt value from all chambers results 0.006±0.002 kg N2O-N kg N^{-1} , lower than IPCC EF1 and in line with values reported by IPCC (2019) for N additions in wet climates, quantified in 0.006 kg N2O-N kg N^{-1} with an uncertainty range of 0.001 – 0.011 kg N2O-N kg N^{-1}. This value of IPPC refers to an average value obtained from many studies (IPCC, 2006) that, on the basis of the specific experimental plan, can include or not N mineralized from mineral soil as a result of loss of soil carbon (i.e., N2O 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 factor without background emission (i.e from a no nitrogen 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, EFf, ranged between 0.0002 and 0.0037 g N2O–N g N^{-1}, with a mean value of 0.0019±0.0015 kg N2O–N kg N^{-1}. These results were from 97% to 38% lower than IPCC EF, respectively for zone A and B. Zone B EFf were comparable to other presented in literature. In their study, Marques et al. (2018), obtained an EFf 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 of 0.54%. In a review of emissions factors from organic amendments additions, Charles et al. (2017) calculates a global mean EFf for organic fertilizers of 0.82 and describes compost as low risk amendments with a mean EFf or 0.27, similarly to our results. On the contrary Gu et al (2019), in a review of N2O emissions from orchards, found an average emission factor uncorrected for control plots (EFt) of 1.76% of the applied fertilizer N, more than double of EFt 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 before the experiment, the emissions related only to fertilizer application is about 45% of the total emissions.

EFt for tillage treatment reacts differently: in Zone A the EFt decreases with incorporation while in Zone B EFt 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 N2O emissions after tillage were reported (Drewer et al., 2017) while, in other context, no relevant effect of tillage on N2O emissions (Bosco et al., 2015) were underlined. Garland et al. (2011) reported no significant differences in N2O 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 N2O 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).

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, EFf 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 a low soil organic carbon content, could lead to a lower loss of N due to N2O release to atmosphere.

4 Conclusions

Results obtained in the present study represent the first high temporal resolution monitoring of N2O 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 N2O measurements) which allowed the calculation of site-specific emission factors on the basis of a robust time series.

A seasonal related trend was visible for both peak and background N2O fluxes, with majority of emissions during growing season until early autumn. A strong relationship between meteorology and N2O 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 N2O fluxes were measured in fertilizer plots, where high emissions occurred during the first 6-7 days after treatment, followed by a decrease in N2O fluxes.

Annual cumulated N2O emission ranged between 0.54 and 0.73 kg N2O-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 N2O 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 EFt 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 EFf, 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 N2O fluxes, and to better interpret soil management effects. The calculation of the EFf, corrected with the control plots, allowed an interpretation of the effect of tillage and fertilizer incorporation, finding EFf of plot where fertilizer was not incorporated on average 48±31% lower 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 N2O emissions estimation. IPCC reference value was confirmed to be a good estimator of the overall N2O 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.

Funding

This work was supported by the European Union Project LIFE VITISOM (LIFE15 ENV/IT/000392).

Acknowledgment

The authors thank all Project partners and in particular the group of research of Prof. Fabrizio Adani who carried out chemical soil analysis.

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CHAPTER 4 – Specific objective 6 - Contribution to the protection of biodiversity, enhance ecosystem services and preserve habitats and landscapes

4.1 Impact of Environmental Conditions and Management on Soil Arthropod Communities in Vineyard Ecosystems

Chapter 3.1 has been published in Sustainability 2021, 13, 11999. DOI: 10.3390/su132111999

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Abstract

The importance of soil biodiversity and soil-based ecosystem services in the context of viticulture has recently been emphasized. Over 85% of soil fauna species richness is represented by edaphic arthropod communities. Edaphic arthropod responses to soil characteristics and management practices can be considered as good bioindicators of soil quality. Here, 168 soil samples that were collected from 2014 to 2019 in several vineyards of different Italian wine-growing areas were analyzed to explore how arthropod communities respond to several factors that are characteristic of vineyard ecosystems. The analysis of the combined effects of the primary abiotic variables (the chemical and physical characteristics of soil) and management practices (organic vs. conventional, soil inter-row management) on soil biological quality (assessed by QBS-ar index) identified soil temperature and soil texture as the abiotic factors exerting the most significant effect on the QBS-ar values. Organic vineyards exhibited higher QBS-ar values compared to those of conventionally managed vineyards, and subsoiling negatively influenced the soil biological quality.

Keywords: soil biological quality; vineyard; organic management; soil temperature; soil texture; subsoiling

1. Introduction

Soil biota is the primary actor in soil ecological processes and plays a pivotal role in the provisioning of soil-based ecosystem services [1,2]. Soil contains a vastly diverse range of organisms, which include microorganisms, small and large invertebrates, and small mammals [3]; however, over 85%

of the species richness of the soil fauna is represented by edaphic arthropods [4]. Therefore, arthropod community diversity can be considered as a good bioindicator of soil quality [5,6]. Numerous studies have investigated the relationships among arthropod diversity and abundance and environmental factors (e.g., soil abiotic variables and meteorological factors) and agronomic practices (e.g., canopy and soil management) [7–16]. The importance assigned to soil arthropods requires the identification of ready-to-use tools for assessing arthropod biodiversity [17]. The QBS-ar index is an acronym of soil biological quality-arthropods (in Italian "Qualità Biologica del Suolo") and is one of the most frequently applied indexes for the evaluation of edaphic arthropod communities in the agricultural sector. This index was proposed by Parisi [18], and its application proved to be useful in discriminating different disturbance levels related to different land use [19,20] or management systems [21,22]. QBS-ar applications are quite easy, as high taxonomic skills are not required. This index focuses on the identification of biological forms that are based on specific functional traits (e.g., pigmentation level, body dimensions) that are linked to different adaptation levels to the soil environment. The index is based on the principle that the greater the sensitivity of a soil arthropod taxon to variability and perturbation of soil conditions, the greater the importance of that taxon as an indicator of soil biological quality. The QBS-ar has already been applied in research examining forests [23,24] and several agricultural agroecosystems [10,25–27]. Current research shows a growing interest in investigating soil biological quality in vineyards. The wine-growing sector represents one important agricultural compartment, and it covers approximately 7.3 million hectares worldwide, with approximately 3.3 million located in Europe [28]. Sustainability within the wine-growing sector is becoming a major issue. In particular, available knowledge demonstrates that chemical and physical characteristics, soil environmental conditions, and management affect soil vineyard biodiversity; the relationship between vineyard management and soil arthropods represents a key element in promoting the transition to an ecologically and economically sustainable viticulture [29]. Ghiglieno et al. [30] explored the effect of abiotic variables, such as meteorological conditions and the chemical–physical composition of vineyard soils on the QBS-ar index. Regarding vineyard management, some effects of different inter-row management techniques on edaphic arthropod communities have been previously characterized [31–35], while differences in soil biological quality between conventionally and organically managed vineyards remain largely unexplored [30,34,36]. Therefore, the current understanding of how arthropod communities respond to the complexity of interaction factors characterizing vineyard ecosystems is still far from being complete. This study aimed to explore the combined effects of the primary abiotic variables (soil chemical and physical characteristics) and management practices (organic vs. conventional, soil inter-row management) on the soil arthropod community (QBS-ar index). The analysis is based on data collected from several field studies conducted from 2014 to 2019 in different Italian viticultural areas. The knowledge acquired from the multifactorial analysis of the responses of edaphic fauna to several abiotic variables and agronomic practices is crucial for the definition of sustainable soil management practices and, thus, for a sustainable wine-growing system.

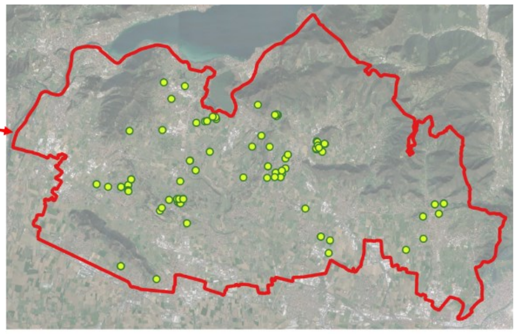
2. Materials and Methods

2.1. Study Sites Description

A total of 168 soil samples were collected from 2014 to 2019 in five different Italian wine-growing regions. Figure 1 presents the locations of the wine-growing areas and the number of samples collected in each area. Over 65% of the investigated vineyards were located in the Franciacorta DOCG area. Approximately 48% of samples were collected during spring, while 52% were collected during autumn.

2.2. Abiotic Variables

Soil environmental variables. Soil moisture and soil temperature data were gathered from the fifth generation of European ReAnalysis (hereinafter, ERA5-land) hourly database. The ERA5-land provides globally complete and consistent datasets at a high spatial (0.1° x 0.1°) and temporal (hourly) resolution [37] that are computed at different depth levels for soil-related variables. We extracted the data for the first two soil layers (average depths of 3.5 and 17.5 cm) and interpolated them linearly to obtain hourly soil moisture (M) and hourly soil temperature (T) data at a soil depth of 15 cm. Bilinear interpolation using climate data operator (CDO) commands [38] was performed.



Region	Viticultural area	Total samples
Lombardy	**a** - Franciacorta DOCG	115
	b - Oltrepò Pavese DOC/DOCG	47
Friuli Venezia Giulia	**c** - Friuli Grave DOC	1
Emilia Romagna	**d** - Colli Piacentini DOC	3
Sicily	**e** - Agrigento Province DOC	2

Figure 1. Location of the five wine-growing areas included in the study (left). In the highlighted area of Franciacorta DOCG (right), the sampled vineyards are indicated by yellow dots.

Based on M and T, we computed a set of soil environmental indicators. The average of the daily minimum, mean, and maximum soil temperature (T_min, T_med, and T_max, respectively) and the average soil moisture (M_med) were calculated for two time intervals that were included during the 7-day period prior to the sampling date (referred to as short-term period: indicator prefix 'short_') and the 30-day period prior to the sampling date (referred as medium-term period: indicator prefix 'medium_'). Moreover, cumulative degree day (DD) indicators were calculated during the medium-term period based on the following thermal thresholds: T ≥ 30 °C (DD_hot), T ≤ 10 °C (DD_cold); 18 °C ≤ T < 30 °C (DD_warm), 10 °C ≤ T < 20 °C (TL), and T ≥ 20 °C (TH). Furthermore, we included within the analysis the indicators associated with soil moisture, as suggested by Ghiglieno et al. [39] for our assessment during the medium-term period:

$$\mathrm{MD} = \frac{1}{24}\sum |\mathrm{M_i} - 0.35|, \qquad 0 \le \mathrm{M} \le 0.35 \qquad (1)$$

$$\mathrm{MH} = \frac{1}{24}\sum (\mathrm{M_i} - 0.35), \qquad \mathrm{M} > 0.35 \qquad (2)$$

MD is the sum of the daily absolute deviations of soil moisture from the threshold value, when M is lower than 0.35. MH is the cumulative daily soil moisture that exceeds 0.35. Chemical and physical characterization of soils. Soil samples were collected at a depth of approximately 0–20 cm; leaf litter layer was excluded. All samples were mixed homogeneously, air-dried, and passed through a 2 mm sieve for chemical analysis. Soil chemistry was characterized according to the Italian regulation (DM 13 September, 1999), including soil texture (sand, silt, clay g/kg of soils), pH, organic matter content (expressed in g kg−1 of soil) (SOM), available P expressed as P2O5 (mg kg−1 of soil), available K expressed as K2O (mg kg−1 of soil), and available Mg expressed as MgO (mg kg−1 of soil). These variables were categorized according to the following criteria: soil texture [40] (p. 125), pH [41] (p. 66), organic matter content [42] (p. 31), available P [43] (p. 4), available K (previously converted from K to K2O, conversion factor 1.2046) [42] (p. 45), and available Mg (converted from Mg to MgO, conversion factor 1.6579) [42] (p. 45).

2.3. Vineyard Age and Management Variables

Vineyard age. Vineyard age was categorized into four classes based on the number of years that the vines had been planted at the time of sampling, and these categories included vineyards up to 3 years old, vineyards of at least 4 and at most 10 years old, vineyards of at least 11 and at most 20 years old, and vineyards older than 20 years.

Vineyard management. Vineyard management was categorized into two main groups that included conventionally managed vineyards (hereinafter 'conventional') and organic managed vineyards in compliance with the European Regulation on organic farming (regulation (EC) no. 2018/848 and subsequent amendments and additions) (hereinafter 'organic'). Organic vineyards were further subdivided in three groups according to the time elapsed at the time of sampling since the start of the conversion period. The sub-groups included 'organic ≤ 3' vineyards that were within the 3-year conversion period provided by European regulations on organic farming, and '3 < organic ≤ 9' and 'organic > 9' groups that included certified organic vineyards for which between 4 and 9 years or greater than 9 years (respectively) have elapsed, including conversion period. Soil management. Three aspects of soil management were considered, including tillage (subsoiling), fertilization, and grass cover. Subsoiling referred to the presence or absence of deep tillage (approximately 30 cm) in the autumn preceding sampling. Fertilization indicated whether fertilization had been performed in the autumn prior to sampling. Grass cover was classified into six classes of the prevailing plant species that were identified in the vineyard grass community during the year of sampling: seeded mixture prevailing legumes (SML), seeded mixture prevailing species other than legumes and graminaceous (SMO), spontaneous grass cover prevailing legumes (SpL), spontaneous grass cover prevailing graminaceous (SpG), spontaneous grass cover prevailing species other than legumes and graminaceous (SpO), and grass cover absence, where continuous tillage was performed with the aim of eliminating spontaneous grass cover (Tillage).

2.4. Soil Biological Quality Evaluation (QBS-ar)

A cubic sample of soil (with a dimension of 10 x 10 x 10 cm) was collected in each vineyard at the same depth as that described for chemical and physical soil analysis. Arthropods were extracted by placing the soil sample in a Berlese–Tüllgren funnel under a 60 W incandescent bulb, and this caused soil arthropods to migrate toward the damp portion of the soil sample (away from the light) and to fall through the funnel cavity into a preserving solution (2/3 alcohol and 1/3 glycerol). The biological forms, taxonomic entities, and biological stages were determined according to the QBS-ar method [44].

2.5. Data Analysis

A multiple linear regression (MLR) model was applied with the aim of analyzing the linear relationships among the response variable (QBS-ar) and the explanatory variables; these variables include factors related to soil environmental indicators (see Table 1), soil chemical and physical categorical variables, and management variables (see Table 2). Considering the large set of potential predictors, a bidirectional stepwise selection [45] was applied to select the best subset of explanatory variables that could explain the variance of the response variable based on the minimization of the Akaike information criterion [46]. Statistical analysis was performed using R software (version 4.0.4), MASS package.

Table 1. Descriptive statistics for continuous factors related to vineyard age, soil chemical characteristics, soil environmental indicators, and QBS-ar in 168 soil samples collected from five different Italian wine-growing regions from 2014 to 2019.

Factors	Units	Mean±SD*	Range [minimum, maximum]
Vineyard age	years	13.30±6.70	1.00-41.00
MH	Pure number	0.55±0.47	0.00-2.34
MD	Pure number	1.50±1.32	0.02-4.80
TL	°C	110.99±40.31	53.42-183.47
TH	°C	38.82±31.52	0.53-99.81
medium_T_min	°C	17.44±3.57	10.51-24.08
medium_T_max	°C	22.13±3.81	14.98-29.22
medium_T_med	°C	19.31±3.53	12.87-26.53
medium_M_med	m^3/m^3	0.31±0.06	0.19-0.42
DD_hot	°DD	0.29±0.95	0.00-6.23
DD_cold	°DD	0.09±0.36	0.00-3.31
DD_warm	°DD	79.44±64.83	0.00-219.90
short_T_min	°C	16.90±3.08	10.64-23.74
short_T_max	°C	21.65±3.41	16.26-30.81
short_T_med	°C	19.32±3.02	13.73-27.08
short_M_med	m^3/m^3	0.32±0.05	0.16-0.41
QBS-ar		113.45±46.06	11.00-226.00

*SD: Standard deviation

Table 2. Frequency distribution of categorical factors related to vineyard age, soil chemical and physical variables, vineyard management, and soil management in 168 soil samples collected from five different Italian wine-growing regions from 2014 to 2019.

Factors	Units	Categories	Freq. dist.	Factors.	Units	Categories	Freq. distr.
Vineyard age (VA)	years	0 < VA ≤ 3	3.57%	Vineyard management		conventional	19.64%
		4 ≤ VA < 10	28.58%			organic ≤ 3	33.34%
		11 ≤ VA ≤ 20	55.95%			3 < organic ≤ 9	28.57%
		VA > 20	11.90%			organic < 9	18.45%
Soil texture	g/kg	clay/clay loam/silty clay	10.13%	P	mg P_2O_5/kg	very low<14	13.69%
		silty clay loam	20.83%			low 14÷28	29.76%
		loam	27.98%			medium 28÷45	16.67%
		silt loam	16.06%			high 45÷70	24.40%
		sandy loam	25.00%			very high>70	15.48%

Soil organic matter (SOM)	g/kg	Low 8÷12 Medium 12÷20 Good 20÷40	16.67% 23.21% 60.12%	pH		acid 5,5÷6,0 sub-acid 6,1÷6,7 sub-alkaline 7,3÷7,9 alkaline 8,0÷8,6	13.69% 20.83% 25.60% 39.88%
Mg	mg MgO/kg	very Low<83 low 83÷166 medium 167÷249 good 250÷332 rich 333÷414 very rich>414	4.17% 32.74% 22.62% 10.71% 5.95% 23.81%	K	mg K_2O/kg	low 48÷96 medium 97÷145 good 146÷217 rich 218÷289 very rich>289	22.62% 23.21% 32.74% 15.48% 5.95%
Subsoiling		yes no	38.69% 61.31%	Grass cover		SML SMO/SpL SpG SpO Tillage	17.26% 5.36% 46.43% 17.00% 1.78%
Fertilisation		yes no	36.31% 63.69%				

"*" P2O5 mg kg-1; "**" g kg-1; "***" MgO mg kg-1; "****" K2O mg kg-1.

3. Results

3.1. Descriptive Analysis

Descriptive statistics and frequency distributions. The descriptive statistics for the continuous factors related to vineyard age, soil environmental indicators, and QBS-ar included in the multiple linear regression model are reported in Table 1, and the frequency distributions of categorical factors are reported in Table 2. The QBS-ar index exhibits great variability and ranges from 11 to 226. Taxa identification and EMI attribution. Table 3 shows taxa identified in the analyzed samples. A total of 25 taxa were identified from the QBS-ar method application. The range of EMI scores associated to each taxon is also reported.

Table 3. Taxa identified in the analyzed samples and the associated range of EMI scores.

Factors	Coefficient estimates	Std. Error	p-value
Management: organic ≤ 3	28.793	11.136	0.011 *
Management: 3 < organic ≤ 9	23.060	9.361	0.015 *
Management: organic > 9	8.250	11.717	0.001 **
Subsoiling	-13.482	6.446	0.038 *
Soil texture: Loam	17.374	6.744	0.011 *
Soil texture: Clay Loam	41.305	9.836	< 0.001 ***
Soil texture: Silty clay loam	45.873	8.145	< 0.001 ***
TL	0.273	0.142	0.057
TH	0.992	0.231	< 0.001 ***
short_T_med	-9.470	1.659	< 0.001 ***
medium_DD_warm	0.352	0.084	< 0.001 ***

"***" $p<0.001$; "**" $p<0.01$; "*" $p<0.05$; "." $p<0.1$

3.2. Linear Regression Analysis

The stepwise multiple linear regression model exhibits a good fit to the data, where it explains half of the variability of the QBS-ar values (adjusted R-squared value = 0.477). The explanatory variables that were statistically significant were vineyard management, subsoiling, soil texture, TL, TH, Short_T_med, and DD_warm (Table 4), while TL (cumulative daily soil temperature degrees exceeding 10 °C, when T was between 10 °C and 20 °C) possessed a p-value that was slightly higher than 0.05. The effects of each factor should be interpreted based on the consideration that all the other variables are equal. The results are presented in the following sections.

Table 4. Table presenting factors that significantly influenced QBS-ar.

Factors	Coefficient estimates	Std. Error	p-value
Management: organic ≤ 3	28.793	11.136	0.011 *
Management: 3 < organic ≤ 9	23.060	9.361	0.015 *
Management: organic > 9	8.250	11.717	0.001 **
Subsoiling	-13.482	6.446	0.038 *
Soil texture: Loam	17.374	6.744	0.011 *
Soil texture: Clay Loam	41.305	9.836	< 0.001 ***
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TH	0.992	0.231	< 0.001 ***
short_T_med	-9.470	1.659	< 0.001 ***
medium_DD_warm	0.352	0.084	< 0.001 ***

"***" *p*<0.001; "**" *p*<0.01; "*" *p*<0.05; "." *p*<0.1

3.3. Effect of Abiotic Variables

Environmental soil indicators. All environmental indicators that significantly influenced QBS-ar were related to soil temperature (Table 3). QBS-ar values were positively influenced by TH ($p < 0.001$), medium_DD_warm ($p < 0.001$), and TL ($p = 0.057$), and they were negatively affected by short_T_med ($p < 0.001$). Chemical and physical soil variables. The linear regression model identified a significant relationship between soil texture and QBS-ar. In particular, the QBS-ar on average is higher in soils possessing loam, clay loam, and silty clay loam textures than in soils exhibiting the other texture categories (clay, silty clay, silt loam, sandy loam), and these data are described in Figure 2.

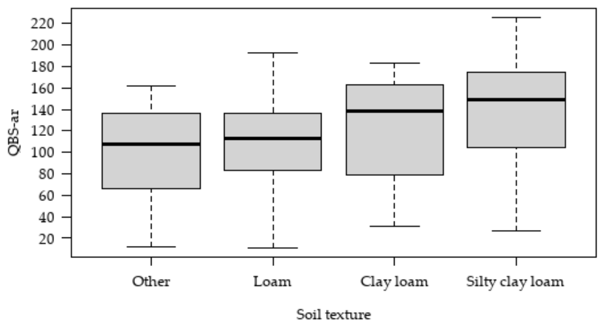

Figure 2. The boxplots provide the frequency distributions of QBS-ar values in the 168 soil samples divided according to soil texture categories. The category “Other” includes clay, silty clay, silt loam, and sandy loam soils.

3.4. Effect of Management Variables

Vineyard management. The model results revealed that the expected value of QBS-ar in soil sampled from organic-managed vineyards was higher than was that in soils sampled from conventionally managed vineyards. This positive effect is already statistically significant in the first 3 years of adoption of the organic management protocol ($p = 0.011$), that corresponds to the period of conversion from conventional to organic management. The positive influence of organic management was maintained during the periods encompassing 3–9 years after adoption ($p = 0.015$) and beyond 9 years from adoption ($p = 0.001$). Soil management. Subsoiling was the only soil management practice that significantly influenced QBS-ar ($p = 0.038$). Subsoiling reduced the value of QBS-ar with respect to soils where subsoiling has not been applied (Figure 3).

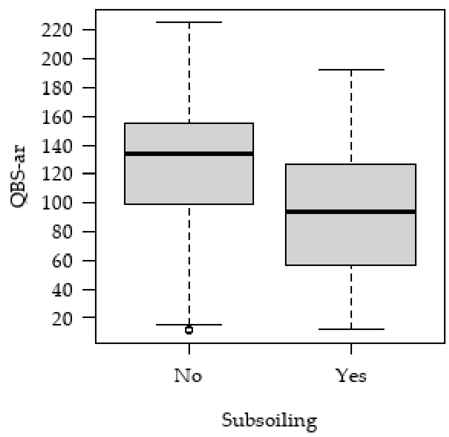

Figure 3. The boxplots provide the frequency distributions of QBS-ar values in the 65 soil samples where subsoiling was performed during autumn prior to sampling (right) and in the 103 soil samples where subsoiling was not performed (left).

4. Discussion and Conclusion

The QBS-ar index has been described by many authors as a useful indicator for discriminating among different soil disturbance levels and soil biological quality [19,22,44,47,48]. QBS-ar has been used in both semi-natural habitats and agroecosystems [10,21,23–27]. Previous studies performed in low disturbed environments, such as grasslands and woodlands, exhibited average QBS-ar values ranging between 140 and 173 [21]. Experiments examining vineyards revealed high variability among QBS-ar values that ranged from 98 to 203 depending on the farming systems [34]. In our study, the average value for the QBS-ar index is equal to 113.5 (±46.1), with a minimum value of 11 and maximum value of 226. These results are in agreement with those of Menta et al. [22] and suggest that vineyard ecosystems can potentially reach QBS-ar values similar to or even higher than environments with lower disturbance levels. Results obtained from our analysis revealed that the effects of environmental soil indicators on QBS-ar were predominantly associated with soil temperature. This is fully in agreement with scientific evidence emphasizing the important effects of soil temperature on edaphic arthropod survival, development, and reproduction [49,50]. In particular, soil temperature in the range of 10 °C–20 °C, as evaluated in the medium-term period, has positive effect on QBS-ar. This positive effect is of greater intensity if the soil temperature ranges from 18 °C to 30 °C. These findings are in agreement with those of previous studies that identified that the optimal temperature range for development and growth was between 20 °C and 30 °C [51,52]. Analysis of the effect of soil environmental conditions in the short-term period (7 days before sampling) revealed a negative relationship between average temperature (short_T_med) and QBS-ar. This result may be related to the ability of soil organisms during the short-term period to mitigate the effect of high temperature by migrating to deeper soil layers, where they are then not identified in the analyzed soil sample [53–55]. In our study, soil moisture that was assessed both in the medium and short periods did not exhibit a significant relationship with QBS-ar. The average values for soil moisture in our samples are 0.31 and 0.32 m3 m-3 in the short-term and medium-term periods, respectively. These values are very close to the threshold of 0.35 m3 m-3, which is considered as the optimal value for survival and reproduction for some edaphic species [56,57]. Therefore, in the sampling conditions of our study, soil moisture stress conditions that may have influenced QBS-ar likely did not occur. Soil texture was the only physical soil parameter that affected QBS-ar, where it caused an increase in this index in soils with loamy soil texture or with loam in association with fine soil fractions (clay loam and silty clay loam). The influence of soil texture on some specific taxa of soil arthropods has been demonstrated by other authors [58,59], although arthropod responses to soil texture variations are not unique. For example, Van Capelle et al. [60] observed that all Collembola life-forms (both atmobionts and euedaphic) were equally promoted in finer texture conditions and that loamy texture reduced the presence of these taxa. Results of experiments investigating the role of management in influencing edaphic arthropod community responses have been of particular importance for the definition of sustainable agronomic practices to preserve and/or increase soil biological quality. Arthropod communities are positively influenced by organic management with respect to conventional management. This is in agreement with previous studies performed in a vineyard environment [30,34,39]. Regarding the timing of the adoption of organic management, QBS-ar value is significantly improved during the first 3 years of adoption according to other experiences carried out in vineyard ecosystems [39]. Results obtained from soil management variable analysis highlighted the negative effect of subsoiling on the QBS-ar index. This soil management practice led to a decrease in QBS-ar values, thus supporting the scientific evidence that emphasizes the sensitivity of edaphic arthropods to soil tillage in the short term [21,32]. However, a more detailed investigation of the role of tillage requires consideration in regard to the long-term effects of agronomic practices on soil arthropod responses, as suggested previously [61,62]. This study, in relation to the large number of observations considered and the variability of the geographical context observed, provides relevant knowledge regarding the effects of soil abiotic conditions and management practices in the vineyard ecosystem on edaphic arthropods. The opportunity to consider different variables related to different dimensions, such as environmental and management, represents an element of innovation that supports the comprehension of how arthropod communities respond to the complexity of interaction factors characterizing vineyards.

Author Contributions: Conceptualization, I.G., P.D., M.T., L.V. and G.G.; data curation, I.G., A.S., M.T., M.V., G.S. and G.G.; formal analysis, A.S. and M.V.; investigation, I.G., P.D., M.T. and L.V.; methodology, I.G., A.S., P.D. and G.S.; supervision, G.G.; visualization, I.G., A.S. and F.G.; writing—original draft, I.G., A.S., F.G. and G.S.; writing—reviewing, and editing, G.G. All authors have read and agreed to the published version of the manuscript.

Funding: The results obtained during 2019 in the Franciacorta DOCG and Oltrepò Pavese DOC/DOCG wine-growing areas were funded by the Project F.A.Re.Su.Bio (European Agricultural Fund for Rural Development—PSR Lombardy Region—Measure 16.1.1). The other results received no external public funding.

Acknowledgments: This paper was awarded (ex-aequo) with the Best Paper Awards presented by a PhD student or a recently graduated PhD fellow, at the XIII Italian National Congress on Biodiversity “Biodiversità 2021”, 7–9 September 2021, Foggia (Italy). The authors would like to thank all of the wine companies that assisted with the data collection. We thank the ‘Consorzio per la tutela del Franciacorta’ for playing a key role in the development of new operational protocols for sustainable viticulture, by promoting and encouraging collaboration between universities and productive excellence. We thank the Chemical Laboratory and Organic Agriculture Unit of the Technology Transfer Center (CTT) of the Fondazione Edmund Mach (San Michele all’Adige, Trento, Italy) for performing chemical analyses of soil and for arthropod group identification.

Conflicts of Interest: The authors declare no conflict of interest.

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4.2 Characterisation of Franciacorta vineyard agroecosystem to support biodiversity and ecosystem services

Chapter 4.2 has been published in BIO Web of Conferences 2022, 44, 02003 DOI:10.1051/bioconf/20224402003

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Abstract

Vineyard is a multifunctional ecosystem associated to a multitude of environmental benefits and ecosystem services. Despite the increased research efforts on the analysis of biodiversity patterns and ecosystem services in vineyard, the lack of approaches to multifunctionality suggests to develop integrated approaches allowing to manage the complexity of vineyard landscape. The present study introduces an innovative methodology aimed at developing a unitary framework able to describe vineyard ecosystems biodiversity considering both local (morphological characteristics, internal ecological infrastructure, and management) and landscape (land-use) components. The case of Franciacorta wine-growing area is described considering a total of 112 ha located in three wine companies. Four informative layers related to different components of Franciacorta territory were defined: i) morphology; ii) internal ecological infrastructure; iii) landscape composition; iv) management. This study provides a useful instrument to increase knowledge about vineyard system biodiversity and to protect ecosystem services provision in Franciacorta territory. The methodology applied can easily be extended to the whole wine-growing compartment.

1 Introduction

Viticulture is among the oldest and most representative forms of agriculture, covering about 7.3 million hectares worldwide [1]. Vineyards strongly shape the appearance of landscapes in a territory [2, 3]. The vineyard is a multifunctional ecosystem [4] associated with a multitude of ecosystem services, performing important economic, cultural, and ecological roles. Winkler et al. [5] identified 27 ecosystem services classes associated with vineyards; among them the most investigated resulted: cultivated crops, sequestration, pest control, disease control, scientific, and heritage, cultural services. Biodiversity represents a key actor in the provisioning of these ecosystem services in the vineyard ecosystem, both at local (vineyard and the areas adjacent to vineyards) and, more extensively, at landscape level [6]. In this context, research efforts on the relationships between biodiversity patterns and ecosystem services in vineyards increased [5]. However, most of the studies addressed specific issues related to biodiversity while few studies adopted approaches to multifunctionality in vineyards [4]. The complexity of the vineyard landscape imposes to perform innovative integrated approaches allowing to define a systemic analysis of vineyards agroecosystems [7], including both local and landscape elements, evaluated at different temporal and spatial scales.

In the present study, an innovative methodology is proposed to develop a unitary framework able to describe vineyard agroecosystem's biodiversity considering all local (morphological characteristics, internal ecological infrastructure, and management) and landscape (land-use) components. The methodology is applied to the case study of the Franciacorta wine-growing area (Lombardy, Italy). Franciacorta is one of the most important Italian wine- growing regions for sparkling wine production. There, farmers are aware of issues related to biodiversity and eco-tourism represents an important resource. Results obtained from this preliminary study set the ground for specific monitoring activities addressing the main components of biodiversity, providing a useful instrument for wine growing companies and the whole Franciacorta territory to increase knowledge about vineyard agroecosystem and to protect ecosystem services provision. This case study represents a virtuous example for the wine-growing compartment promoting a multifunctional perspective of the vineyard ecosystem, supporting long-term sustainable use of natural resources.

2 Materials and methods

2.1. Study area

The research was promoted by the Consorzio per la tutela del Franciacorta. Franciacorta territory includes a total of 3299 ha of vineyards, 2902 of which are dedicated to Franciacorta DOCG sparkling wines production. To perform a preliminary characterisation of the landscape of Franciacorta wine territory, an area of 112 ha of vineyards was considered.

2.2 Environmental Units identification

The analyzed area was split into several units, hereinafter Environmental units (EUs), defined as a whole vineyard or portion of vineyard homogenous in terms of four agronomic characteristics: planting year, planting density, cultivar, and training system. EUs identification was performed using the Geographic Information System (GIS). In particular, the area of each EU was defined as a polygon based on the classification of the vineyards in the land-cover/land-use map of the Lombardy region

(DUSAF v6.0 2018, ‘Destinazione d’Uso dei Suoli Agricoli e Forestali’, freely available online from the geoportal of the Lombardy region ‘Geoportale della Lombardia’ – GOL [8]).

2.3 Environmental Units characterisation

EUs were characterized by local and landscape components organized in four different informative layers: i) morphology; ii) internal ecological infrastructure, iii) landscape composition; iv) management.

2.3.1 Morphological characterisation

In the morphological layer, altitude, slope, aspect, row orientation, and solar irradiance were used to characterize the EUs.
Altitude (meters above sea level) was calculated using the elevation raster, with pixel 5 m x 5 m, freely available online in GOL. Slope (expressed in degrees) is the mean angle of inclination to the horizontal of the EU. Aspect (expressed in degrees, from 0° to 360° starting from the North) is the compass direction that the EU slope faces. Slope and aspect values were computed using an open-source GIS software [9] based on the GOL elevation raster. Solar irradiance was calculated using the model r.sun (based on Krcho [10] later improved by Jenco [11]) that estimates the daily sum of solar irradiation [Wh*m- 2*day-1]. Model for solar irradiance was implemented in an open-source GIS software [12] using topographic factors obtained from the GOL elevation raster. We computed this model for the June 2020 solstice (171st day of the year). Values of altitude, slope, aspect, and solar irradiance were calculated as the average value of all raster pixels inside the polygon of the EU. Row orientation was obtained by digitilising one or many rows of vines in each EU. Cosine of the angle between azimuth and each row (expressed in degrees) were obtained in the GIS environment [9]. High values (near 1) indicate N-S orientation while low values (near 0) E-W orientation.

2.3.2 Internal ecological infrastructures

EUs internal ecological infrastructures were characterised based on photointerpretation from of high scale (1:2.000) regional orthophotos (GOL) and manual digitalisation of structural elements.
Two types of structural elements were considered: punctual (points) and areal elements (polygons) [6]. Punctual elements (e.g. isolated trees) were digitalised, counted, and reported as the number of elements per m2 of EU area. Areal elements (e.g. Grassland) were digitalised as polygons, computed through polygon area, and reported as m2 of areal elements per m2 of EU area. Each areal element was classified using EUNIS habitat type classification [13] as suggested by Kratschmer et al. [14].

The characterisation of internal ecological infrastructures was conducted in the EU area plus a small buffer zone with a radius of 5 meters around the EU.

2.3.3 Landscape composition

Landscape composition was characterised in a buffer area of a radius of 500 meters around the EU. The percentage of buffer area covered by each land-use category identified using DUSAF was calculated.

2.3.4 Management practices

Information about the management practices of each EU was collected through a survey carried out at each of the wine companies involved in the project. The survey was referred to the agricultural year 2019-2020 and included information on management regime, canopy management, and ground management. In the study, six variables related to inter-row ground management were considered. Sown cover crop, fertilisation, fertilisation with pellet fertiliser, fertilisation with organic fertiliser as compost or manure, mowing managed in alternated rows were represented by binary variables (0= no adoption of the practice, 1= adoption). Times of tillage was identified as an integer variable (form 0= no-tillage to 5= 5 times tillage).

2.4 Data analysis

To identify homogeneous EU units according to the characteristics defined for each information layer, a cluster analysis was performed. The adequate clustering model and the optimal number of clusters for each layer were selected evaluating the silhouette plot (function eclust, package factoextra [15] of the software R). The Euclidean distance and the Ward method were used for the analysis.

3 Results and discussion

3.1 Environmental Unit identification

In the 112 ha of vineyards, 108 EUs were identified. The average area of EUs resulted equal to 0.96±0.76 ha ranging from 0.09 ha to 4.96 ha.

3.2 Environmental Unit characterisation

3.2.1 Morphological characterisation

Franciacorta morphology is heterogeneous [16], as shown by the variability of all morphological characteristics in the EUs (Table 1).

Table 1. Descriptive statistics of morphological variables in the 108 EUs.

Variables	Units	Mean±SD*	Range [min, max]
Altitude	m a.s.l.	229.81±37.37	133.71-318.22
Aspect	°	181.32±64.93	44.64-311.22
Slope	°	6.75±5.80	0.51-31.06
Solar irradiance	$Wh*m^{-2}*day^{-1}$	8819.29±70.89	8554.91-8907.06
Row orientation	pure number	0.68±0.32	0.01-1.00

* SD: standard deviation

Clustering analysis identified five groups of EUs homogeneous by morphological characteristics (Fig. 1).

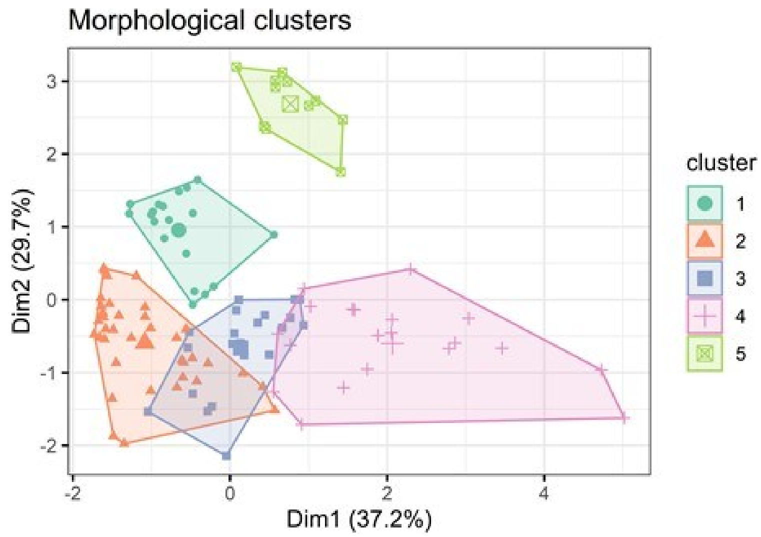

Fig. 1. Cluster plot related to morphological variables.

All morphological variables are well discriminated among clusters. For instance, the differences in slope and aspect values according to clusters are reported in Fig. 2.

We focused on slope and aspect due to their fundamental role in the determination of hydraulic condition [17] and evapotranspiration in vineyards [18].

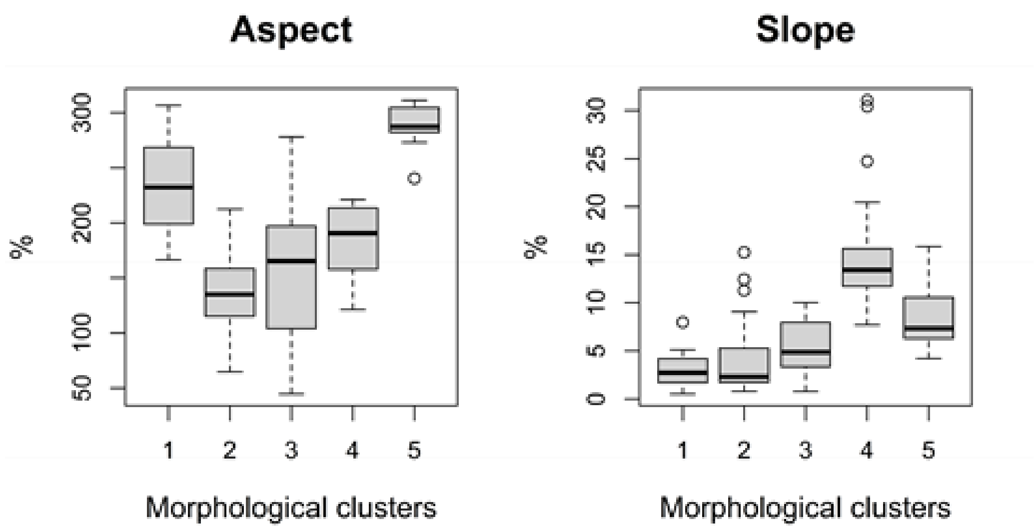

Fig. 2. Boxplots representing the distribution of values of Aspect (left) and Slope (right) in the 108 EUS according to the five clusters identified.

3.2.2 Internal ecological infrastructures

Ecological infrastructures play an important role in maintaining biodiversity in vineyard agroecosystems [19]. EUs characterisation allowed to identify eight internal ecological infrastructures (Table 2).

Table 2. Descriptive statistics of internal ecological infrastructure variables.

Punctual elements (number*m^{-2})			
Code	**Definition**	**Mean±SD***	**Range [min, max]**
Isolatedtrees	Number of isolatedtrees	0.000±0.000	0.000-0.003
Discontinuous row	Number of trees in adiscontinuous row	0.000±0.001	0.000-0.007
Areal elements (m^{2}*m^{-2})			
EUNIS Code	**Definition**	**Mean±SD***	**Range [min, max]**
E	Grasslands (excludingvineyard interrow vegetation)	0.091±0.160	0.000-1.033
G2.91	Olea europaea groves	0.031±0.110	0.000-0.736
G5	Lines of trees, smallanthropogenic woodlands	0.035±0.062	0.000-0.325
J2	Low density buildings	0.001±0.003	0.000-0.023
J4	Transport networks and other constructed hard- surfaced areas	0.016±0.062	0.000-0.581

J5	Highly artificial man- made waters and associated structures.	0.000±0.001	0.000-0.011

* SD: standard deviation

The 108 EUs were classified into two clusters according to internal ecological infrastructures (Fig. 3).

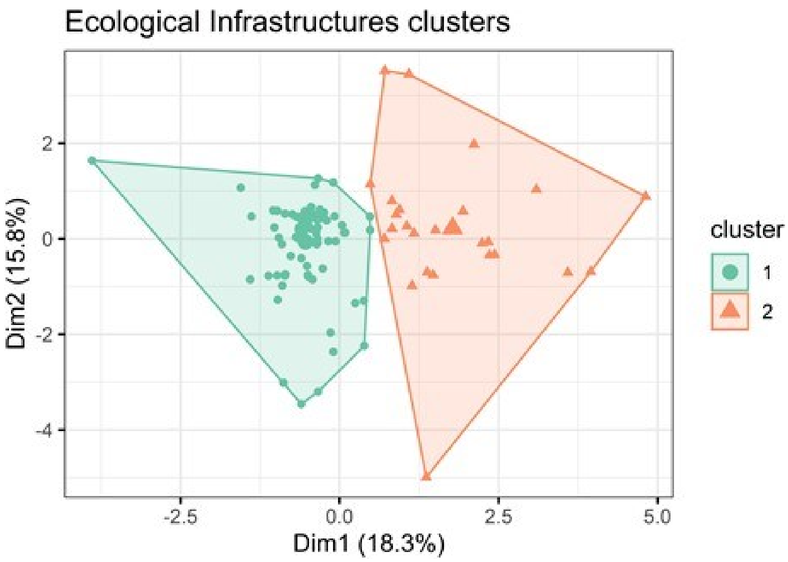

Fig. 3. Cluster plot related to internal ecological infrastructure variables.

Cluster 1 includes 83 EUs characterised by a higher abundance of internal ecological infrastructures compared to EUs included in Cluster 2. This is particularly evident considering the presence of Isolated trees and Grassland (Fig. 4). These ecological infrastructures are related to important ecosystem functions as pest control and pollination services. Assandri et al. [20] emphasised the role of isolated trees in providing nesting sites, while Kratschmer et al. [14] reported an increase in the abundance of solitary wild bees in vineyards with a higher presence of isolated trees. Grasslands (which include grass strips and floral strips) provide benefits to many mobile ecosystem service providers as predators, parasitoids and pollinators [19].

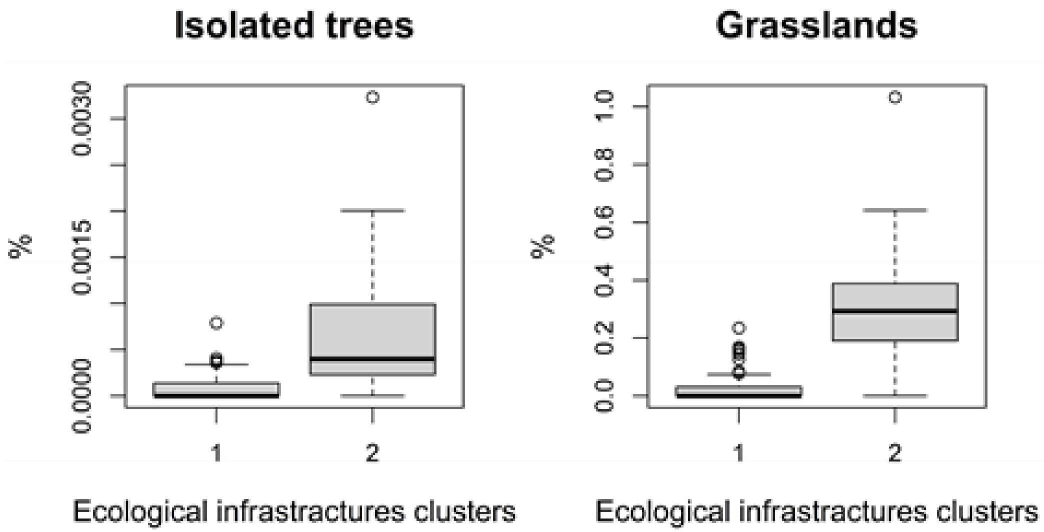

Fig. 4. Boxplots representing the distribution of values of Isolated trees (left) and Grassland (right) in the 108 EUs according to the two clusters identified.

3.2.3 Landscape composition

A higher level of landscape heterogeneity can reduce biodiversity decline in vineyard ecosystems [21], contrasting the landscape simplification deriving from the intensification of monoculture. In the buffer areas around the 108 EUs, 19 different land-use categories were identified (Table 3).

The land-use categories most present in the landscape of the EUs were non-irrigated arable land, vineyards, pastures, residential or industrial units, broad-leaved forest.

EUs were classified into three clusters according to landscape composition (Fig. 5).

Table 3. Descriptive statistics of landscape variables in the buffer areas of the 108 EUs.

Code	Definition	Mean±SD*(%)	Range [min,max] (%)
AGRC01	non-irrigatedarable land	20.52±12.98	0.00-48.82
AGRC02	horticulturalcrops	0.17±0.37	0.00-1.80
AGRC03	protected crops	0.01±0.07	0.00-0.54
AGRC04	floriculturalcrops	0.74±1.16	0-4.13
AGRC05	vineyards	36.30±12.61	11.81-63.56
AGRC06	fruit trees, berry plantations	0.15±0.36	0.00-2.41
AGRC07	olive groves	1.10±1.34	0.00-4.99
AGRC08	other orchards	0.59±0.86	0.00-2.33
AGRC09	pastures	4.06±5.26	0.00-26.64
ANTR01	residential or industrial units	13.84±7.51	2.43-35.37
ANTR02	isolate buildings	0.70±0.62	0.00-4.15

ANTR03	road, rail networks and associated land	0.51±1.04	0.00-4.72
ANTR04	green urban areas	1.03±1.10	0-4.60
ANTR05	other artificial units	1.13±1.85	0.00-11.48
BOSC01	broad-leaved forest	16.65±13.11	0.00-71.28
IDRC01	water bodies	0.07±0.12	0.00-0.39
SNAT01	near-rivers broad-leaved woodlands	2.08±2.07	0.00-9.51
SNAT02	inland marshes	0.02±0.21	0.00-2.23
SNAT03	transitional woodland-shrub	0.32±0.52	0.00-1.87

* SD: standard deviation

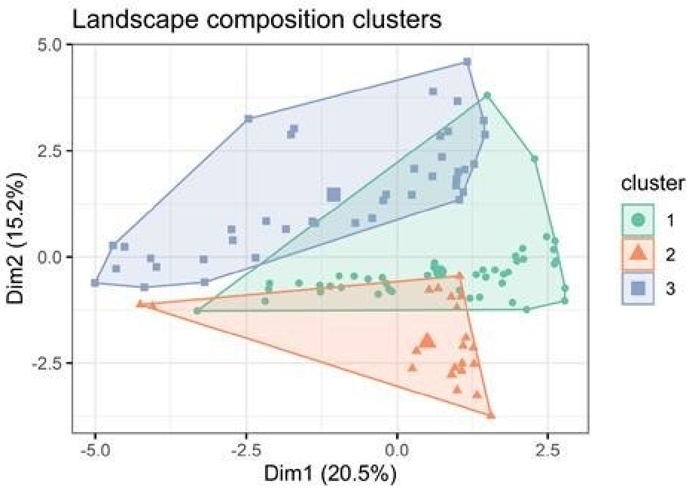

Fig. 5. Cluster plot related to landscape variables.

The distributions of the percentage of buffer area covered by vineyards and broad-leaved forests (Fig. 6) are an example of the composition differences between the EU landscapes. Although EUs characterised by landscapes mainly occupied by vineyards (Cluster 2) can be negatively influenced in their biodiversity [6], this general consideration should be deepened including information about internal vineyards characterisations and other landscape components. Forest areas represent a potential source habitat for many functional guilds and are usually linked to a greater landscape heterogeneity [22].

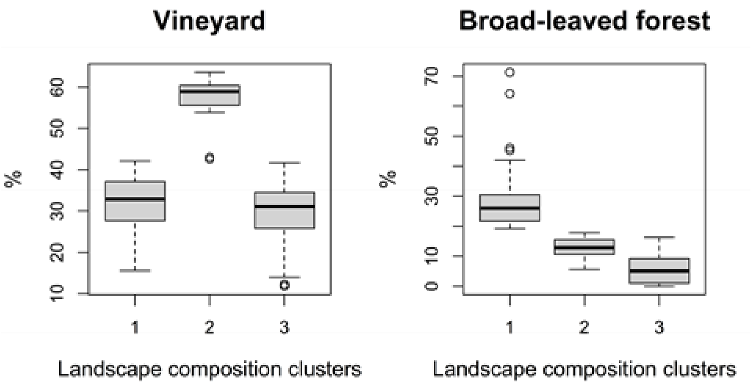

Fig. 6. Boxplots representing the distribution of values of Vineyard (left) and Broad-leaved Forest (right) in the 108 EUs according to the three clusters identified.

3.2.4 Management

Many studies focused on the role of vineyard management in influencing biodiversity and related ecosystem functions [6]. Specifically, the role of ground management practices has been emphasised [4]. Two groups of EUs were identified considering management practices (Fig. 7).

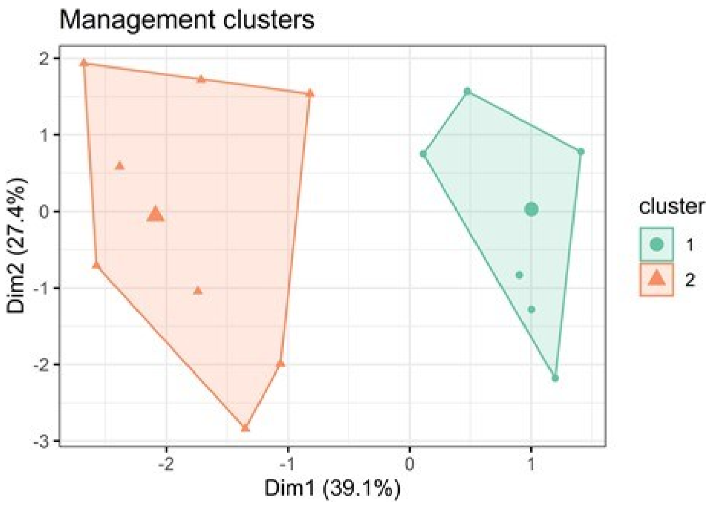

Fig. 7. Cluster plot related to Management variables.

Fig. 8 shows the frequency distribution of the six management practices in the two clusters. Cluster 1 included EUs not tilled or tilled once, while cluster 2 included EUs tilled from 0 to 5 times.

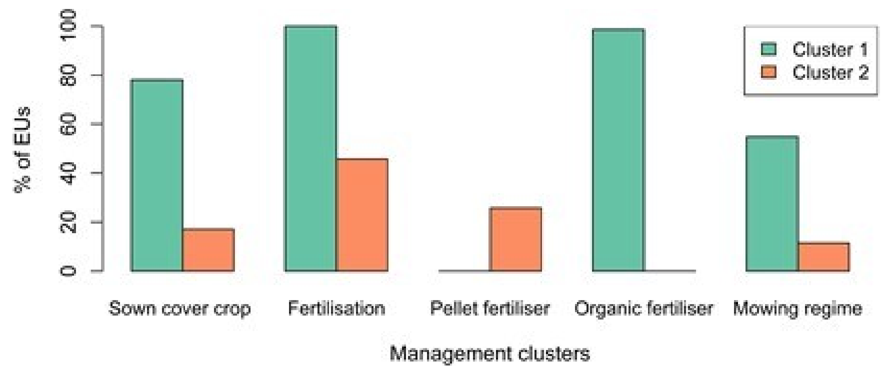

Fig. 8. Distribution of EUs (%) in each cluster according to inter- row ground management binary variables.

4 Conclusion

In this study, an innovative methodology to develop a framework for a systemic approach to vineyard agroecosystem is presented. This integrated approach to the assessment of vineyard biodiversity allows managing the complexity of the vineyard landscape.

The case study of the Franciacorta wine-growing area is reported. Different components of local (within vineyard) and landscape biodiversity are presented in separated informative layers to obtain a detailed description of Franciacorta wine-growing area territory.

This study set the ground for carrying out specific monitoring activities addressing the main components of biodiversity, providing a useful instrument to increase knowledge about vineyard system biodiversity.

We thank the 'Consorzio per la tutela del Franciacorta', which promoted this Project, and the wine Companies assisting with data collection.

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CHAPTER 5 – Concluding remarks

The Thesis aims at proposing an attempt to integrate the results obtained by the research activities in my PhD program, defining a methodological scheme supporting sustainability analysis in viticulture. The Specific Objectives (SOs) of the new CAP 2021-2017 that are mainly related to environmental sustainability (i.e., SO-4, SO-5, SO-6) were considered in structuring the framework. Research activities reported in Chapter 2, 3, 4 deal with some Specific Activities (SAs) related to the considered SOs (i.e., SA-4.1; SA-5.2 and 5.4 and SA-6.2 and 6.3). The SAs considered here concern important challenges in viticultural sector, and in general in agriculture, such as the impact on climate change and the strategies for adaptation and mitigation; the problem of nutrient loss (leakage and emissions in atmosphere) and the erosion of biodiversity.

Research activities performed enable to identify a set of possible interventions dealing with the challenges listed above.

In Chapter 2.1 the Carbon Footprint (CF) of organic and conventional vineyard management are compared. No significant differences between the two management strategies are revealed considering the overall CF. Fuel consumption for field operations represents the main source of greenhouse gas (GHG) emissions. This result suggests that effort must be dedicated to precisely evaluate the fuel consumption associated to each field operation for adopting strategies reducing the energetic costs. CF analysis also reveals the importance of nitrogen fertilizing in increasing GHG emissions mainly in relation to the release of nitrous oxide in the atmosphere. N_2O can be in fact considered one of the main air pollutants, together with ammonia and other nitrogen oxides. Agronomic practices that allow to reduce N_2O emissions consistently contribute to the reduction of air pollution. Strategies for efficient fertilization also reduce dispersion of nitrogen through leakage, with benefits in terms of N uptake by the vine. Moreover, N_2O is a powerful GHG with a Global Warming Potential around 280; consequently, strategies reducing the dispersion of this gas represent a key element in CF reduction protocols. Considerations about the possibility of reducing nitrous oxide emissions are addressed in Chapter 3 where results obtained from a high temporal resolution monitoring of N_2O fluxes from organic fertilization in vineyard are reported. This study highlighted that in plots where organic fertilizer was not incorporated into the soil N_2O emissions are lower than in plots where fertilizer was incorporated.

Chapter 2.2 explores the effect of canopy artificial shading on sparkling wine production facing problems related to climate change. The parameters investigated are the anticipation of grapes ripening, the acidic conservation and the polyphenolic composition. The results obtained confirmed the role of artificial shading as one of the effective practices for the adaptation to climate change thanks to the reduction of the temperature of the shaded grapes.

The complexity of interaction of factors characterizing vineyards leads to consider the combined effects of different factors on biodiversity response. In Chapter 4.1 and Chapter 4.2 this complexity is addressed through two different point of view. Chapter 4.1 includes considerations about edaphic arthropods biodiversity as arthropod community plays a pivotal role in the provisioning of soil-based ecosystem services and represents a good bioindicators of soil quality. The positive effect of organic management of vineyard is reported with respect to conventional management as well as a negative effect of subsoiling in the short term is highlighted. Chapter 4.2 the overall vineyard agroecosystem complexity is considered. A methodology to develop methodology to develop a systemic approach to vineyard biodiversity is proposed. The characterization of three winegrowing companies located in the Franciacorta area is reported structuring different components of local (within vineyard) and landscape biodiversity in separated informative layers. The analysis of information acquired allowed to reveal the variability of the territory both in terms of biodiversity composition (i.e. ecological infrastructure and landscape) and in terms of soil management strategies adopted. This variability suggests to plan specific monitoring activities addressing the main components of biodiversity, providing a useful instrument to increase knowledge about vineyard system biodiversity.

A synthesis of main results obtained from research activities included in this Thesis are reported in **Figure** *3*:

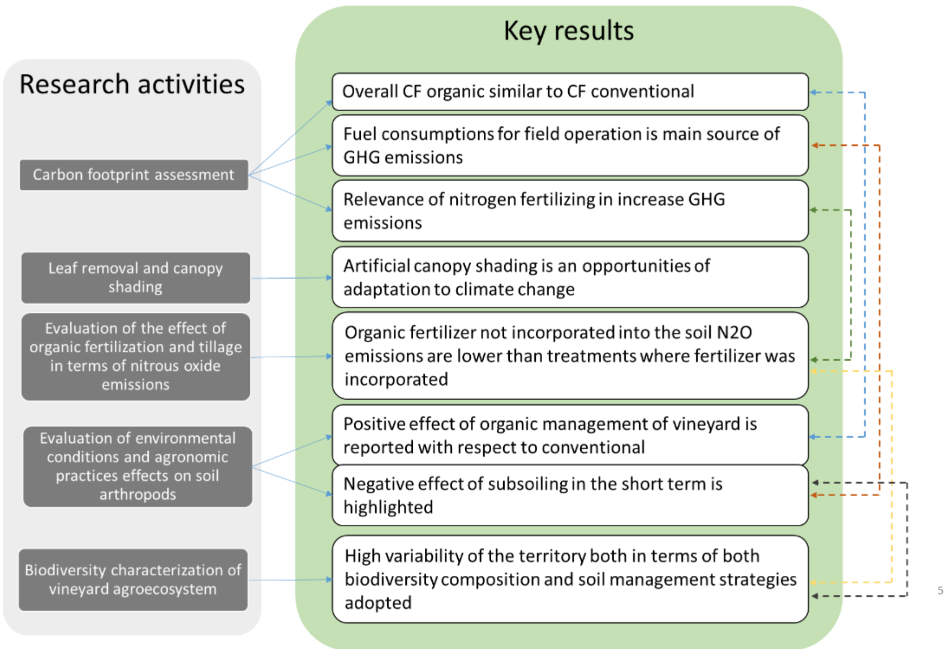

Figure 3 Synthesis of main results obtained from research activities carried out during PhD. Dashed lines indicate possible interactions among results obtained.

Dashed lines in Figures 3 represent and attempt of integration of key results obtained by the research activities. The integration of key results can be considered as a proposal of methodological scheme to allow a full assessment of sustainability analysis in viticulture.