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**Ph.D. Dissertation**

***Distribution of biological products by autonomous platforms for weed control in Mediterranean agricultural systems***

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This work is also part of the activities of the project “Advanced Technologies for LANDSmanagement and Tools for Innovative Development of an EcoSustainable agriculture” (ATLANTIDE). The general objective of ATLANTIDE is the complete integration between theoretical knowledge and technologies for the definition and implementation of agricultural production models that aim to efficiently combine inputs (water, fertilizers, pesticides, energy, time) with outputs (increase efficiency, improvement of quality, reduction of production losses, reduction of resource use, reduction of land use, reduction of the ecological footprint). The team of the ATLANTIDE project is made up of three Partners with strong complementarities: Topcon Agriculture S.p.A-Private Company, Abinsula Srl-Sardinian Small-Medium Enterprise, and the Center for Innovative Agriculture, University of Sassari-Research Organization (Coordinator).

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## **Chapter 1. General introduction**

Over recent decades, global demographic expansion has emerged as one of the main factors on socio-economic and environmental systems at the planetary scale. United Nations projections indicate that the global population has already exceeded eight billion individuals and is expected to continue growing in the next decades [1]. United Nations projections indicate that the world population has already exceeded eight billion individuals and is expected to continue growing in the coming decades [2]. In fact, FAO statistical models suggest that the population growth curve, which has been consolidating since the mid-20th century, will maintain an exponential trend, with estimates leading to a world population of around nine billion by 2050 [3].

Analyses of FAO annual reports and medium- to long-term demographic forecasts consistently highlight crop management systems - and more broadly, the primary production sector - as vital for maintaining global food security and, thus, human well-being worldwide [4], [5]. This outlook raises critical concerns about the planet's ability to provide a food supply that is not only quantitatively sufficient but also safe and environmentally sustainable [6].

The agriculture, therefore, occupies a central role, tasked with meeting rising demand amid shrinking natural resources. The ongoing loss of arable land, worsening effects of climate change, and growing water scarcity demand fundamental changes in production systems [7]. As a result, boosting agricultural productivity through sustainable intensification is essential to ensure food availability for a growing global population while protecting ecosystem integrity.

At the same time, advances in agricultural mechanisation have led to an increase in the use of machinery in agricultural production. This development has facilitated a substantial increase in unit yields, but has also had ecological consequences, mainly in the form of environmental pollution with significant implications for ecosystem integrity [8]. Conventional agricultural management systems, consolidated during the Green Revolution, have been characterised by intensive mechanisation and the systematic application of synthetic mineral fertilisers and pesticides [9]. These practices have enabled significant increases in productivity and contributed to the stabilisation of global food supplies. However, numerous scientific studies indicate that the excessive use of chemicals has led to a significant environmental impact, including the eutrophication of surface waters, groundwater contamination, loss of biodiversity, and degradation of soil biological fertility

[10], [11]. In addition, the emergence of multiple resistances among pathogens and pests has necessitated the use of new active compounds, thus creating a self-perpetuating cycle of dependence on chemicals [12], [13].

### **1.1. Organic products for an ecological management**

In recent decades, traditional chemical crop management has gradually been supplemented by organic management [14], driven by farmers' increasing awareness of environmental sustainability and the protection of biodiversity [15]. Organic plant protection products (PPPs) play a central role in this, offering a robust strategy for mitigating the environmental impact of conventional agricultural practices involving chemical pesticides [16].

These products are used at crucial stages in crop management, such as fertilisation, controlling plant diseases, and managing weeds. One relevant PPP is pelargonic acid [17], [18], a naturally derived substance found in some plants of the Asteraceae family. Among the plants that synthesise this substance, many are of the *Cynara* genre, which concentrates these molecules in their achenes [19]. Pelargonic acid is a saturated, nine-carbon-atom fatty acid used as a natural herbicide that acts on contact, causing the dehydration of weeds tissues [20].

Research on pelargonic acid has been advanced through this PhD programme conducted in collaboration with Novamont Spa. Among its research initiatives, Novamont Spa focuses on the production, characterization, and development of pelargonic acid-based plant protection products aimed at biological weed control [21].

Pelargonic acid is particularly relevant in integrated weed management strategies as it enables farmers to control weed growth without compromising soil quality or posing risks to human health. Furthermore, unlike the synthetic herbicides commonly used in conventional agriculture, the rapid biodegradability of pelargonic acid reduces the risk of environmental accumulation and facilitates its inclusion in agronomically sustainable production systems [22]. Therefore, it follows that organic PPPs can be integrated into critical crop management operations, supporting plants during crucial growth and development stages.

### **1.2. Biostimulant support**

Among the organic PPPs, a highly relevant product application is represented by biostimulants, which play a central role in this scenario [23], [24]. Recently subject to a unified regulatory definition under Regulation (EU) 2019/1009, these products include substances and microorganisms that do not directly supply nutrients or protect plants, but

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rather stimulate physiological processes and improve metabolic efficiency [25]. Studies in the scientific literature have shown that biostimulants can:

- improve nutrient use efficiency [26], thereby reducing losses due to leaching
- improve crop tolerance to abiotic stresses, particularly drought and heat stress [27]
- modulate enzyme activity and photosynthetic processes, thereby directly affecting productivity [28]
- Strengthen mutualistic interactions with the rhizosphere to improve soil fertility [29], [30]

The main categories of biostimulants include seaweed extracts, rich in bioactive compounds with hormone-like activity; protein hydrolysates, containing bioactive peptides capable of modulating growth processes[31]; humic and fulvic acids, which improve nutrient availability and soil physical structure [32]; yeast extracts, which provide signaling molecules and metabolic precursors that enhance plant vigor[33]; and microelements, essential cofactors that regulate enzymatic activity and optimize physiological processes [34].

The application of yeast extracts, for instance, has been shown to stimulate root development and enhance plant resilience under abiotic stress conditions, while macroalgal extracts have demonstrated significant effects in improving drought tolerance in fruit and vegetable crops [34]. Protein hydrolysates have also been reported to increase net photosynthesis and biomass accumulation in high-intensity crops, such as wheat and tomato [35].

From a management perspective, biostimulants represent a convergence point between conventional and organic systems. In integrated and conventional systems, they enable a gradual reduction of chemical inputs while maintaining competitive yields; in organic systems, they compensate for the limited availability of authorized products, expanding the suite of tools available to farmers [36].

Thus, the integration of organic principles and the strategic use of biostimulants outline an innovative pathway toward sustainable agriculture, simultaneously addressing production efficiency, food security, and the conservation of natural resources.

### **1.3. PA and monitoring system importance**

Over the past decades, cultivation and crop management techniques have undergone substantial evolution, driven by a shift toward more efficient and rational utilization of production resources and supported by technological innovations introduced to the market. This progression occurs within a context of growing awareness regarding the need to reduce

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production costs, enhance input-use efficiency, and mitigate the environmental impacts of agricultural practices.

In this context, precision agriculture (PA) represents one of the most advanced expressions of agricultural evolution [37]. The fundamental principle of this approach can be summarised as follows: "To do the right thing, on the right place, at the right moment" meaning to manage crop operations in a site-specific way, depending to the heterogeneity on field. The objective of this approach is the targeted and differentiated application of inputs and agronomic practices in response to field variability.

This model is employed to adapt field operations to the actual conditions of crops and soil, thus achieving a twofold objective. The first of these objectives is to optimise crop yields and quality [38]. The second is to reduce waste and environmental dispersion of potentially impactful substances, such as nitrogen fertilisers and plant protection products [39]. Consequently, PA enhances the technical and economic efficiency of the production process whilst concurrently promoting environmental sustainability, thus having a beneficial effect on the conservation of agricultural and natural ecosystems.

Currently, field monitoring systems for site-specific crop analysis are predominantly based on sensor technologies designed to observe and record spatial and temporal variability within cultivated fields [40]. These systems facilitate the study of a wide range of parameters, including soil and water characteristics, physiological and morphometric crop traits, and environmental factors such as temperature, humidity, and precipitation.

Among mobile sensors, proximal and remote sensing technologies play a particularly important role. The primary distinction between these approaches lies in acquisition distance: remote sensing is defined when the sensor is located at a distance greater than approximately two meters from the target, whereas proximal sensing operates below this threshold.

Depending on the type of analysis and the required acquisition frequency, sensors can be categorized as either stationary—permanently installed in the field—or mobile, meaning they are transported across the plot or mounted on dedicated platforms [41], [42]. These platforms, traditionally represented by tractors or electrically and mechanically powered quadricycles, have increasingly been complemented by autonomous ground vehicles (UGVs), which are assuming an increasingly prominent role among terrestrial systems for crop monitoring and field management [43].

The application of these technologies represents an innovative alternative to traditional, largely destructive analytical methods that require sampling and subsequent laboratory processing. In contrast, proximal and remote sensor systems enable non-destructive

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monitoring of crops, acquiring physiological and agronomic information directly from the plant under real field conditions, thereby providing a more accurate and dynamic representation of crop status.

Remote sensing systems include a range of technologically advanced platforms, as unmanned aerial systems (UAS), manned aircraft equipped with dedicated instrumentation, and satellites. Each platform exhibits specific characteristics in terms of spatial, temporal, and spectral resolution [44]. The use of these tools facilitates the analysis of extensive agricultural areas within relatively short timeframes, supporting the development of management strategies targeted to intra-field variability. Among these technologies, UAS and satellites are currently the most widespread and operational. UAS offer high operational flexibility, extremely high-resolution acquisitions, and customizable deployment, while satellite constellations provide temporal continuity and broad spatial coverage, enabling consistent monitoring of large geographic areas at performant resolutions [45].

Despite their widespread adoption, remote monitoring systems present specific operational limitations. Satellite-derived information is conditioned by orbital revisit times and cloud cover at the time of sensor passage, factors that can significantly reduce data quality and temporal continuity [46]. The use of modern UAS, equipped with sensor auto-calibration capabilities, partially mitigates these limitations, enabling data acquisition under variable cloud conditions; however, their limited flight autonomy and low operational altitude constrain coverage to relatively small areas. Nonetheless, remote sensing remains a cornerstone tool for crop monitoring and the validation of management strategies, as extensively documented in the literature.

In association with remote sensing, proximal sensing systems provide a critical contribution to field data acquisition. These systems include diverse sensor categories capable of measuring heterogeneous soil, environmental, physiological, and productive parameters. Sensors based on reflectance and fluorescence analysis are particularly relevant for monitoring plant physiological activity. The processing of these signals allows the extraction of vegetation indices sensitive to specific biochemical and physiological processes, providing high-spatial-resolution information down to the scale of individual leaves or fruits, with direct applications in site-specific management strategy development [47].

Although proximal sensing provides high accuracy at the point scale, it exhibits limitations when applied over large areas, primarily due to longer acquisition times compared with satellite or airborne remote sensing, resulting in reduced operational efficiency for extensive fields. Among proximal technologies, fluorescence sensors are distinguished by their ability to rapidly generate vegetation indices that assess plant physiological dynamics [48]. These

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indices facilitate monitoring of key parameters, including chlorophyll content, polyphenol presence, nitrogen nutritional status, and water stress, at both leaf and fruit levels, representing essential tools for implementing precision agriculture practices oriented toward productive efficiency and sustainability [49].

#### **1.4. Digitalisation and future agriculture perspectives**

The agronomic data information base from crop monitoring systems can be enhanced by georeferencing measurements using satellite positioning. This allows for more accurate spatialisation of the acquired parameters and optimises the process of planning crop operations. Evaluating geo-localised variables using dedicated geographic information systems (GIS) and integrating geostatistical analysis with site-specific data enables the estimation of field variability, the creation of vigour maps, and the provision of an initial layer of information for interpreting and planning subsequent crop activities [50]. Vigour maps can therefore serve as fundamental digital layers for executing pre-sowing crop operations and throughout the entire growing season.

Information derived from vigour maps is typically used to generate distribution or prescription maps, which are displayed on advanced agricultural machinery associated with Agriculture 4.0. Field data must be tailored to the specific context of each farm by adjusting distribution and operational parameters to match the available equipment. Prescription maps mark the final stage of operational planning and guide the execution of agricultural tasks such as tillage, fertilisation, weeding, pruning, or harvesting. This approach establishes a digital framework that supports the precise execution of complex agricultural operations and assists operators in all practical aspects of their work [51].

In fact, integrating standardised communication protocols between tractors and operating machines — with the ISOBUS protocol as the main interconnection system — enables prescription maps to be integrated into tractor terminals. This automates data transfer and optimises input management while reducing operational inefficiencies [52].

In line with the latest technological developments and experiments, modern robotic agricultural management systems are showing a growing interest in the operational synchronisation between unmanned aerial system (UAS) and the most advanced autonomous ground vehicles (UGVs). The purpose is to achieve highly efficient management of the site-specific distribution of PPP and, more generally, of production inputs [53]. UGVs, thanks to their ability to move autonomously in the field with high precision, represent a strategic asset for precision agriculture. They help reduce the need for manual labor, enhance operator safety, and ensure targeted interventions only in areas where

they are truly required, thereby minimizing product waste and environmental impact. Current precision farming technologies aim to establish fully interconnected systems that link field equipment with farm management centers, digitizing monitoring data for reprocessing and the automatic generation of prescription maps [54].

The integration of aerial and ground platforms with the latest multi- and hyperspectral sensors, high-accuracy RTK-based positioning, and artificial intelligence platforms enables the near real-time collection and analysis of agronomic data, supporting more informed crop management decisions. Recent advances have also led to the development of digital models that not only process field data but also interpret it through artificial intelligence and digital twin approaches. These models can identify patterns, predict outcomes, and support increasingly precise data-driven decision-making. Digital twin technologies further extend this potential by enabling the real-time simulation and optimization of processes within virtual environments. Together, these innovations are transforming the way agricultural data is collected, analyzed, and applied, offering powerful tools to improve efficiency, sustainability, and strategic planning across the sector.

These fully digital systems integrate autonomous ground and aerial platforms into the crop management chain with the aim of implementing fully automated, high-precision operations for delicate tasks such as weed management, plant disease control, and fertilisation [55].

These systems, central to Agriculture 5.0, present a significant technological challenge in the digitisation, planning, and automation of crop operations [56]. The role of the agricultural operator shifts from performing manual tasks to managing complex digital systems that rely on continuous, high-precision information flows and demand advanced analytical and decision-making skills [57].

### **1.5. Dissertation objectives**

This PhD thesis investigates the potential and effectiveness of proximal and remote sensing systems in different agricultural scenarios, particularly in crop management and production processes. The research aims to support the development of advanced decision-support systems (DSS) for optimising control strategies involving new organic pesticide products. Specific attention is devoted to the optimisation of targeted distribution strategies for biologically derived plant protection products (PPPs), with the aim of increasing their efficiency and reducing their environmental impact.

The research is organised into three chapters, each demonstrating how multitemporal surveys combined with advanced digital processing techniques can provide robust, efficient, and effective tools for monitoring and managing both perennial (grapevine) and horticultural

(artichoke) crops. The findings highlight the reliability of these approaches for precision crop management and their suitability for organic agricultural practices.

### 1.6. Dissertation structure

The present dissertation include a first general introduction, three chapters, and a overall conclusion.

The three central chapters report three articles published, and are reported as follows:

Chapter 2 explore a proximal sensing application on a horticultural cultivar to observe the sensivity of fluorimetry of different cultivation management approaches.

Chapter 3 examine the effects of varying biostimulant application rates on vineyard from differend monitoring tecniques, using traditionam analysis method, proximal and remote sensing.

Chapter four summarise in a three-year experiment the capacity of a different monitoring system to detect different vigour areas, and trough the application of geostatistic, to provide the best output results as a DSS for target intervention.

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## Chapter 2. Proximal and Remote Sensing Monitoring of the ‘Spinoso sardo’ Artichoke Cultivar on Organic and Conventional Management

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**Abstract**

The development of new techniques to improve crop management, especially through precision agriculture methods and innovations, is crucial for increasing crop yield and ensuring high-quality production. The horticultural sector is particularly vulnerable to inefficiencies in crop management due to the complex and costly processes required for producing marketable products. Optimal nutritional inputs and effective disease management are crucial for maintaining commercial standards. This two-year study investigated the physiological differences between organic and conventional crop management of the Sardinian ‘Spinoso sardo’ artichoke ecotype (*Cynara cardunculus* var. *scolymus* L.) by integrating a multiplex force-A (MFA) fluorometer and unmanned aerial systems (UASs) equipped with a multispectral camera capable of analysing the NDVI vegetation index. Using both proximal and remote sensing instruments, physiological and nutritional variations in the growth cycle of artichokes were identified, distinguishing between traditional and two organic management practices. The two-year MFA experiment revealed physiological variability and different trends among the three management practices, indicating that MFA proximal sensing is a valuable tool for detecting physiological differences, particularly in chlorophyll activity and nitrogen content. In contrast, the UAS survey was less effective at distinguishing between management types, likely due to its limited use during the second year and the constrained timeframe of the multitemporal analysis. The analysis of the MFA fluorimetric indices suggested significant differences among the plots monitored due to the ANOVA statistical analysis and Tukey test, showing greater adaptability of the conventional system in managing production inputs, unlike the organic systems, which showed higher variability within the plots and across the survey years, indicating aleatory trends due to differences in crop management.

**Keywords:** precision agriculture; horticulture; fluorometer; UAS; organic management

## 1. Introduction

In recent decades, innovations in agricultural practices have led to significantly higher crop yields per unit area compared to the previous century [1]. This evolution is related to the gradual rise in the global population [2] and, consequently, increased demand for essential goods, all aimed at maximising production efficiency and ensuring food security [3]. In the agricultural scenario, horticultural crops are crucial, accounting for more than a billion tonnes in 2022 [4]. Traditionally, conventional horticultural crop management involves numerous interventions in soil management, plant nutrition, and disease control [5], with massive treatments to achieve production goals and maintain quality standards [6]. Recently, the emergence of organic management for horticultural crops has led to a significant reduction in production inputs, tillage, and the total elimination of chemical plant protection products [7]. Artichoke cultivation (*Cynara cardunculus* var. *scolymus* L.) represents a typical species of the Mediterranean area in the Asteraceae family, derived from domesticated forms of the wild cardoon (*Cynara cardunculus* var. *sylvestris* Lam.); it occupies a relevant place among vegetable crops [8], counting several varieties and management methods found in the regions where it is cultivated [9]. Artichoke cultivation is considered a high-value agricultural activity, attributable to its substantial yield and economic return per hectare [10], and, depending on the region, it exhibits a wide range of differentiated ecotypes. With regard to its commercial diffusion in Sardinia, one of the principal ecotypes in Sardinia is the “Spinoso sardo”, due to its wide diffusion on the island. Among artichoke ecotypes, the “Spinoso sardo” variety is an autumn–winter re-flowering variety [11]. Traditionally, it is planted with semi-dormant offshoots during the summer and is subject to a forcing technique. This method uses early awakening through irrigation to start the production cycle sooner, allowing the plants to produce first-order flower heads by October or November. Therefore, the forcing technique anticipates the plant’s cycle, allowing for an earlier commercial output. It highlights the significance of cultivation techniques and how different management methods can impact the plant’s physiological development [12].

Conventional artichoke management involves supplementing the soil with mineral fertilisers, weed control, phytiatric treatments, and, in some cases, the removal of senescent crop residues for potential energy use at the end of the season, despite their low energy yield [13]. This cultivation system is typically based on continuous artichoke monoculture. Traditionally, artichoke cultivation depends on conventional soil management and uniform fertilisation practices throughout the growing season. As a nutrient-absorbing species,

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artichoke requires substantial inputs of nitrogen and phosphorus–potassium fertilisers, which are typically supplied through mineral fertilisers applied during soil preparation and throughout the growing season [14]. These practices influence both the physiological crop activity and the chemical–structural composition of the soil. In recent years, organic management systems have emerged as a sustainable alternative to conventional cultivation [15]. This approach enhances crop nutrition through intercrops cultivation, which is subsequently incorporated into the soil, promoting natural mineralisation processes and reducing dependency on synthetic fertilisers [16–19]. While the organic system offers more sustainable management and less environmental impact than traditional systems, organic cultivation presents the risk of unpredictable production from both a nutritional and phytosanitary point of view, as the control of production inputs is more limited and potentially less efficient. Nonetheless, traditional cultivation methods, as with many other cropping systems, often lack strategies for the selective application of inputs based on the heterogeneous characteristics within a field. Uniform field management—encompassing tillage and nutrient application—frequently fails to account for spatial variability in soil fertility and crop needs [12,20]. The gradual and site-specific management of agricultural inputs is one of the key innovations introduced by precision agriculture (PA). PA seeks to address intra-field variability by employing advanced technologies and analytical methods for crop monitoring, providing the basis for decision support systems (DSSs) that guide more efficient and sustainable management strategies. Crop monitoring is a critical component of DSS development [21], enabling real-time data acquisition through dedicated sensors. This data is then processed into actionable information, allowing operators to make informed decisions. A central objective of such monitoring is to generate spatial information that distinguishes local field conditions, thereby facilitating the optimisation of input use. This helps to prevent the over- or under-application of resources in areas where conventional, uniform management might lead to inefficiencies. Proximal sensing systems have emerged as a highly effective tool for localised crop monitoring [22]. By providing high-resolution, site-specific data, these systems capture the physiological variability within the field and enable timely, targeted interventions [23]. The proximal sensing application strategy could integrate and enhance the evaluation of site-specific input applications, such as localised nitrogen fertilisation, water management, and plant protection product (PPP) application [24]. These tools, equipped with the latest technologies, offer rapid analysis and immediate access to critical information, enhancing decision-making processes at the field level. Proximal sensing can provide information ranging from physiological to quantitative variables. Among the various existing instruments, fluorimetric sensors deserve particular

mention, as they can rapidly obtain non-destructive information on the crop's physiological variables. This application is applied in numerous cropping systems, from herbaceous to tree crops. Fluorimetry has multiple applications in horticulture as well, enabling the analysis of different physiological characteristics of crops both in the field and in the laboratory [24,25]. In the field of artichoke cultivation, fluorimetry has been used to analyse chlorophyll, chlorosis disease [26], and biomass composition [27,28]. In parallel, remote sensing technologies, using satellite platforms, manned aircraft, and unmanned aerial systems (UASs), have expanded the scale and efficiency of monitoring practices through multiple sensors and vegetational indices [29]. In fact, the application of these techniques has contributed to more rapid crop analysis, reducing the operational costs of acquiring physiological data in the field. Traditionally, analyses conducted for product evaluation involved destructive sampling, which negatively affects production. Over the past decade, these tools have become integral to agricultural surveillance, enabling the collection of data across large areas with increasingly reliable results [30], regardless of variations in ground resolution associated with these different systems [31].

Understanding field variability, achieved through site-specific physiological evaluation of the crop, could enable more efficient management of production resources. This process would allow for a more targeted and potentially localised assessment of individual plants, considering their physiological needs to optimise crop management.

The present study involved non-destructive sampling of 'Spinoso sardo' Sardinian ecotype artichoke leaves during the 2018/2019 and 2019/2020 seasons, covering the vegetative growth and reproductive phases of the plants, monitoring three different cultivation management systems, classified as conventional or organic, using a proximal fluorimetric sensor. The primary purpose is to evaluate the ability and efficiency of fluorimetric indices in detecting any physiological differences in leaves based on the different cultivation techniques adopted. Secondly, due to the high intensity of the work involved in MFA analysis on a wide range of samples, an aerial survey was performed using a UAS to acquire the NDVI vegetation index. The UAS analysis was performed to observe the dynamics of the remote sensing application and compare it with fluorimetric data, evaluating the potential for using a UAS as a substitute instead of MFA.

## **2. Materials and Methods**

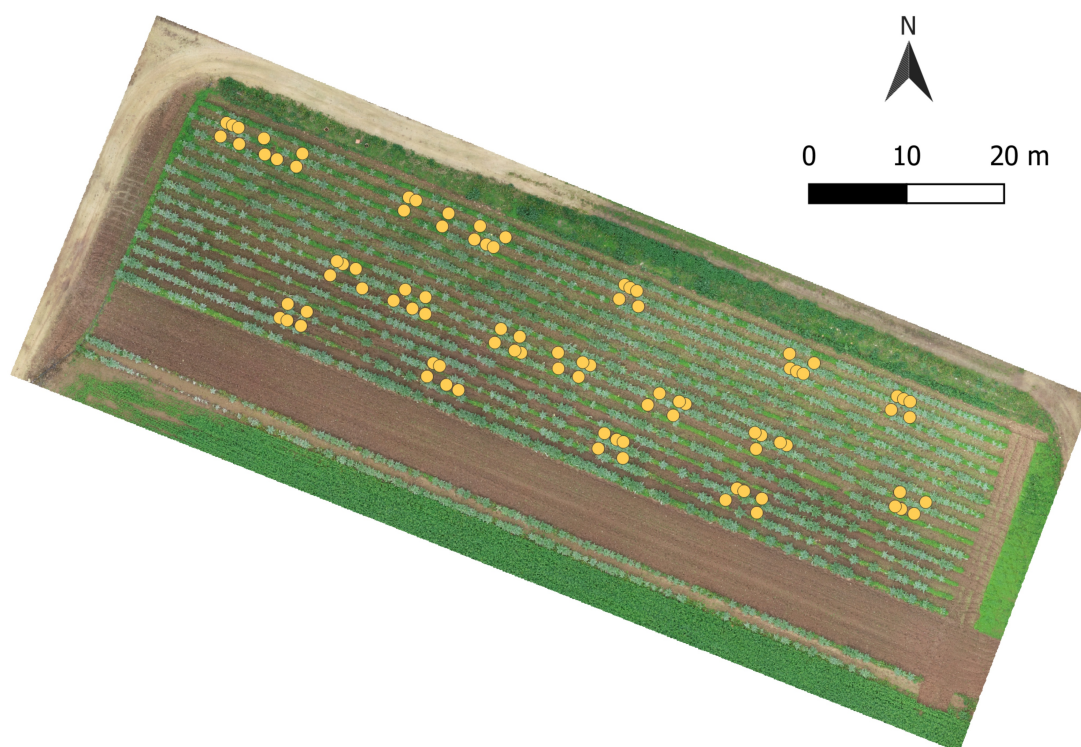
### **2.1. Site and Experimental Design**

The experiment took place at the experimental fields of the Agriculture Department, University of Sassari, located in Ottava (SS, Italy, 40°46'31" N; 8°29'12" E, WGS84

Coordinate System, 81 m above ground level) during the 2018/2019 and 2019/2020 seasons. These two seasons were characterised by a similar temperature trend, with a 10% reduction in natural water supply from precipitation, settling at around 450 mm.

The monitoring operation was performed in an ongoing experiment plot [16] identified in Figure 1, covering an area of approximately 2500 m<sup>2</sup>. The artichoke plants were distributed in rows oriented in a northwest–southeast direction, with a row spacing of 0.7 m × 1.4 m (along the row and between rows, respectively), resulting in a density of 9524 plants/ha. To minimise the canopy border effect, two rows were planted on the borders, and two additional rows were placed between the crop management sections to prevent contamination. Artichokes were planted in July using semi-dormant offshoots as propagation organs. The adopted experimental design considered three different types of agronomic management for the artichoke, as follows:

- Conventional (CON) management plot;
- Organic plot with annual presence of artichoke (ORG-I);
- Organic plot, alternated annually with cauliflower (ORG-II);



**Figure 1.** Experimental field under analysis. The plants monitored during the first survey year are marked in yellow. Sampling operations covered specific parts of the field, where the operator visually assessed the location of the measurements around some checking points placed along the field.

The experimental design compares conventional (CON) practices with two alternative organic management types, where artichoke succession occurs annually and biennially in

the field (ORG-I and ORG-II, respectively). The management of CON involves monocropping in the same plot, with conventional agricultural practices including mineral fertilisation, weeding, phytiatric treatments, and the soil incorporation of senescent dried crop residues toward the end of the crop cycle during the spring season.

The artichoke-growing cycle on the annual organic management ORG-I was interrupted early at the end of the marketable harvest period (mid-April), and the fresh residues were chopped and ploughed into the soil. To restock the soil's nitrogen, a short-cycle legume, French bean (*Phaseolus vulgaris* L. cv. *Bronco*) (Monsanto Agricoltura Italia SpA, Milan, Italy), was planted in the ORG-I plot. The French bean was interrupted at the reproductive stage, when the plants produced the first pods (end of flowering). The biomass was incorporated in a fresh state to increase the soil's nutrient content. Fresh residues from this bean crop were also incorporated into the soil at the end of June, before the new growing season for artichoke began.

The biennial rotation of artichokes was managed using cauliflower (*Brassica oleracea* L. var. *botrytis* cv. *Nautilus*). Artichoke and cauliflower species were cultivated alternately in the two plots in the two survey years. *Pisum sativum* L. cv. *Attika* (Limagrain Verneuil Holding, France), used as a legume cover crop, was sown in the inter-row spaces of artichoke and cauliflower in February. At the end of the primary crop's marketable harvest period, and when the peas were flowering (mid-April), artichoke, cauliflower, and fresh pea residues were incorporated into the soil.

According to organic farming principles, no phytosanitary treatments or chemical fertilisation were applied in the ORG-I and ORG-II management types, and the irrigation criteria followed the ongoing experiment [32,33]. The surveys were conducted during the crop's critical phenological phases, with monitoring operations scheduled according to the weather trend on a three-week basis.

Table 1 reports the details of the survey days during the experiment and their corresponding BBCH (Biologische Bundesanstalt, Bundessortenamt, and Chemical industry) code identification [34].

**Table 1.** Experimental survey days with the associated day of year (DOY) and BBCH scale.

Date	DOY	BBCH
21 December 2018	355	59
16 January 2019	16	59
13 February 2019	44	61
07 March 2019	66	67

22 March 2019	81	69
29 November 2019	333	55
27 December 2019	361	59
16 January 2020	16	59
24 February 2020	55	69

Fluorimetric data acquisition involved five representative plants of “Spinoso sardo” positioned around several checkpoints distributed along the field, as some crop failures were found along the rows due to the abortion of the offshoots. As shown in Figure 2a, the monitorable plants have large, well-expanded leaves, apparently not diseased and of normal development. The non-monitorable plants, as observed in Figure 2b, have poor development, with inadequately expanded leaves that cannot be observed with the monitoring instrument.

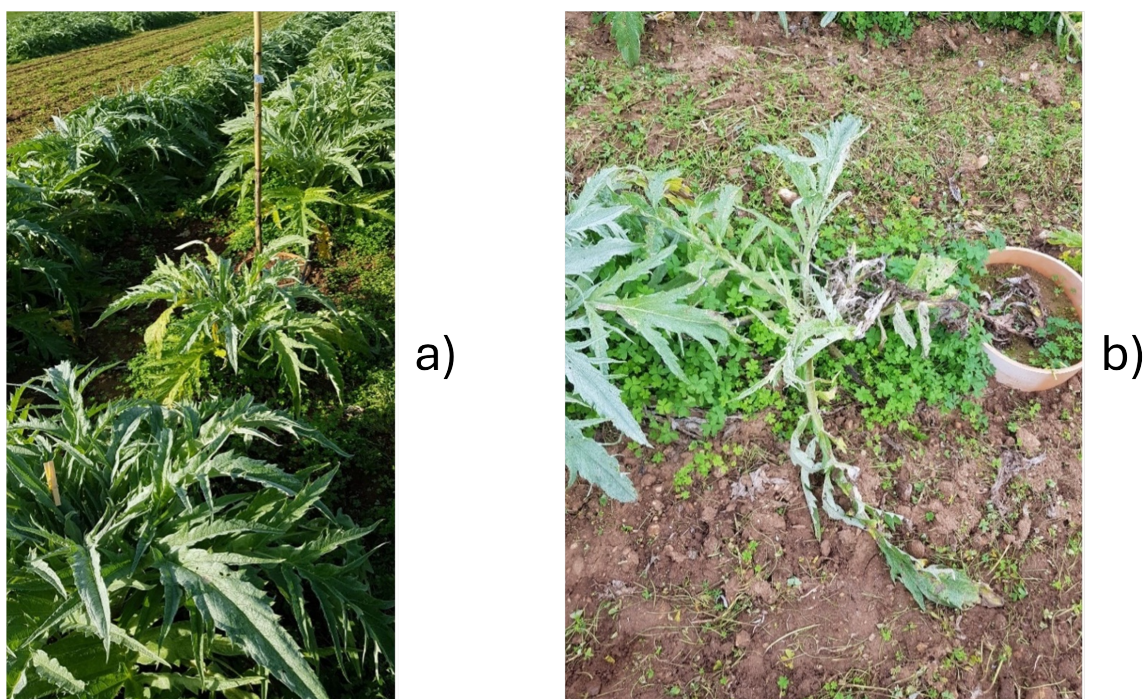
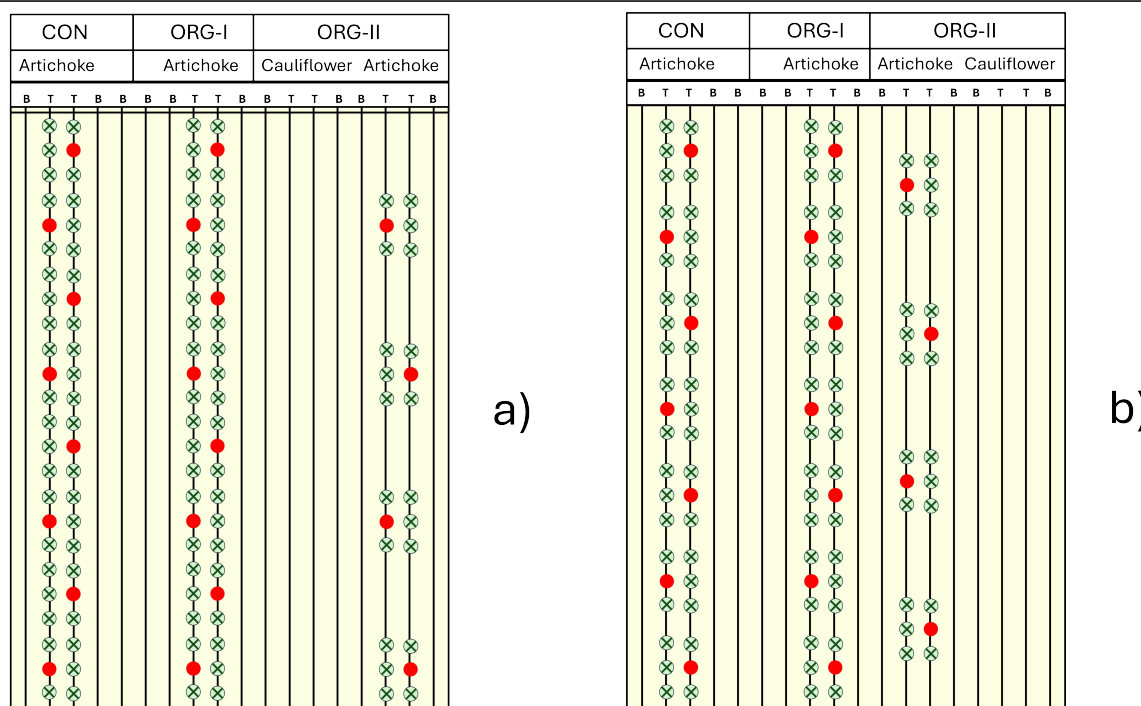


Figure 2. Example of sample selection during the sampling preparation phase. On the right, well developed plants are adequate for multi-temporal monitoring (a); on the left, plants are unsuitable for monitoring (b). The preliminary identification of the plants permitted their subsequent monitoring along the artichoke production cycle.

MFA field measurements were taken on clear days after the morning dew on the plants had evaporated. Figure 3 shows the thesis arrangement in the field; the ORG-II thesis appears on different plots in alternate years with the cauliflower thesis due to their biennial rotation. Figure 3 displays the plots involved in the survey, with experimental rows (Thesis, “T”) spaced by intermediate border rows (“B”) to isolate the different treatments.



**Figure 3.** Experimental design adopted for 2018/2019 (a) and for 2019/2020 (b). From left to right, the monitored thesis (T) separated by the border (B) rows are as follows: Conventional (CON) plot; organic plot (ORG-I) in a monocropping and annual rotation with French bean; organic plot (ORG-II) in a biennial rotation with cauliflower, as observable in both designs. Checking points are marked with red circles, and artichoke plants are marked with green crossed-out circles.

## 2.2. Fluorimetric Analysis

The instrument adopted for the non-destructive monitoring of plants is a hand-held multi-parameter fluorescence device capable of observing the physiological evolution of the crop and extrapolating its main nutritional characteristics [25]. The instrument used was the ForceA Multiplex 3 portable fluorometer (MFA, Orsay, France). The detection system comprises three light-emitting diode (LED) channels that emit pulsed radiation at wavelengths in the visible spectrum (RGB): 470 nm (blue), 516 nm (green), and 635 nm (red), respectively. The instrument features six LEDs that operate at a wavelength of 375 nm in the ultraviolet spectrum [35]. All the detectors are enclosed in a plexiglass structure to protect and isolate input and output radiation. At the centre of the sensor array, there are three sensors responsible for fluorescence detection in three delimited ranges, as follows: far-red fluorescence (FRF), near-red fluorescence (RF), and blue–green fluorescence (BG-F). The signals processed by MFA provide a total of 24 specific indices, which estimate the main physiological properties of the crop [36]. For this research, the fluorimetric analysis involved the SFR-G, SFR-R, NBI-G, NBI-R, FLAV, and BRR-FRF indices, evaluating chlorophyll, nitrogen, and flavonoid content, over the water stress and other abiotic stress indices. According to the literature, the choice of these indices is based on their ability to

investigate the main physiological characteristics of the plant, providing a comprehensive view of the plant's state [37]. The measurements include the sample fluorescence ratio with excitation channels in the green and red wavelengths (SFR-G and SFR-R) for assessing chlorophyll content and yield [37], and the nitrogen balance index, which utilises excitation channels in red and green (NBI-G and NBI-R) to monitor total nitrogen levels [38]. The flavonoid index (FLAV), defined as the logarithm of the ratio of red to UV excitation of chlorophyll fluorescence in the RF, is widely used in leaf surveys for flavonoid estimation [39]. Furthermore, the blue-to-red emission ratio index (BRR-FRF) is a comprehensive indicator. Depending on the specific crop analysed, it can identify stress, nutritional deficiencies in field crops, the presence of pathogens, or grape ripening [40].

In the experimental design, the MFA instrument was used to monitor the artichoke vegetative architecture, dividing it into three different layers, as follows: pre-senescent adult leaves (first stage), adult leaves (second stage), and newly formed leaves (third stage), respectively. The MFA analysed each leaf at three different points—the apical, central and basal parts—for a total of nine leaves per plant, at a 10 cm distance between the sample and the sensors. The application of a three-layer analysis was necessary to obtain a homogeneous multitemporal analysis; during the growing period, and until its final stages, all the measurements focused on the same kind of leaves, guaranteeing the uniform analysis of the plant.

The indices used in the MFA monitoring are listed below.

$$\text{SFR} - \text{G} = \frac{\text{FRF}_G}{\text{RF}_G}, \quad (1)$$

$$\text{SFR} - \text{R} = \frac{\text{FRF}_R}{\text{RF}_R}, \quad (2)$$

$$\text{FLAV} = \log \left( \frac{\text{FRF}_R}{\text{FRF}_{UV}} \right), \quad (3)$$

$$\text{BRR} - \text{FRF} = \frac{\text{YF}_{UV}}{\text{FRF}_G}, \quad (4)$$

$$\text{NBI} - \text{G} = \frac{\text{FRF}_{UV}}{\text{RF}_G}, \quad (5)$$

$$\text{NBI} - \text{R} = \frac{\text{FRF}_{UV}}{\text{RF}_R}, \quad (6)$$

### 2.3. UAS Survey

During the 2019–2020 season, MFA monitoring was combined with a UAS survey to observe the different management types through a vegetation index. To avoid environmental variability in remote and proximal sensing data acquisition, the surveys were conducted on the same day. The aerial system involved in the monitoring operations was a commercial UAS Phantom 4 Pro (DJI, Shenzhen, China) with an RGB CMOS 1-inch 20-megapixel

camera, equipped with a multispectral red–green–near-infrared (RGN) Survey 3 (Mapir) sensor mounted on the UAS frame. The Mapir calibration target was positioned on the ground due to reflectance corrections for raw data acquisition. The flights were planned on sunny days, in order to maintain a constant meteorological situation and ensure homogeneous and adequate image acquisition conditions. All the surveys were performed at 40 m above ground level (AGL), with a ground sampling distance (GSD) of 1.096 cm. To acquire a high-resolution orthomosaic reconstruction, the overlap values were 75% and 85% for frontal and side directions, respectively. The RGB camera was necessary to reconstruct and segment the canopy into three dimensions, allowing for the digitalisation of individual artichoke plants. This operation facilitated plant-specific analysis using the structure-from-motion system Agisoft Metashape (St. Petersburg, Russia). Specifically, the RGB orthomosaic, combined with the height values obtained from the digital terrain model (DTM) and the digital surface model (DSM), enabled the identification of individual plants using the canopy height model (CHM) segmentation technique. The reconstructed plants allowed the analysis of vegetative crops through the normalised difference vegetation index (NDVI) derived from the bands collected by the RGN sensor, enabling the assessment of physiological variability between the three management methods adopted. Below is the equation for the extraction of the NDVI index.

$$\text{NDVI} = \frac{\text{NIR}(850 \text{ nm}) - \text{Red}(550 \text{ nm})}{\text{NIR}(850 \text{ nm}) + \text{Red}(550 \text{ nm})}, \quad (7)$$

#### 2.4. Data Processing and Software Elaboration

The raw data from MFA and UAS were pre-processed prior to statistical analysis. Regarding the fluorimetric analysis, the three MFA measurements taken from each leaf were averaged. The result was then averaged across the three leaf layers to yield a single value for each plant, representing its overall trend. The CHM technique for the canopy reconstruction involved subtracting the DTM layer from the DSM layer to isolate the heights of the plants effectively [41]. The digital layer segmentation process was performed on QGIS using the following formula:

$$\text{CHM} = \text{DSM} - \text{DTM} \quad (8)$$

The reconstruction and extraction of individual plants through the CHM model enabled the calculation of the NDVI vegetation index for the examined plants, as the multispectral sensor was externally implemented in the UAS frame, and the NIR RAW data did not contain GNSS positioning information, which required the overlaying of the CHM model.

The preliminary dataset construction and processing allowed the association of the fluorimetric measurements with the corresponding vegetation indices for individual plants. The MFA data were processed using the open-access software R for statistical analysis and reconstruction of the relative graphs. After the preliminary statistical analysis check for normality distribution, the analysis of variance (ANOVA) was used to identify significant differences among the studied groups, and subsequently, the Tukey test [42] was performed to assess the subdivision between the CON, ORG-I, and ORG-II groups. The statistical results were integrated by incorporating the respective graphs for each variable, which illustrated the evolution of indices over the monitoring period.

The indices derived from the MFA were categorised into the following four groups: (a) date, (b) management, (c) checking points, and (d) index. The “date” refers to the measurement days, “management” pertains to the type of cultivation practice applied, and “checking points” indicate the relative positions of the plants to the sample points. The “index” represents the value of interest, averaged across different leaf stages and plants at the various checking points. Among these variables, “management” serves as the key discriminating factor, as it represents the primary source of variation in the fluorimetric indices. Additionally, potential interactions between “management” and “date” (date  $\times$  management), as well as between “management” and “checking points” (management  $\times$  checking points), were explored to assess whether the survey dates or the spatial arrangement of the checking points in the field could contribute to variability in the fluorimetric indices. The NDVI data from UAS followed the same MFA statistical analysis. They were then compared with the MFA indices using a correlation matrix in R through the ggcorrplot and emmeans libraries to observe any correlations between UAS and MFA data.

### **3. Results**

#### **3.1. Fluorimetric Analysis**

The present subsection explores the statistical results of the MFA survey in the 2018/2019 and 2019/2020 research years.

##### **3.1.1. First Survey Year**

The ANOVA analysis for the SFR indices displayed in Table 2 observed a highly significant statistical difference, evidencing a different canopy evolution among the management during the survey period. Table 2 also reports the Tukey test analysis to identify any affinities between ORGs and CON based on the chlorophyll detection indices. The comparison

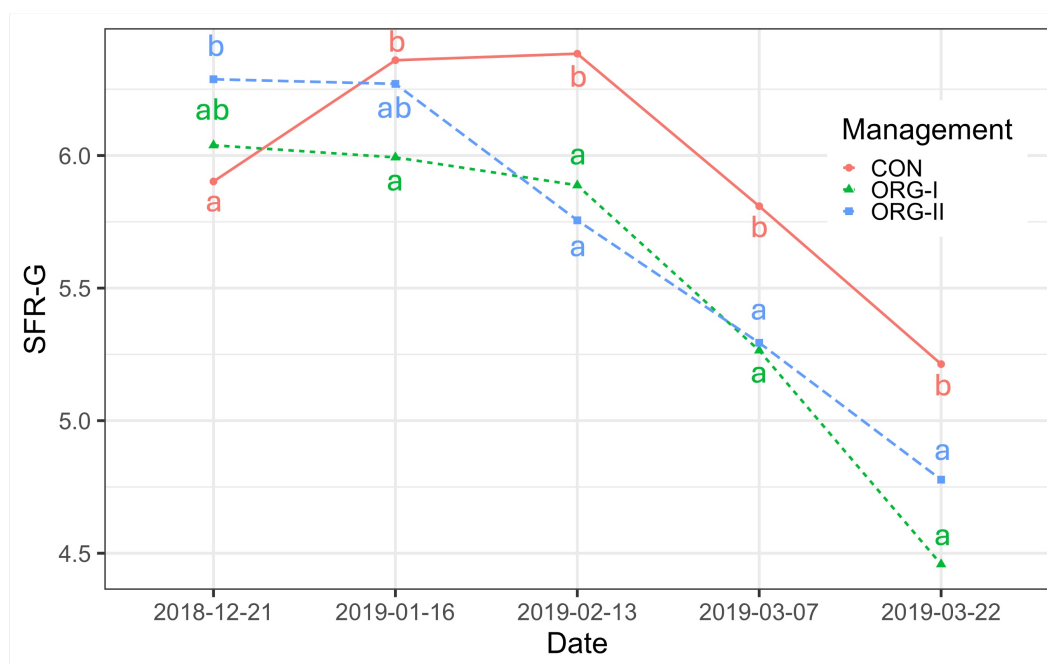
between ORG-I and ORG-II did not indicate a significant difference, but a difference emerged when they were compared with CON management.

**Table 2.** ANOVA and Tukey test results on SFR-G and SFR-R.

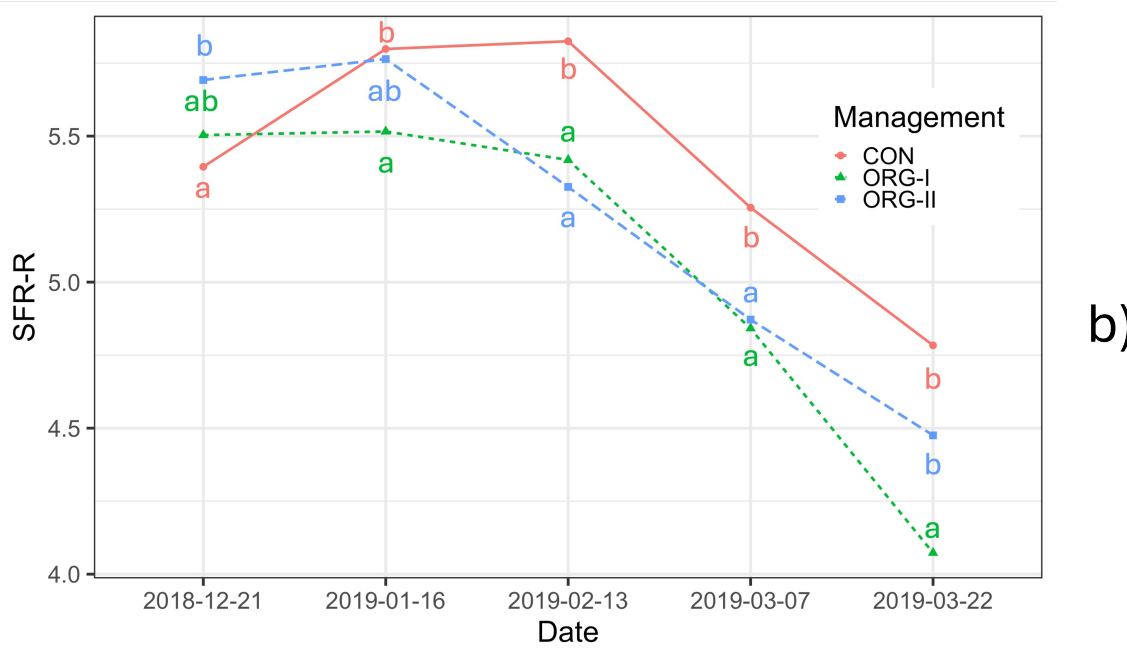
Index	SFR-G		SFR-R	
	F-value	p-value	F-value	p-value
Management	23.609	$1.69 \times 10^{-10}$ ***	22.401	$5.09 \times 10^{-10}$ ***
Date $\times$ Management	5.314	$2.12 \times 10^{-6}$ ***	5.337	$1.97 \times 10^{-6}$ ***
Tukey Test	p-value		p-value	
CON-ORG-I	$<1.0 \times 10^{-16}$ ***		$<1.0 \times 10^{-10}$ ***	
CON-ORG-II	$3.0 \times 10^{-4}$ ***		0.030 **	
ORG-I-ORG-II	0.205 ns		0.080 ns	

The level of significance (*p*-value) is shown in the table, where \* indicates  $p < 0.05$ , \*\* indicates  $p < 0.01$ , and \*\*\* indicates  $p < 0.001$ . ns represents no significant difference.

The results from the three management types were plotted in a line graph to observe the seasonal trend across the survey days. The SFR-G and SFR-R graphs in Figure 4 show that, for the CON management, photosynthetic activity was initially slightly lower than in the ORG-I and ORG-II theses, but rose to higher values starting in January 2019. The ORG management types, however, did not provide a statistical discrimination between them, but the graphs in Figure 4 highlight the SFR index decrease for ORG-II management, which is 23% lower than the other plots.



a)



**Figure 4.** Evolution of SFR indices through the first survey year. On the x-axis are the survey dates, and on the y-axis are the index values. The two graphs show the SFR-G index (a) and the SFR-R (b). The letters in the graphs indicate the differences between the various management systems identified in the Tukey test.

The BRR-FRF index did not provide a statistical discrimination between the treatments during the first survey year, as observed in the ANOVA analysis and Tukey test results. Different from the stress index, the FLAV index showed a significant difference between the ORG-I and ORG-II treatments. The index increased steadily over the days of monitoring, increasing tenfold on the last day. Table 3 shows the results of the ANOVA and the Tukey analysis.

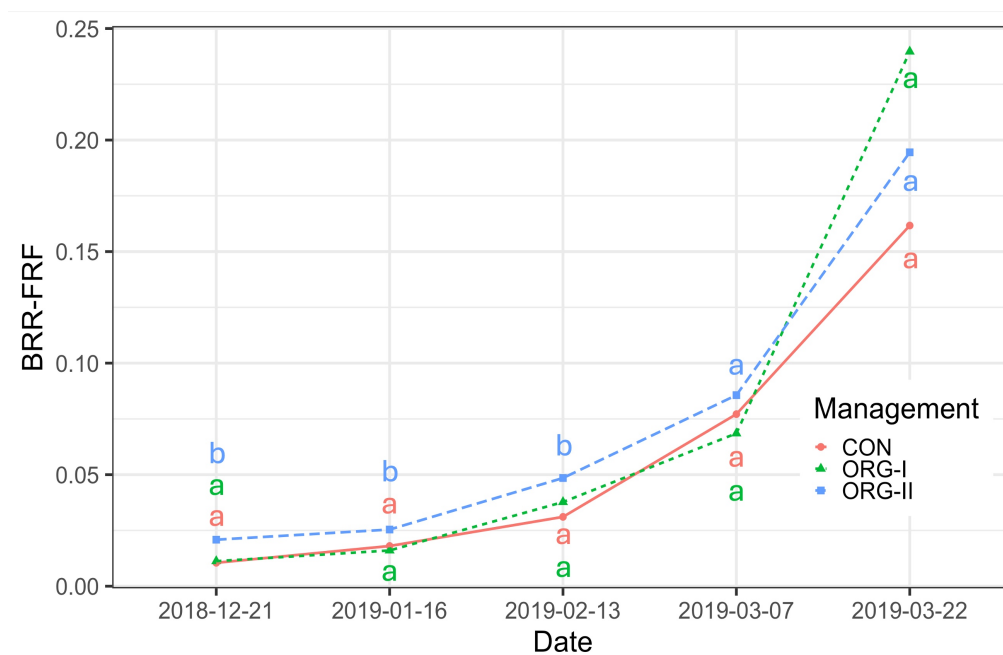
**Table 3.** ANOVA results on BRR-FRF and FLAV.

Index	BRR-FRF		FLAV	
	F-value	p-value	F-value	p-value
Management	1.165	0.313 ns	2.945	0.054 ns
Date × Management	1.140	0.335 ns	1.546	0.139 ns
<b>Tukey Test</b>		<b>p-value</b>		<b>p-value</b>
CON-ORG-I		0.449 ns		0.770 ns
CON-ORG-II		0.371 ns		0.156 ns
ORG-I-ORG-II		0.947 ns		0.044 *

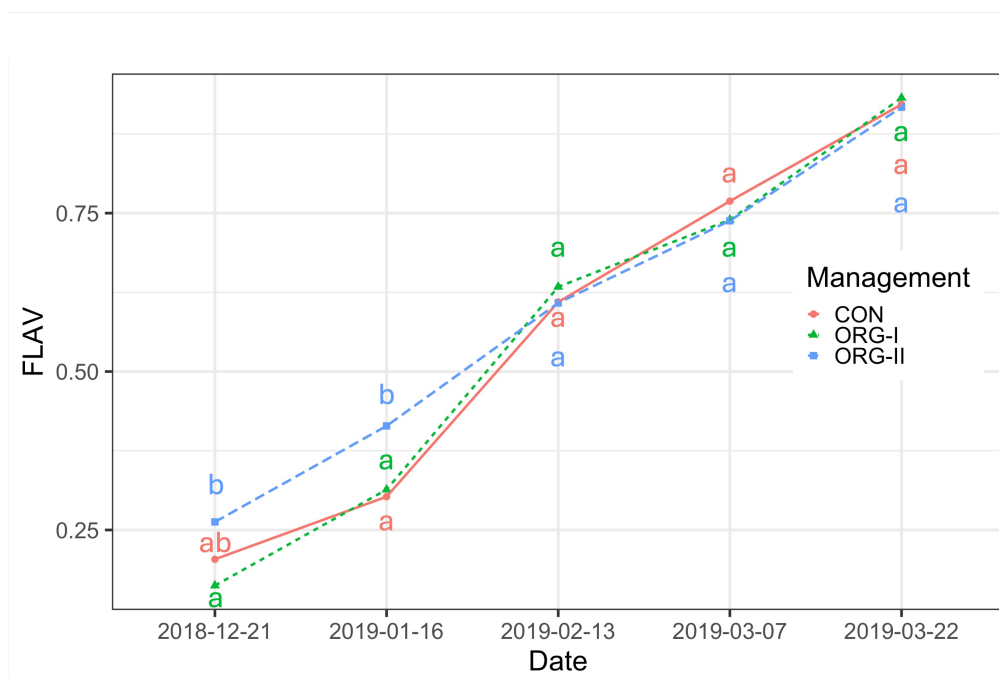
The level of significance (*p*-value) is shown in the table, where \* indicates  $p < 0.05$ . ns represents no significance.

The graphical reconstruction of the stress and flavonol indices during the survey confirms the low level of significance among the management types due to the overlapping of the obtained values. In particular, in the graph in Figure 5a, the trends of the three management

types run side by side during all the sampling dates, separating only at the end of the cycle (22 March 2019). In this survey year, the ORG-I and ORG-II treatments show a much higher stress level than the CON management, especially in the last monitoring survey, despite the FLAV index in Figure 5b, which indicates an increasing and constant trend in all three management types. The ORG-II plot, despite the lack of a significant response, showed higher values than the other management types for most of the time (+11%), a level that the ORG-I management type reached in the last survey.



a)



b)

**Figure 5.** BRR-FRF (a) and FLAV (b) indices, indicating the evolution of stress and flavonol indices during the season. The letters in the graphs indicate the differences between the various management systems identified in the Tukey test.

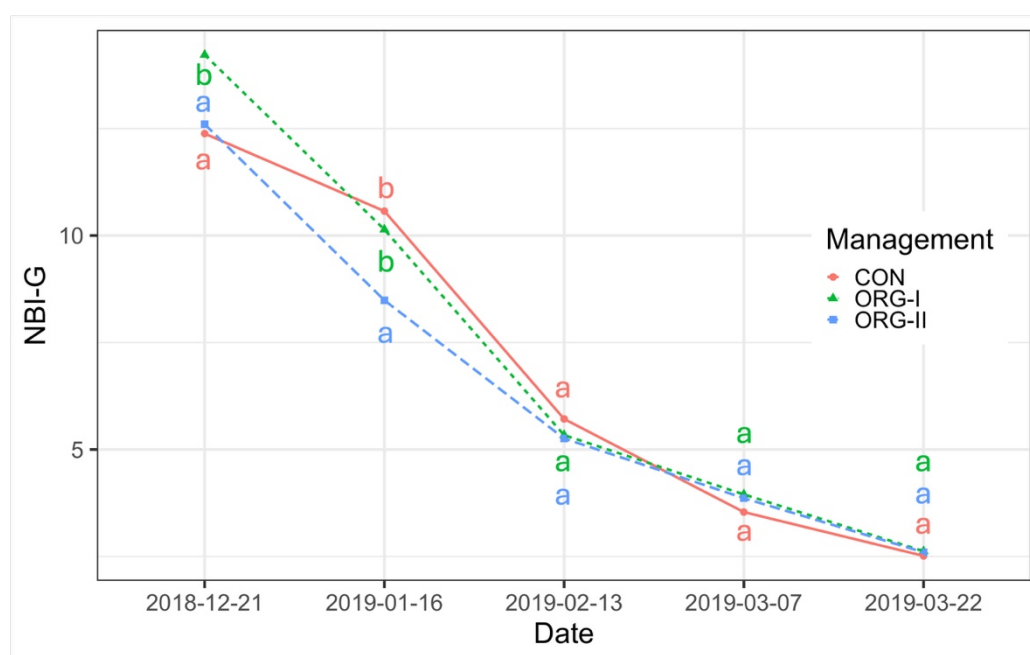
The final aspect considered is the total nitrogen content, observed using the NBI-G and NBI-R indices. The statistical analysis, as with the previous ones, identified fluctuations in the nitrogen fluorimetric indices among the survey dates. Unlike the previous indices, especially those focusing on chlorophyll activity, the Tukey test revealed a significant difference between the ORG-I and ORG-II treatments, while showing no particular differences with the CON thesis. Table 4 shows the ANOVA and Tukey test statistical results.

**Table 4.** ANOVA results on NBI-G and NBI-R.

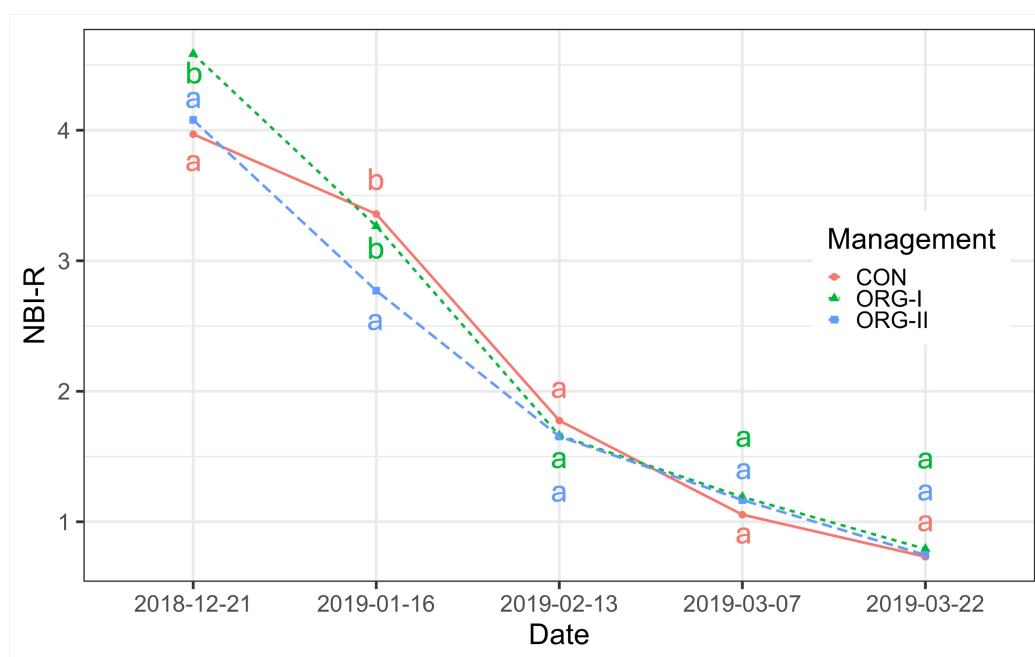
Index	NBI-G		NBI-R	
	F-value	p-value	F-value	p-value
Management	6.468	0.002 **	5.662	0.004 **
Date × Management	3.460	0.001 ***	2.786	0.005 **
<b>Tukey Test</b>	<b>p-value</b>		<b>p-value</b>	
CON-ORG-I	0.132 ns		0.089 ns	
CON-ORG-II	0.118 ns		0.277 ns	
ORG-I-ORG-II	0.001 ***		0.003 *	

The level of significance (*p*-value) is shown in the table, where \*\* indicates  $p < 0.01$ , and \*\*\* indicates  $p < 0.001$ . ns represents no significance.

The fluorimetric data in Figure 6 indicate that the nitrogen indices show a constant inflexion throughout the survey dates, with continuous overlapping of values between the three management types, consolidating what was identified by the statistical analyses. These graphs, despite the low test significance, reflect what is indicated by the SFR, BRR-FRF, and FLAV indices. For each NBI index, the decrease fell to more than 80% during the first monitoring year, according to the other fluorimetric indices observed above.



a)



b)

**Figure 6.** NBI-G (a) and NBI-R (b) on leaves during the first survey year. The letters in the graphs indicate the differences between the various management systems identified in the Tukey test.

### 3.1.2. Second Survey Year

The second monitoring year is characterised by different evolutionary dynamics compared to the previous one. Regarding the chlorophyll indices SFR-G and SFR-R, the ANOVA analysis in Table 5 reports a significant difference among the three management systems, highlighting statistical variability across the survey dates. According to the Tukey test, the primary differences are observed between the CON and ORG-I management systems due to the high significance values and, for the SFR-R index, between the CON and ORG-II management systems. For both fluorimetric indices, no significant differences are reported between ORG-I and ORG-II.

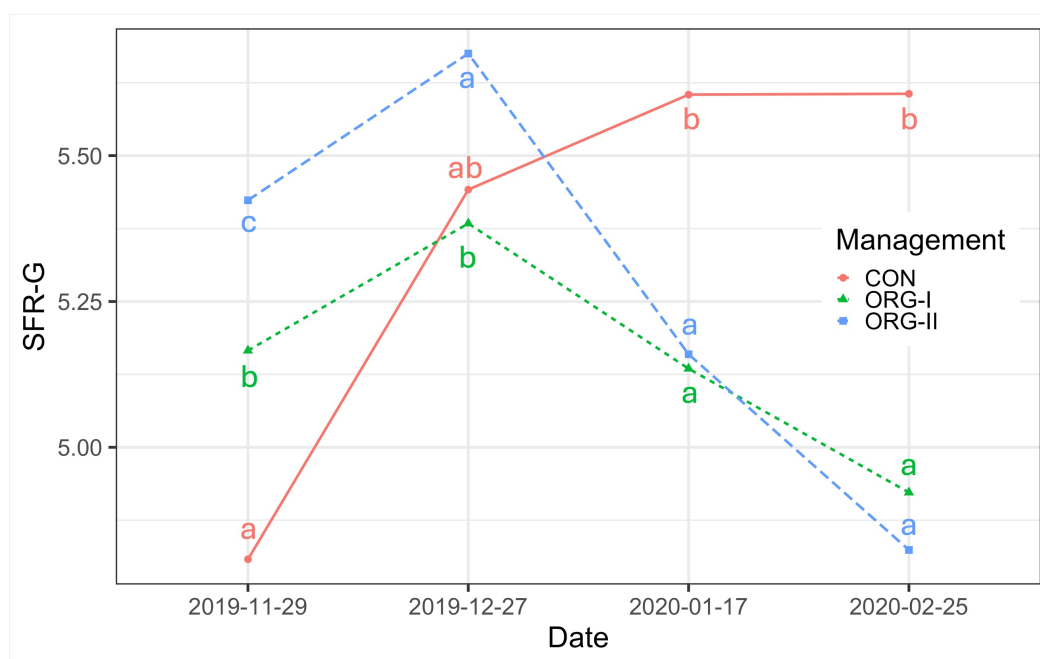
**Table 5.** ANOVA results on SFR-G and SFR-R.

Index	SFR-G		SFR-R	
	F-value	p-value	F-value	p-value
Management	7.388	<0.001 ***	7.622	<0.001 ***
Date × Management	17.743	$2.000 \times 10^{-16}$ ***	17.798	$2.000 \times 10^{-16}$ ***
<b>Tukey Test</b>	<b>p-value</b>		<b>p-value</b>	
CON-ORG-I	< 0.001 ***		< 0.001 ***	
CON-ORG-II	0.342 ns		0.039 *	
ORG-I-ORG-II	0.133 ns		0.677 ns	

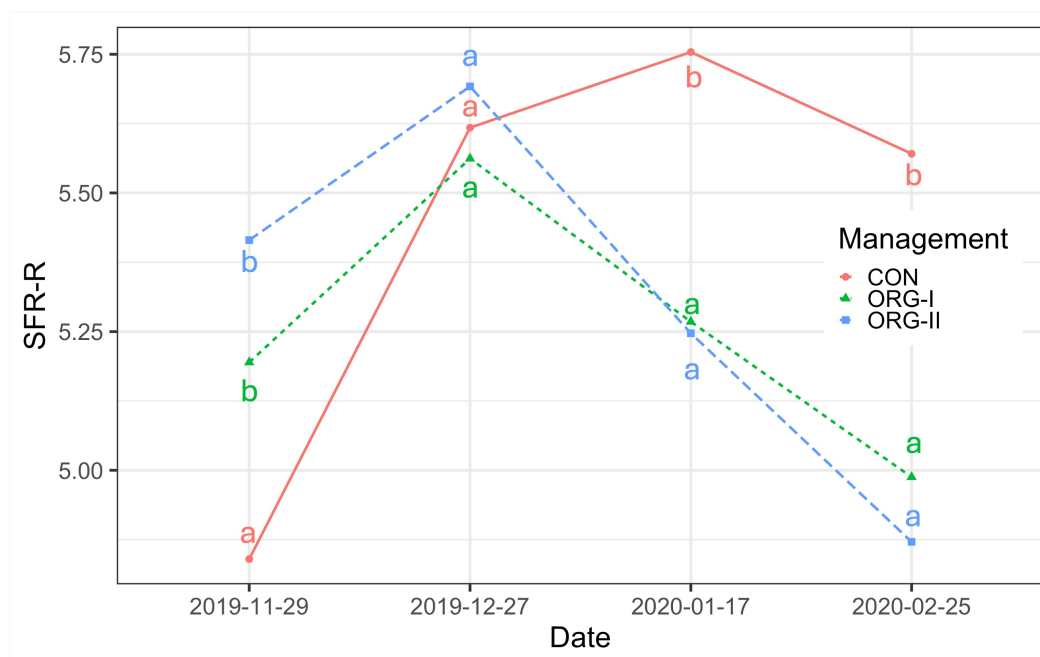
The level of significance (*p*-value) is shown in the table, where \* indicates  $p < 0.05$ , and \*\*\* indicates  $p < 0.001$ . ns represents no significance.

The graph construction of the SFR indices in Figure 7 shows the evolution of the three theses during the multi-temporal analysis. The ORG management types display high chlorophyll

values in the early stages of the cycle, peaking in December and then undergoing a slow but continuous decrease until the end of the plant's physiological cycle. The CON thesis, on the other hand, shows the opposite trend to the ORG theses, starting with values consistently lower than those of the ORG types in the early growth stages, then experiencing a rapid escalation (+16.6% and 18.9% for SFR-G and SFR-R, respectively) until it surpasses both organic theses in January (+8.9% and 9.4% for SFR-G and SFR-R), remaining steady until the end of the cycle.



a)



b)

**Figure 7.** The SFR-G (a) and SFR-R (b) index trends during the 2019–2020 survey. The letters in the graphs indicate the differences between the various management systems identified in the Tukey test.

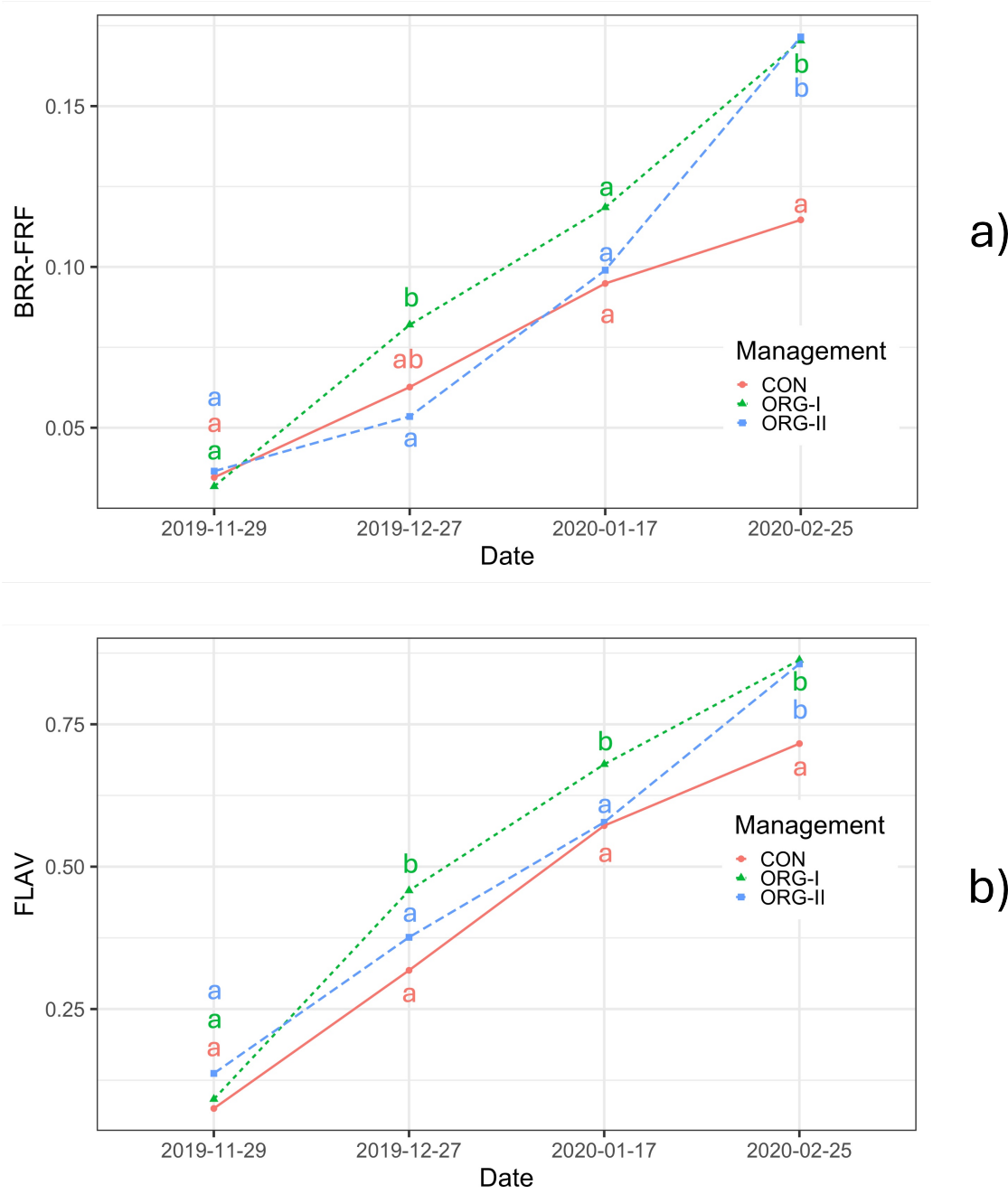
The spatial–temporal variability of the BRR-FRF-2 and FLAV-2 indices is presented in Table 6, revealing significant differences among the management systems, in contrast to the results from the previous year. ANOVA analysis of the management and date variables indicated a significant separation among the management systems, unlike the 2018/2019 season. All pairwise comparisons performed using the Tukey test showed significant differences between the CON and ORG management systems, while no statistical significance was found between ORG-I and ORG-II. Table 6 summarises the analysis of BRR-FRF and FLAV indices.

**Table 6.** ANOVA results on BRR-FRF and FLAV.

Index	BRR-FRF		FLAV	
	F-value	p-value	F-value	p-value
Management	15.676	$3.12 \times 10^{-7}$ ***	32.590	$1.19 \times 10^{-13}$ ***
Date × Management	6.148	$3.96 \times 10^{-16}$ ***	4.150	$4.89 \times 10^{-4}$ ***
<b>Tukey Test</b>	<b>p-value</b>		<b>p-value</b>	
CON-ORG-I	< 0.001 ***		< 0.001 ***	
CON-ORG-II	0.006 **		< 0.001 ***	
ORG-I-ORG-II	0.192 ns		0.207 ns	

The level of significance (*p*-value) is shown in the table, where , \*\* indicates  $p < 0.01$ , and \*\*\* indicates  $p < 0.001$ . ns represents no significance.

The graphical results of the BRR-FRF and FLAV indices provide a valuable overview of the temporal evolution of the three management systems. The BRR-FRF index (Figure 8a) shows that the values of the three plots are similar during the early growth stage but begin to diverge from late December until the end of the monitoring period, with the ORG systems displaying significantly higher stress levels compared to the CON management (+49.3%). Similarly, the FLAV index (Figure 8b) exhibits the same trend, indicating that ORGs show higher values than the CON management. Throughout the production cycle, the CON system consistently maintains lower levels of stress and flavonoids than the ORG systems (−28.3%). These findings align with the SFR indices, where the lower stress and flavonol values observed in the CON management complement the higher chlorophyll content.



**Figure 8.** The stress index BRR-FRF (a) and the flavonol index FLAV (b) during the second survey year. The letters in the graphs indicate the differences between the various management systems identified in the Tukey test.

According to the previous indices, the statistical analysis of the NBI-G and NBI-R MFA indices demonstrated significant differentiation among the management systems. The ANOVA analysis revealed high variability in both the management and date variables, as indicated by the significance values. The Tukey test confirmed a distinction between the CON and ORG systems, similar to the BRR-FRF and FLAV indices, with the CON system being statistically different from the ORG system. However, no significant differences were observed between ORG-I and ORG-II. The ANOVA and Tukey statistical results are reported in Table 7.

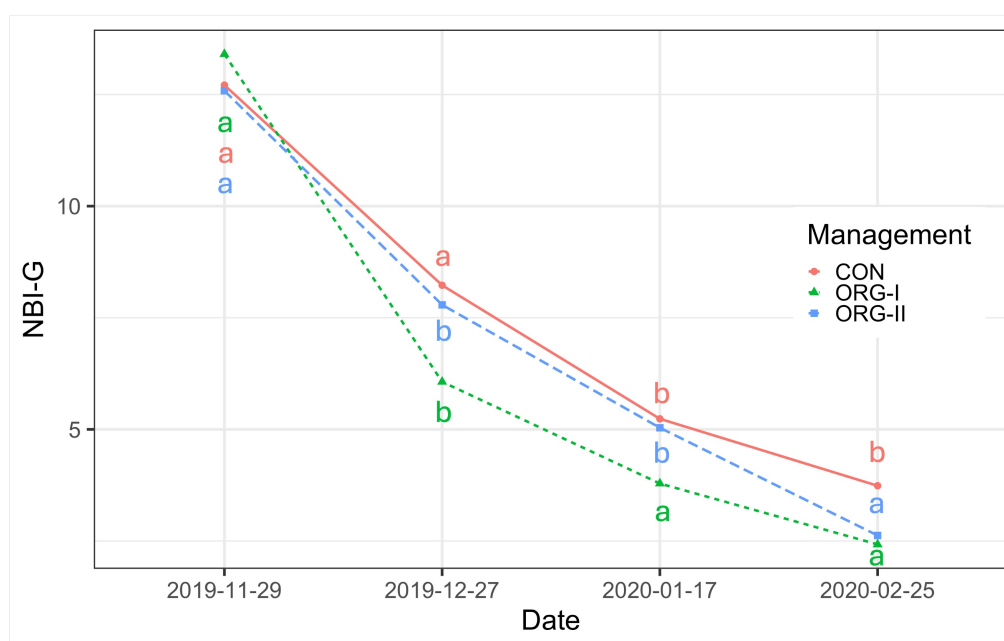
**Table 7.** ANOVA results on NBI-G and NBI-R.

Index	NBI-G		NBI-R	
	F-value	p-value	F-value	p-value
Management	15.02	$5.69 \times 10^{-7}$ ***	15.119	$5.19 \times 10^{-7}$ ***
Date $\times$ Management	5.42	$2.31 \times 10^{-5}$ ***	5.667	$1.27 \times 10^{-5}$ ***
<b>Tukey Test</b>	<b>p-value</b>		<b>p-value</b>	
CON-ORG-I	< 0.001 ***		< 0.001 ***	
CON-ORG-II	0.017 *		0.007 **	
ORG-I-ORG-II	0.115 ns		0.214 ns	

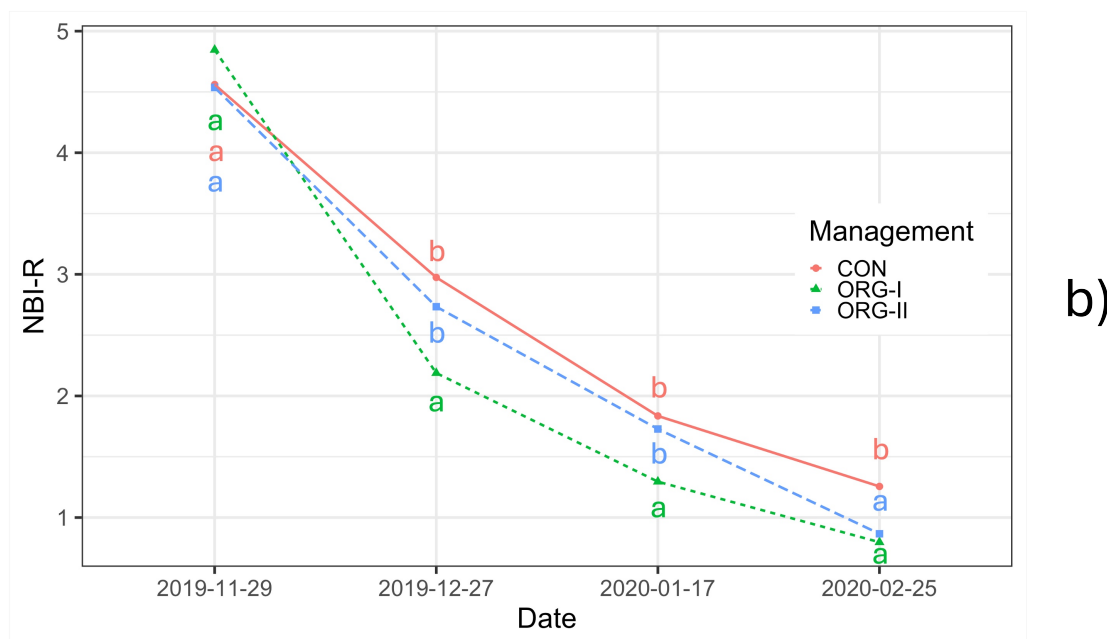
The level of significance (*p*-value) is shown in the table, where \* indicates  $p < 0.05$ , \*\* indicates  $p < 0.01$ , and \*\*\* indicates  $p < 0.001$ . ns represents no significance.

The NBI-G and NBI-R graphs in Figure 9 show a general decline in nitrogen content, which gradually decreases throughout the growth stages, averaging a 77% reduction across the management systems. As indicated by the Tukey test, although ORG-I and ORG-II exhibit relatively similar MFA values, both differ significantly from the CON system, consistent with previous observations from other indices. Notably, on the first monitoring day, the CON system displays lower NBI values compared to ORG-I but rises to higher values on all subsequent survey dates. Figure 9 resumes the MFA management evolution during the surveys.

This observation reflects what was highlighted in the SFR, BRR-FRF, and FLAV indices, where the CON thesis consistently maintains higher levels during the intermediate and final phases of the artichoke's biological cycle, suggesting a higher nitrogen content than that of the ORG types.



a)



**Figure 9.** Trends of the NBI-G (a) and NBI-R (b) indices during the second survey year. The letters in the graphs indicate the differences between the various management systems identified in the Tukey test.

### 3.2. UAS Survey

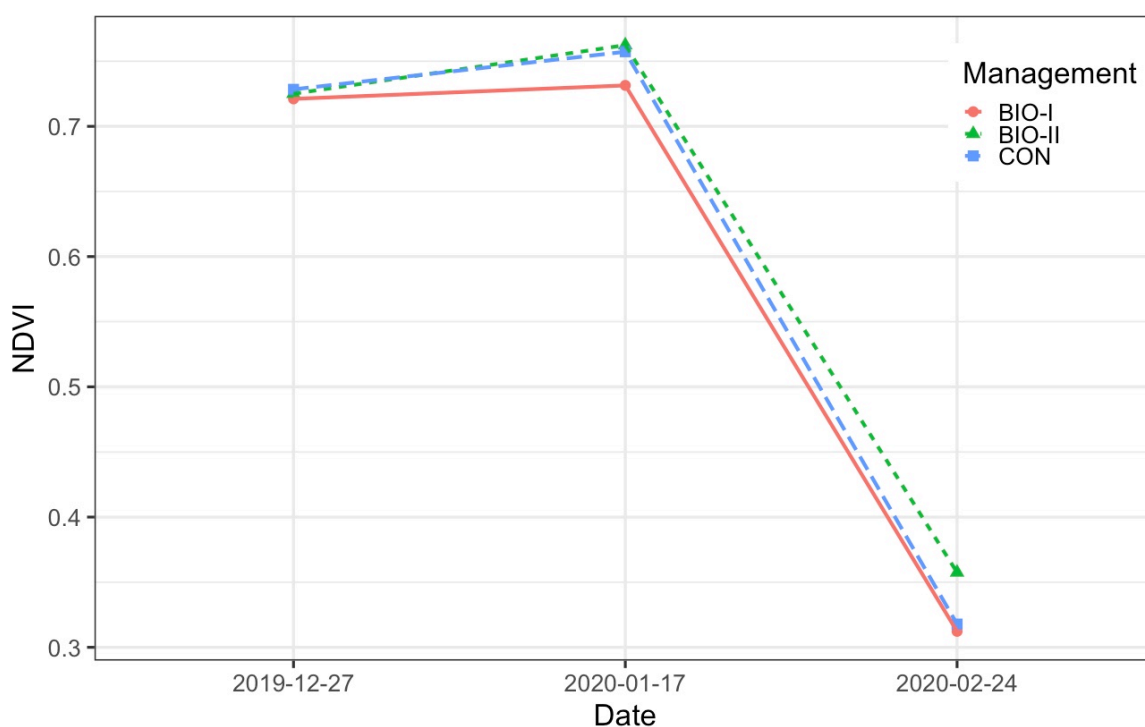
The remote analysis performed by the UAS allowed the acquisition of vegetative vigour using the NDVI vegetation index. The plant segmentation performed in QGIS with the CHM technique enabled analysis exclusively on the plants, without the soil and the weeds within the field. The multi-temporal survey was conducted on the last three dates of the second year of monitoring, during the period of maximum vegetative–reproductive activity. The ANOVA analysis performed on the three management types did not observe a comparable trend to the fluorometric indices, as it did not discriminate particular differences between the plots. As observable in Table 8, no statistical difference was found between the treatments during the season, while instead, the ANOVA analysis evidenced a variability among the checking points (management×checking points), suggesting an internal heterogeneity on the three plots. Further analysis of singular surveys found that only on the last date (2020-02-24) was there significant variability between plots, with the ORG-II management differentiating itself from the others (+13.5%).

**Table 8.** ANOVA analysis on the NDVI index, where  $p$ -value  $< 0.05$ . The high  $p$ -value for the management variable suggests that the UAS did not evidence particular differences between the treatments, but rather indicated significant differences within the plots.

Index	NDVI	
	F-value	p-value
Management	5.952	0.002 ***
Date × Management	1.954	0.116 ns
Tukey Test		p-value
CON-ORG-I		0.835 ns
CON-ORG-II		0.676 ns
ORG-I-ORG-II		0.985 ns

The level of significance ( $p$ -value) is shown in the table, where \*\* indicates  $p < 0.01$ , and ns represents no significance.

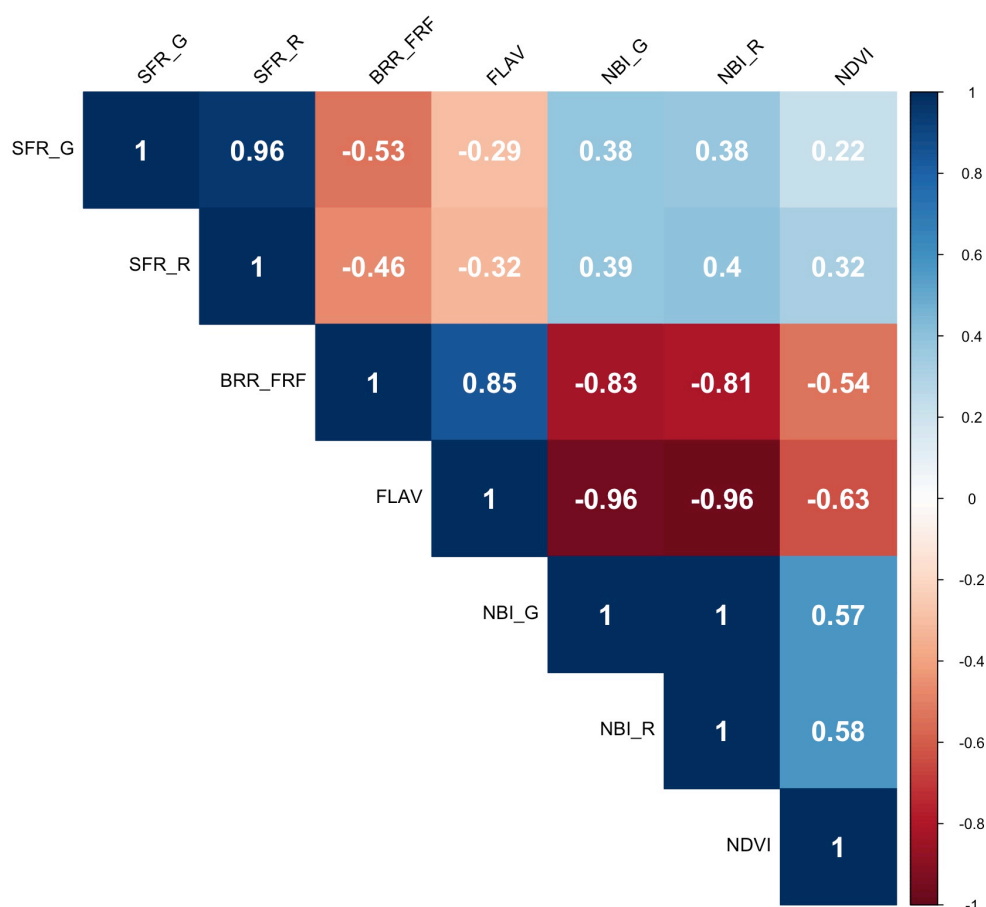
The NDVI data development is summarised in Figure 10, comparing the three plots during the survey period. The data graph confirms what was observed in statistical analysis, where the CON and ORGs NDVI values follow a uniform development.



**Figure 10.** NDVI indices performance from the UAS survey. The field evolution across the three management plots did not identify any differences between treatments, pointing to a uniform tendency.

### 3.3. Correlation Matrix

Lastly, a correlation matrix was created between the NDVI and the MFA-generated index, as shown in Figure 11. The results show that the NDVI, SFR, and NBI indices perform unidirectionally, indicating a direct proportional correlation among them. As observed in the graphs in Figures 5 and 8, the BRR-FRF stress index and FLAV show an inverse correlation with the other indices. Specifically, high correlation values were observed between the NBI indices and the FLAV and BRR-FRF indices, suggesting that total leaf nitrogen content is closely related to stress and vegetative decay, with this result being associated with the more moderate relationship detected with the SFR data.



**Figure 11.** Matrix correlation of all variables considered in the experiment, converging MFA and UAS indices. The palette legend illustrates the proportional correlation (blue) to inverse correlation (red) between the indices. The white numbers in the matrix represent the correlation coefficients.

## 4. Discussion

The fluorimetric analysis conducted with the MFA in the three management areas provided a similar overview during the two seasons. Statistical analysis of the chlorophyll indices (SFR) showed a different evolution between the ORG and CON theses. The survey performed with the MFA revealed that the traditional CON management starts with lower SFR values. It reverses the trend toward December and maintains a higher level of

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photosynthetic activity until the senescence phase compared with the ORG management. This phenomenon can be explained by the effect of the agronomic strategy that was applied. The CON field relied on chemical fertilisation multiple times throughout the year, ensuring a continuous nutrient uptake. In contrast, the ORG-I and ORG-II plots only received fresh residues for nutritional supplements, as expected under organic conduction. In line with this assumption, leaf nitrogen analysis using the NBI indices led to similar conclusions, suggesting different developments between organic and conventional management. In fact, during the mid-periods of the two-year study, the nitrogen indices in the CON treatment were continuously higher than in the ORGs. This phenomenon suggests that the effect of fertilisation in the CON treatment extended the phenological phases, showing higher photosynthetic activity and total nitrogen content than in the ORGs. The NBI data did not identify a substantial and stable difference between the theses during the two monitoring years. This result may be explained by the plant's ability to manage nitrogen, as the uniform course of the three theses suggests that crop management did not affect leaf nitrogen uptake and management. It is important to note that the ORG-I and ORG-II management types can be compared with the CON during the first and second years of monitoring, respectively. This similarity is particularly evident during the winter period (December–January), i.e., at the crucial time of harvesting. It could suggest variable nitrogen management in the two ORG theses, depending on soil conditions and the availability of productive soil inputs, particularly due to the drier weather trend in the 2019–2020 season. This result was also observed for stress and flavonoid indices, which continued regularly in the three management types throughout the monitoring period, except peaking at higher values for the two organic management types than the CON one on the last monitoring day in 2020. The BRR-FRF stress index trend significantly reflected the decline of the artichoke cycle, particularly in the ORG management, showing higher values in the cycle ending, while the chlorophyll and nitrogen indices showed decreasing values. The lower BRR-FRF and FLAV values in the CON thesis over the two seasons suggest that CON plants managed to extend their vegetative cycle, compared to the organic theses.

The MFA sensor and associated indices proved to be efficient tools for monitoring the physiological status of artichoke plants, revealing statistically significant differences among the three crop management systems. The non-destructive methods agree with existing literature, confirming that the assessment of physiological variables in fruits and vegetables is highly correlated with conventional destructive analysis methods [43]. This finding underscores the potential of non-destructive sensors in horticulture, enabling timely and targeted agronomic interventions through spatial and temporal management.

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According to Kim et al. (2022) [44], integrating proximal and remote sensing techniques, such as using a fluorometer in combination with a UAS-mounted NIR sensor, can be a valuable approach for crop monitoring and management, provided proper calibration within the experimental design. However, in this experiment, the UAS-based aerial analysis did not reveal significant differences between the plots. This suggests a lower sensitivity of the UAS in detecting variations among the different management types, making its performance less comparable to that of the MFA. A first consideration is that the application of the UAS was conducted only in the second year of monitoring, reducing the possibility of detecting a significant difference between the three management types throughout the two-year experiment. As shown in Figure 9, NDVI values remained similar between treatments, following the same trend until the end of the analysis. Additionally, remote sensing measurements from UAS captured the entire plant, unlike the MFA survey method, which focused exclusively on leaves. By including all leaves and flower heads, UAS altered the NDVI values of representative leaves in monitored plants [45]. This highlights the broader function of the NDVI index, which serves as a general measure of vegetative vigour and should be considered differently depending on the research conducted. Another consideration for UAS monitoring is based on the multitemporal survey during the artichoke growth cycle, as the NDVI data were collected when the artichoke had already reached the vegetative and reproductive growth peak. Since important differences were observed in the early stages, one or more overflights in the early stages of the cycle would have helped to reinforce the UAS observations, possibly in support of additional vegetation indices to obtain a more complete view of the progress of the three treatments. Furthermore, the UAS was only used for a few months during the two-year experiment, so a more complete trend could not be followed as in the case of the MFA. Over the temporal limitation, another aspect has to be considered. The experiment, therefore, was conducted at a single site, where the pedological and climatic conditions play an important role in physiological development and the respective fluorimetric response.

Overall, the MFA system was able to distinguish the CON thesis from the ORGs during the survey period. However, proximal monitoring does not offer significant advantages in speed and convenience, as it requires the operator to spend several hours performing the monitoring.

Additionally, to ensure reliable results, more than a thousand singular measurements with the MFA are provided to achieve a sufficient sample size. Furthermore, the MFA's interaction window should always be occupied by the sample to avoid interference from external elements, such as impurities, weeds, etc., which could lead to inaccurate readings

of the physiological parameters. Due to these operational requirements, the MFA analysis can be employed as a high-performance sensor for conducting site-specific field investigations, following an initial crop assessment using an alternative wide-range system analysis.

## 5. Conclusions

The MFA showed a significant characterisation of plant physiology between the three crop management types analysed. The parameters of chlorophyll, stress, flavonoids, and, to a limited extent, nitrogen were adequate to observe the progression of the various theses throughout the entire crop season. The statistical analysis showed that the fluorimetric indices systematically correlated with the chlorophyll content over the two years between the ORG-I and ORG-II theses, suggesting a homogeneous trend, as indicated by the low significance value of the Tukey test. The different performance of the NDVI compared to the fluorimetric indices suggests that two high-performance instruments, such as the UAS and the MFA, do not always provide comparable results, as has been observed in other research contexts [46], as they perceive the vegetative characteristics of the crop differently. Consequently, the UAS cannot replace proximal sensing monitoring techniques for evaluating physiological differences in artichoke management. Future research activities will focus on characterising the fluorimetric indices provided by the MFA with the quantitative values of chlorophyll, nitrogen, and flavonoids to construct the respective agronomic calibration curves by correlating the indices obtained from the MFA with their equivalent quantitative values. This correlation, associated with crop control and the management of production inputs, could provide a valuable monitoring and decision-support tool for field operators to better control the main physiological characteristics of the artichoke, ultimately enhancing precision in cultivation management and input optimisation. The integration of advanced technologies, such as multi-sensor systems combining thermal imaging, hyperspectral data, and LiDAR, can capture a more comprehensive physiological and structural profile of the crop. These approaches could contribute to the development of more precise, repeatable, and immediate decision-support tools for the sustainable management of artichoke cultivation. Future research activities on artichoke management will also consider different fields and agroclimatic conditions to test and validate proximal and remote sensing applications through a longer multitemporal analysis, examining the responses from the two approaches during all the artichoke stages.

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**Data Availability Statement:** The data that support the findings of this study are available from the corresponding author upon reasonable request.

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## Abbreviations

The following abbreviations are used in this manuscript:

AGL	above ground level ANOVA analysis of variance
BBCH	Biologische Bundesanstalt, Bundessortenamt, and CHemical industry
BRR-FRF	blue-green to far-red fluorescence ratio
CHM	canopy height model
CON	conventional management
DOY	day of year
DSS	decision support system
FLAV	flavonol
MFA	multiplex force-A
NBI-G	nitrogen balance index on green excitation channel
NBI-R	nitrogen balance index on red excitation channel
NDVI	normalised difference vegetation index
ORG-I	organic annual management
ORG-II	organic biennial management
PA	precision agriculture

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PPP	plant protection product
SFR-G	sample fluorescence emission ratio on green excitation channel
SFR-R	sample fluorescence emission ratio on red excitation channel
UAS	unmanned aerial system

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## Chapter 3. Multisensor analysis for biostimulants effect detection in sustainable viticulture

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**Abstract**

Biostimulants are organic agents employed for crop yield enhancement, quality improvement, and environmental stress mitigation, reducing, at the same time, reliance on inorganic inputs. With advancements in sustainable agriculture, data acquisition technologies have become crucial for monitoring the effects of such inputs. This study evaluates the impact of four increasing rates of Biopromoter biostimulant application on grapevines: 0, 100 g plant<sup>-1</sup>, 100 g plant<sup>-1</sup> with additional foliar fertilizers, and 150 g plant<sup>-1</sup> with additional foliar fertilizers. The biostimulant was applied via foliar or ground methods, and its effects were assessed using vegetation indices derived from Unmanned Aerial Systems (UAS), as well as proximal and manual sensing tools, alongside qualitative and quantitative production metrics. The research was conducted over two seasons in a Malvasia Bianca vineyard in Sardinia, Italy. Results indicated that UAS-derived vegetation indices, consistent with traditional ground-based measurements, effectively monitored vegetative growth over time but revealed no significant differences between treatments, suggesting either a lack of vegetative indices sensitivity or that the applied biostimulant rates were insufficient to elicit a measurable response in the cultivar. Among the tools employed, only the SPAD 502 meter demonstrated the sensitivity required to detect treatment differences, primarily reflected in grape production outcomes, especially in the second year and in the two theses managed with the highest amounts of biostimulant distributed by foliar and soil application. The use of biostimulants promoted, although only in the second year, greener canopy and higher productivity in treatments where it was delivered to the soil. Further agronomic experiments are required to improve knowledge about biostimulants composition and mode of action, essential to increase their effectiveness against specific abiotic stresses. Future research will focus on validating these technologies for precision viticulture, particularly on long-term benefits.

**Keywords:** Proximal Sensing; Remote Sensing; Unmanned Aerial System; UAV; Precision Viticulture

## 1. Introduction

The agricultural sector is increasingly challenged by climate change, which has caused significant shifts in weather patterns, including rising temperatures and unpredictable rainfall. These changes have necessitated the adoption of sustainable farming practices that prioritize environmental compliance while maintaining crop productivity and quality [1].

In viticulture, climate change manifests through earlier harvest times, reduced yields, and alterations in grape composition, all of which adversely affect the quality of wine [2]. Key abiotic stresses, such as excessive sunlight, water deficits, and high temperatures during the growing season, can severely impact vine health and grape quality, posing a considerable challenge to sustainable viticulture, particularly in Mediterranean area [3]. In these regions, including Italy, vineyards are often cultivated without irrigation support, relying solely on natural rainfall. This reliance makes them highly vulnerable to drought, which can lead to significant reductions in yield and deterioration of grape quality traits, thereby affecting vinification processes and the sensory attributes of the resulting wine [4,5].

Conventional farming practices, particularly the extensive use of chemical fertilizers, are commonly employed to maintain crop yields and economic viability. However, these practices have profound environmental consequences, including soil contamination and reduced fertility, water pollution, and eutrophication [6]. The persistent application of fertilizers and agrochemicals in vineyards often leads to the accumulation of organic pollutants and heavy metals, further degrading soil quality and posing serious environmental and toxicological risks [7,8]. This degradation, coupled with erosion and sustained tillage, exacerbates the challenges associated with maintaining soil health in viticultural systems. Effective management of these issues necessitates the availability of timely, precise, and comprehensive data regarding vineyard conditions and stress factors, underscoring the importance of integrating advanced monitoring technologies in viticulture [9]. Biostimulants have emerged as a promising alternative to conventional agrochemicals, aiming to enhance plant growth and resilience while minimizing environmental impacts.

These substances, applied to soil or foliage, stimulate natural plant processes independent of their nutrient content, thereby improving nutrient use efficiency, enhancing resistance to biotic and abiotic stresses, and promoting overall crop quality [10–14]. The European Union has recognized biostimulants as fertilizing products, reflecting their potential to support sustainable agricultural practices. They can be broadly classified into categories such as humic substances, seaweed extracts, complex organic materials, amino acids, antitranspirants, beneficial elements, inorganic salts, and chitosan derivatives [15]. Among these, seaweed extracts and humic acids have demonstrated significant benefits in

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viticulture. Seaweed extracts, applied as foliar treatments, have been shown to promote root growth, enhance nutrient uptake, and improve vegetative growth, contributing to increased vine vigor and grape quality [16]. Humic acids, on the other hand, enhance chlorophyll content, protein levels, and macro nutrient absorption in leaves, leading to improved plant efficiency and productivity, and rising anthocyanin content in the juice [17]. Fulvic acids, another category of humic substances, positively influence several growth parameters, including bud burst, vegetative development, leaf area expansion, and grape composition, while maintaining acidity levels that are crucial for wine quality.

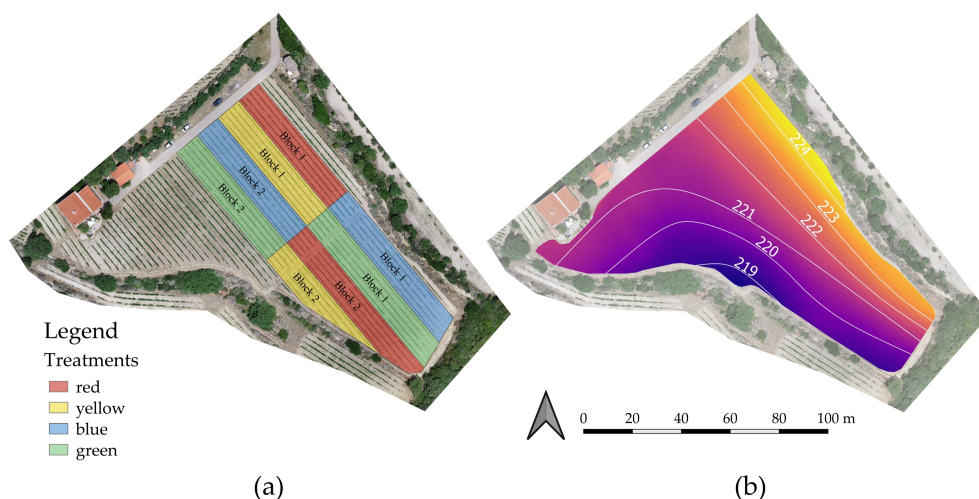
Despite the growing interest in biostimulants within the agricultural industry (specifically on multi-compound products), scientific research has lagged behind commercial developments, partly due to the complexity of understanding their modes of action and the multifaceted nature of their effects on plant physiology. There is a pressing need for comprehensive studies that elucidate the mechanisms by which biostimulants influence plant health and productivity [18]. The advent of digital technologies, such as remote and proximal sensing, offers new opportunities to accurately monitor and quantify the effects of biostimulants on crop performance at different spatial and temporal scales. Traditional methods for assessing plant stress and physiological status, such as the Scholander pressure chamber for Stem Water Potential (SWP) measurement, are limited by their destructive sampling requirements and labor-intensive nature, making them unsuitable for large-scale applications [19]. Similarly, tools like the SPAD 502 meter for chlorophyll content estimation and the Multiplex Research 3 (MFA) fluorimeter for assessing physiological status are valuable but constrained by their inability to cover extensive vineyard areas efficiently [20–23]. Remote sensing technologies, particularly those integrated into Unmanned Aerial Systems (UASs), have become increasingly relevant in precision viticulture. These systems provide non-invasive, scalable solutions for monitoring various vineyard parameters, including plant vigor, nutrient status, and disease incidence, enabling a comprehensive understanding of vineyard dynamics [24,25]. Among all the existing sensors implemented on board, the multispectral camera is the most widely used in viticulture, followed by thermal and LIDAR UAS technologies [26]. The ability to conduct multi-temporal analyses is a key advantage of remote sensing, as it allows for the monitoring of changes in vineyard parameters over time, facilitating the development of historical data series that can improve crop management decisions. By integrating remote sensing with traditional ground-based measurements, it is possible to obtain a more comprehensive and accurate picture of how biostimulants affect vineyard performance, ultimately contributing to more sustainable and effective viticulture practices [27–29].

This study aims to evaluate the sensitivity of remote and proximal sensors, alongside traditional manual observations, in detecting the effects of varying biostimulant application rates on vineyard biomass production, water stress resistance, and grape yield and quality throughout the vegetative and production phases. By employing a combination of these technologies and mainly focusing on the possibility of detecting such effect on a large scale relying only on UAS multispectral technology, this research seeks to enhance the understanding of biostimulant efficacy and provide valuable insights into the development of sustainable precision viticulture strategies that promote environmental sustainability and economic viability.

## 2. Materials and Methods

### 2.1. Experimental site

The study was carried out at the "Columbu" winery in Magomadas (OR), Sardinia, Italy (coordinates: Easting 457074.761, Northing 4456775.910; UTM 32 N, EPSG 32632), in an organic vineyard of cultivar "Malvasia bianca" (controlled designation of origin Malvasia di Bosa DOC), covering an area of 0.81 ha (Figure 1). The vineyard, planted in 2008, grafted onto 140 Ruggeri rootstock and located at 220 meters Above Sea Level (ASL), is characterized by Guyot training system with a  $0.9 \times 2.1$  m spacing configuration, corresponding to 5290 plants per hectare, oriented northwest-southeast. The terrain exhibits a slope perpendicular to the vine rows (Figure 1b). Climate is Mediterranean semi-arid, with hot and dry summers and a scarce annual rainfall. Daily maximum temperatures ( $T_{max}$ ), mean temperatures ( $T_{mean}$ ), minimum temperatures ( $T_{min}$ ) Figure A1, and rainfall from 1 January to 31 December of both years (Figure A2) were obtained by a weather station located nearby (4,6 km) the vineyard.



**Figure 1.** The Randomized Complete Block Design in Magomadas (Sardinia, Italy) with the four treatments repeated in two blocks (a) disposed along the slope gradient expressed in meters Above Sea Level (ASL) (b).

The site has a relatively uniform marly calcareous soil, with an average depth of 60–70 cm, and the following physico-chemical characteristics: sand 67.6% clay 22.8%, silt 9.6%, pH = 8.65 and 0.9 g kg<sup>-1</sup> of nitrogen, 6.5 mg kg<sup>-1</sup> of phosphorus, 8 g kg<sup>-1</sup> of organic matter. The soil is moderately deep soils, medium texture, medium gravelly skeleton common on surface abundant in depth, moderately calcareous on the surface to extremely calcareous at depth, alkaline reaction, moderately rapid drainage. Field data collection was conducted under sunny, clear sky conditions during the 2020 (3 July, 29 July, and 24 August) and 2021 (18 June, 2 August, and 24 August) seasons. Phenological growth stages of the grapevine were detected according to the extended BBCH (Biologische Bundesanstalt, Bundessortenamt and Chemical industry) scale proposed by Lorenz et al. [30].

## 2.2. Experimental design and agronomic management

The experiment consisted of four treatments, each containing increasing concentrations of the Biopromoter biostimulant (based on Eurovix S.P.A. production company recommended rates and the experience in this matter of the research team), replicated in two blocks and a single plot size of 5.5 m (5 rows) x 60 m, arranged perpendicular to the terrain slope using a randomized complete block design (Figure 1a). The vineyard was managed uniformly, except for biostimulant application, which followed four specific supply treatments, in combination with foliar fertilizer application outlined in Table 1 and Table 2.

**Table 1.** The four biostimulant distribution treatments and the related cost ha<sup>-1</sup>.

Treatments	Winter fertilization (g plant <sup>-1</sup> )	Seaweed application season-1)	foliarFertilizers (napplication season-1)	foliarCost (n(Euro)	ha-1
Red	NA*	4	NA	69	
Yellow	100	4	NA	784	
Blue	100	4	4	839	
Green	150	4	4	1240	

\* Not Applied.

**Table 2.** Biostimulant treatments' application specifics according to the BBCH scale.

BBCH [30]	Product	Treatments				Application
		Red	Yellow	Blue	Green	
0	Biopromoter (g)	NA	100	100	150	Ground
	EUROALG S (g L <sup>-1</sup> )	3	3	3	3	Foliar
19	EUROLIGO (g L <sup>-1</sup> )	NA*	NA	3	3	Foliar
	EUROMOLIB (g L <sup>-1</sup> )	NA	NA	3	2	Foliar

53-55	EUROALG S (g L-1)	3.5	3.5	3.5	3.5	Foliar
	EUROLIGO (g L-1)	NA	NA	3.5	3.5	Foliar
	EUROMOLIB (g L-1)	NA	NA	3.5	3.5	Foliar
69-73	EUROALG S (g L-1)	3.5	3.5	3.5	3.5	Foliar
	EUROLIGO (g L-1)	NA	NA	3.5	3.5	Foliar
	EUROMOLIB (g L-1)	NA	NA	3.5	3.5	Foliar
71	EUROALG S (g L-1)	4	4	4	4	Foliar
	EURODUAL (g L-1)	NA	NA	2	2	Foliar
	BIOKALIUM (g L-1)	NA	NA	2	2	Foliar
	EUROMOLIB (g L-1)	NA	NA	NA	1	Foliar
79-81	EUROALG S (g L-1)	5	5	4	4	Foliar
	EURODUAL (g L-1)	NA	NA	3	3	Foliar
	BIOKALIUM (g L-1)	NA	NA	3	3	Foliar
	EUROMOLIB (g L-1)	NA	NA	3	3	Foliar

\* Not Applied.

The organic biostimulant fertilizer utilized in the survey originates from the methodical maturation and stabilization of selected organic substrates, augmented with microbial strains and enzymes. Humic and fulvic acids inclusion, in conjunction with macro and micronutrients, microbial communities, and advantageous enzymes, makes the biostimulant an agent for restoring soil fertility (Table 3).

**Table 3.** Biostimulants composition declared by the production company (Eurovix S.P.A.).

Nutrient	Content (%)
Organic nitrogen (N)	3
Sulphur dioxide (P2O5)	9
Calcium oxide (CaO)	8
Potassium oxide (K <sub>2</sub> O)	1
Magnesium oxide (MgO)	2
Iron (Fe)	2
Biologic Organic carbon (C)	16

### 2.3.UAS platform and implemented sensors

The UAS Phantom 4 Pro (DJI Technology Co., Ltd, Shenzhen, China) equipped with an RGB (Red Green Blue) CMOS 1" 21-megapixel (MP) resolution sensor alongside two 12 MP Survey 3 multispectral cameras (Mapir, San Diego, USA) performed remote sensing data acquisition. The MAPIR calibration target and Camera Control calibration software

(MAPIR, San Diego, CA, USA) enabled raw data surface reflectance correction. UAS flights, in accordance with most research papers on UAS application in viticulture [26], were conducted at an altitude of 45 m Above Ground Level (AGL), with a speed of 2.5 m s<sup>-1</sup>, maintaining 85% front and 75% side overlaps, using the DJI Pilot android app for flight planning and execution, totaling 4 minutes and 15 seconds per flight. To ensure accurate orthomosaic orthorectification, an RTK GNSS Reach RS+ (Emlid Tech Kft., Budapest, Hungary) recorded coordinates for 9 Ground Control Points (GCPs), incorporated inside the structure from motion software Agisoft Metashape (Agisoft LLC, St. Petersburg, Russia), used for the vegetation index digital models obtainment together with QGIS open-source software (ver. 3.30.0, QGIS Development Team) by incorporating the Equations 1 and 2 [26] used for biostimulants effect detection on the canopy. The equations report the name of the implemented spectral bands (where "NIR" refers to the Near Infra-Red spectral band) and the corresponding wavelength in nm inside the brackets.

$$NDVI = \frac{NIR(850\text{ nm}) - Red(550\text{ nm})}{NIR(850\text{ nm}) + Red(550\text{ nm})}, \quad (1)$$

$$NDRE = \frac{NIR(850\text{ nm}) - RedEdge(725\text{ nm})}{NIR(850\text{ nm}) + RedEdge(550\text{ nm})}, \quad (2)$$

#### 2.4. Fluorometer proximal analysis

The proximal sensing tool implemented for fluorometric analysis was the portable UV-Visible MFA (Force A, Orsay, France), able to detect information about the physiological status of vegetation and grapes in a non-destructive way. The system is composed of three light detector channels, able to detect the Far-Red Fluorescence (FRF), the Blue Green Fluorescence (BGF), and the Red Fluorescence (RF) emitted by the tool and reflected by the plant. The light emission derives from an LED system that works at 375 nm (UV Radiation) and six RGB operating at 470 nm (Blue Radiation), 516 nm (Green Radiation), and 635 nm (Red Radiation). The decision to include the MFA in the trials derives from its ability to monitor the vegetation status and bunches' growth-ripening phase without causing any damage to the crop. For this purpose, three indices were selected to recognize any physiological differences between plots [31], focusing on the analysis of stress (Equation 3) and flavonoids (Equation 4) on the upper page of adult leaves and the bunches. The fluorometric indices used and their mathematical function are listed below.

$$BRR - FRF = \frac{YF - UV}{FRF - G}, \quad (3)$$

$$FLAV = \log \frac{FRF\_R}{FRF\_UV}, \quad (4)$$

#### 2.5. Plant water stress estimation

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Scholander pressure chamber (SPC) (PMS Instrument Company, Albany, USA) was used to measure the water tension associated with plant water stress (SWP) [32]. The procedure involved sampling 16 leaves, each enclosed in a mirrored plastic bag for 1 hour (SKB stem water potential bags), with two leaves collected from each plot. The samples of the leaves were then placed in the pressure chamber, gradually increasing the applied negative pressure until the xylem tension was overcome, indicated by the appearance of water on the stem of the leaf. Higher pressure values corresponded to greater leaf tension, reflecting elevated levels of water stress in the sampled plant.

## **2.6. Leaves chlorophyll estimation**

SPAD 502 (Minolta, Osaka, Japan), a non-destructive portable tool to measure the chlorophyll concentration and the derived nitrogen content in leaves [33], was implemented according to the experimental design reported in section 2.2 to sample two plants (five leaves each) in each plot as an indicator of vegetation health status, crop productivity, and photosynthetic efficiency [34].

## **2.7. Quantitative and qualitative harvesting evaluation**

To assess the impact of biostimulant applications on grape production, field sampling was conducted on 23 September 2020 and 28 September 2021. During each of these sampling events, the production quantity was measured as kg per plant for six randomly selected vines from each experimental plot. The individual vine yields were averaged to provide a representative value for each plot's overall productivity.

In addition to yield measurements, grape quality assessments were performed on three dates prior to harvest for each year. Specifically, samples were collected on 9, 17, and 23 September in 2020 and on 7, 13, and 28 September in 2021. For each sampling date, random bunches were harvested from five plants per plot, resulting in a combined sample weight of approximately 1 kg per plot (Figure 1a). This sampling strategy ensured that the grape quality analysis was comprehensive and representative of each plot's overall condition.

Following collection, the grape samples underwent a preparation process in the laboratory [29]. The entire sample from each plot was crushed, and the resulting must was filtered to separate coarse components such as skins and seeds. A 50 ml portion of the clarified liquid was then transferred into Falcon-type plastic tubes for further analysis.

After allowing sufficient time for the solid particles to settle within the tubes, the liquid fraction was subjected to a quantitative and qualitative analysis using a Fourier transform mid-infrared spectrometer (WineScan™ Flex, Foss Italia srl, Italy).

The WineScan™ Flex spectrometer facilitates the precise determination of various grape quality attributes critical to evaluating the qualitative characteristics of Malvasia bianca [35]. In this study, titratable acidity, pH, and total soluble solids (°Brix) were reported as the primary indicators of grape quality, given their significant influence on sensory properties and overall quality profile of the resulting wine. Titratable acidity and pH provide insights into the balance and stability of the wine, while °Brix value, representing the concentration of soluble sugars, is a key determinant of grape ripeness and potential alcohol content. Together, these parameters offer a robust framework for evaluating the effects of biostimulant treatments on grape quality under varying environmental conditions.

## 2.8. Statistical analysis

Survey features were evaluated using Python (Python Software Foundation) scripts through Pandas, Matplotlib, and Seaborn packages for data manipulation and plotting. Analysis of Variance (ANOVA) was performed for each season separately using the statistical package SPSS 16®. When needed, Least Significant Differences (LSD) were calculated at the 5% significance level to discriminate between treatment means and reported inside the plots in the following results section.

## Results

### 2.9. Meteorological trend during the 2-year survey

The meteorological trends displayed significant differences between the two years of the trial.

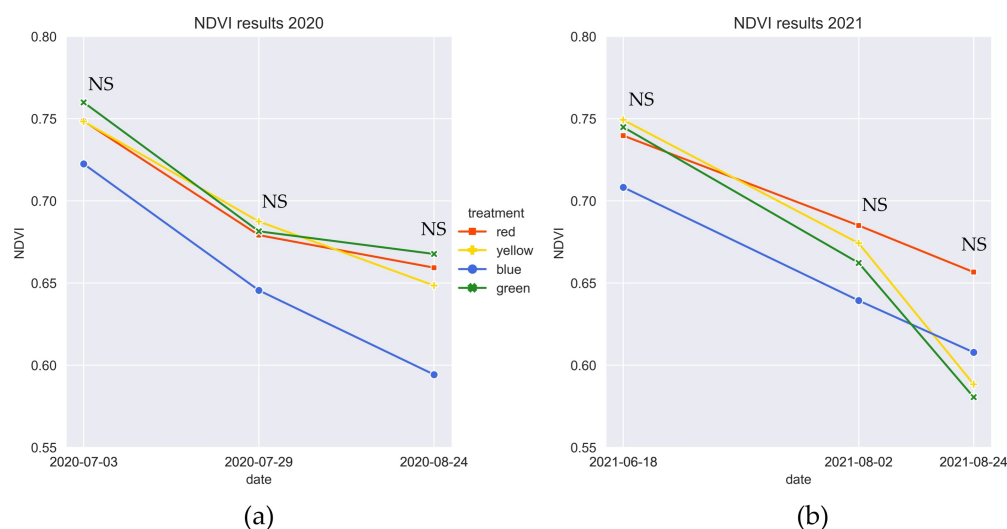
Temperature trends (Figure A1) appeared very similar for the total duration of the survey, defining July and August as the hottest months, with temperatures averaging over 23-24 °C. Although the annual maximum temperature appeared to be higher in 2021 than in 2020 by slightly less than 0.30 °C, considering the most critical period for the grapevine (i.e. the period starting from June to the end of September), the maximum temperature appeared to be higher in 2021 than in 2020 by slightly more than 2 °C. Moreover, assessing daily maximum temperatures, the number of days above temperature exceeding 35 °C was higher in 2021 (13 days) than in 2020 (7 days).

The total rainfall (Figure A2) recorded each year was similar, with 690 mm in 2020 and 761 mm in 2021, higher than the 30-year average of 667 mm from 1980 to 2010 (data not shown). However, the distribution of precipitation throughout the seasons varied. In 2020, the winter and spring seasons experienced low rainfall, with significant accumulation occurring only during harvesting. In contrast, the 2021 season was marked by substantial rain in the first-year quarter, followed by low to moderate rainfall during the spring and summer months.

## 2.10. Vegetative status establishment

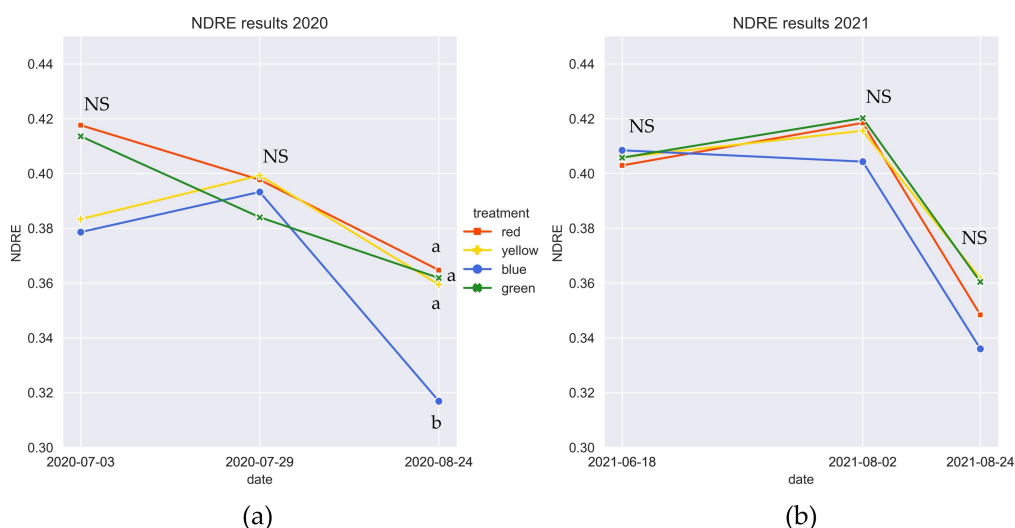
### 2.10.1. UAS monitoring

The NDVI results (Figure 2) for both growing seasons demonstrated a higher vegetative response during the early stages, with values ranging from 0.70 to 0.75 in July. These values indicate substantial vegetative vigor, which progressively declined as the growing season advanced, reaching lower levels between 0.60 and 0.65 in August.



**Figure 2.** NDVI patterns during the 2020 (a) and 2021 (B) growing seasons.

Despite receiving higher nutrient rates, the blue treatment consistently exhibited lower performance compared to the red and yellow treatments throughout both seasons. This anomaly is likely attributable to the specific characteristics of the plot's location in the northwest section of the vineyard (Figure 1a), an area marked by limited nutrient availability and suboptimal soil conditions, predisposing the vines to reduced vegetative growth and vigor. In 2020, the NDRE results (Figure 3a) depicted a general decreasing trend across all treatments. Both the blue and yellow treatments initially started from marginally lower NDRE values and converged toward similar values as the season progressed. However, the blue treatment's performance remained significantly lower, especially on the final assessment day, highlighting the considerable influence of the field's heterogeneity, which disproportionately affected the blue treatment. A comparable trend was observed in the 2021 season (Figure 3 b), although overall performance decreased further compared to 2020.



**Figure 3.** NDRE patterns during the 2020 (a) and 2021 (b) growing seasons.

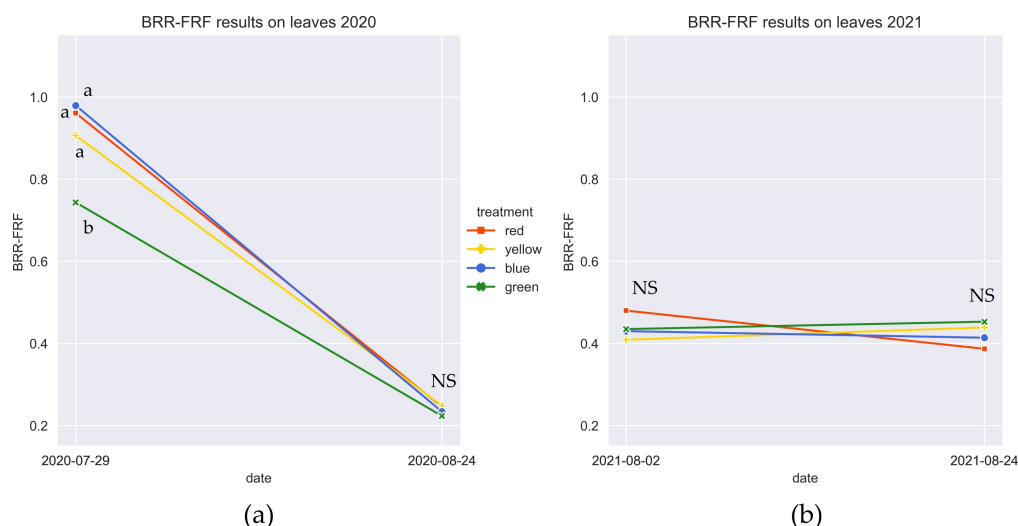
This reduction in vegetative indices can be attributed to adverse climatic conditions, including elevated temperatures and water scarcity, to which the vines were unable to adequately adapt. Additionally, the detrimental impact of nearby wildfires, which reached the vineyard's borders, likely exacerbated the stress conditions experienced by the plants. This hypothesis is supported by the NDVI data from the same year (Figure 2b), where only the red treatment maintained a stable trend throughout the season, whereas the green and yellow treatments displayed a sharp decline towards the end of the season. This overall vegetative decline is further corroborated by the SWP results from 2021 (Figure 6b), which showed a marked reduction in water status compared to the previous year.

Throughout both years, none of the vegetation indices were able to distinctly differentiate between treatments that displayed similar behavior over the growing seasons, with the exception of the blue treatment. This uniformity in response across treatments might be due to the consistent application of biostimulant rates (Table 1), which did not produce the expected differentiation in vegetative response, particularly for the green and blue treatments, which were hypothesized to outperform the others under increased nutrient supply.

### 2.10.2. MFA leaves analysis

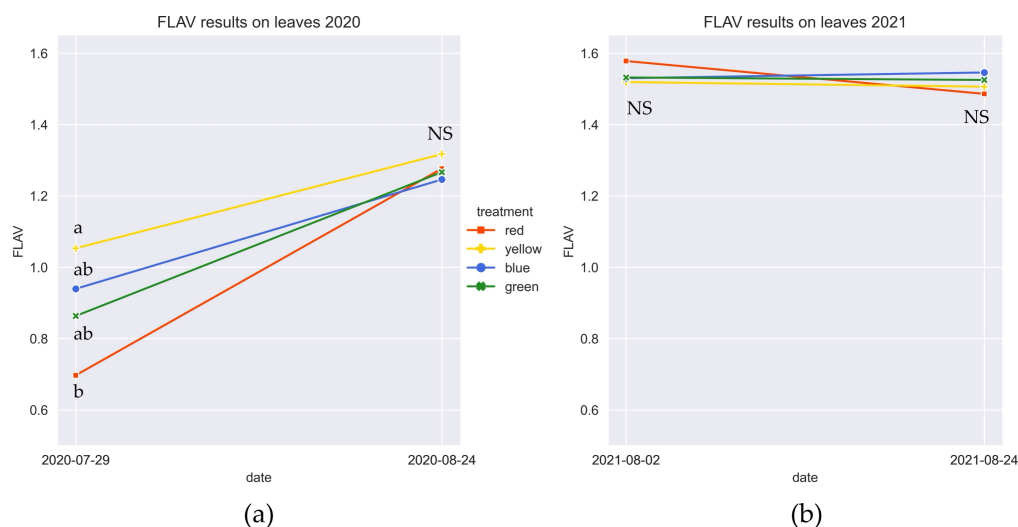
The MFA results for the fluorescence indices (Figure 4) displayed a generally consistent trend among the four treatments, with notable variation between the two years. The indices indicated a more stable, albeit critical, vegetative condition in 2021, mirroring the trends observed in the UAS-derived vegetation indices and indicating a general decline in vegetative vigor in the second year of the study. In 2020, the BRR-FRF index results for

leaves (Figure 4a) exhibited a pronounced decreasing trend across all treatments, with values declining from approximately 0.75-1.0 at the beginning of the season to 0.2 by the end. The green treatment initially recorded high values (0.7) on the first sampling date but converged to similar lower levels as other treatments by the final survey date. In contrast, the 2021 season (Figure 4b) displayed a more consistent BRR-FRF response across treatments, with no significant differences observed and values stabilizing around 0.4 across the two monitoring dates.



**Figure 4.** BRR-FRF results for 2020 (a) and 2021 (b) growing seasons.

The FLAV index results followed a similar pattern to the BRR-FRF index in both years, reflecting the physiological maturation of the vine canopy. In 2020, the total flavonoid content exhibited a steady increase, indicating typical canopy development during the growing season (Figure 5a).



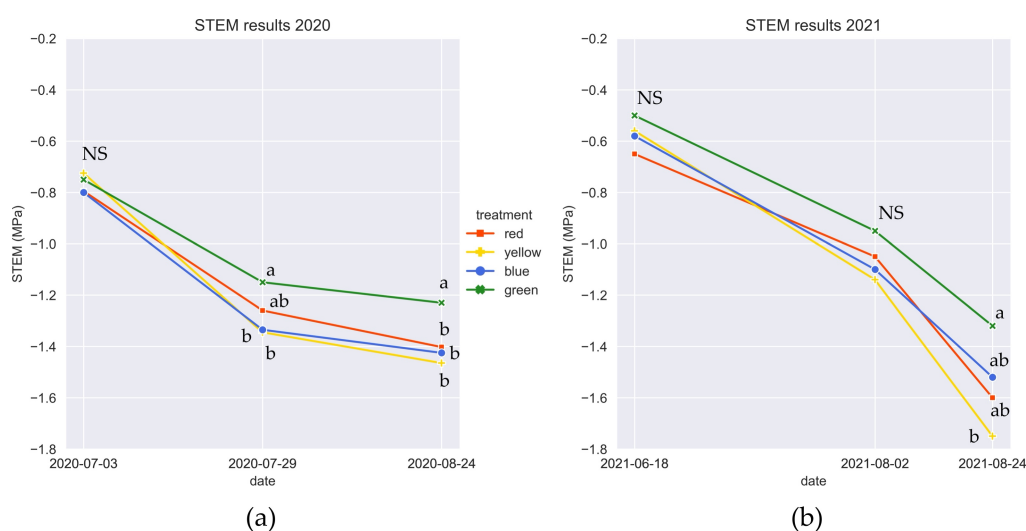
**Figure 5.** FLAV results for 2020 (a) and 2021 (b) growing seasons.

Conversely, the 2021 season (Figure 5b) displayed a nearly constant trend in FLAV values, maintaining elevated levels (around 1.5) compared to the previous year, in line with the BRR-FRF index results. These observations suggest a more critical but stable physiological condition, consistent with the intensified environmental stresses experienced during the second year.

The MFA indices clearly demonstrated significant differences in the first 2020 survey, which is inconsistent with the applied biostimulant rates. In stark contrast, the 2021 season showed no significant difference between treatments.

### 2.10.3. Water stress measurement

Early in the 2020 season, SWP values were uniform across all treatments, declining as water availability diminished (Figure 6a).



**Figure 6.** SWP patterns during the 2020 (a) and 2021 (b) growing seasons.

However, as the season progressed, the green treatment displayed a distinct advantage in maintaining higher water status (-1.2 MPa) compared to other treatments (-1.4 MPa), as showed in the last date of 2021, which reported a significant distinction between treatments, comparable to the applied biostimulant rates.

SWP divergence, not captured by the vegetation indices, likely reflects the ability of certain vines to better withstand water stress in a non-irrigated vineyard system, where only vines in optimal condition can sustain higher SWP values in late phenological stages.

The observed variation may also be related to the biostimulant application rates, which were possibly insufficient to support increased biomass production, particularly in the initial year of treatment.

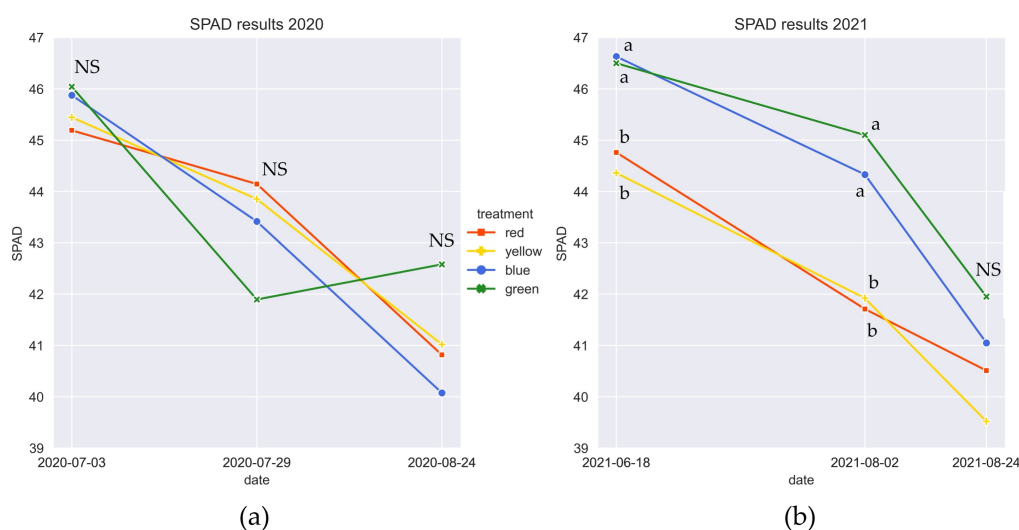
A similar trend was observed in the second year (Figure 6b), with all treatments starting from a better initial condition but converging to similar results. The green treatment,

however, again demonstrated superior water stress resistance, highlighting the cumulative effects of biostimulant applications and their potential role in enhancing drought resilience over time. Despite the continued use of biostimulants, all treatments exhibited heightened water stress sensitivity in 2021, likely due to extreme weather conditions and the unanticipated occurrence of wildfires near the field, as previously discussed.

#### 2.10.4. Leaves chlorophyll content estimation

SPAD measurements, indicative of leaf chlorophyll content, revealed a declining trend without significant differences between the four treatments in the first year (Figure 7a). The green treatment, however, exhibited a variable pattern and improved performance on the final survey date, suggesting a delayed response to biostimulant application.

In 2021, both green and blue treatments showed markedly better SPAD values compared to red and yellow treatments (Figure 7b), consistent with observations from the previous year. These results suggest that the cumulative effect of biostimulant applications becomes more evident after two consecutive years, or alternatively, that the SPAD meter demonstrated a higher sensitivity to subtle differences in vegetative vigor not captured by other remote and proximal sensing methods employed in this study.



**Figure 7.** SPAD patterns during the 2020 (a) and 2021 (b) growing seasons.

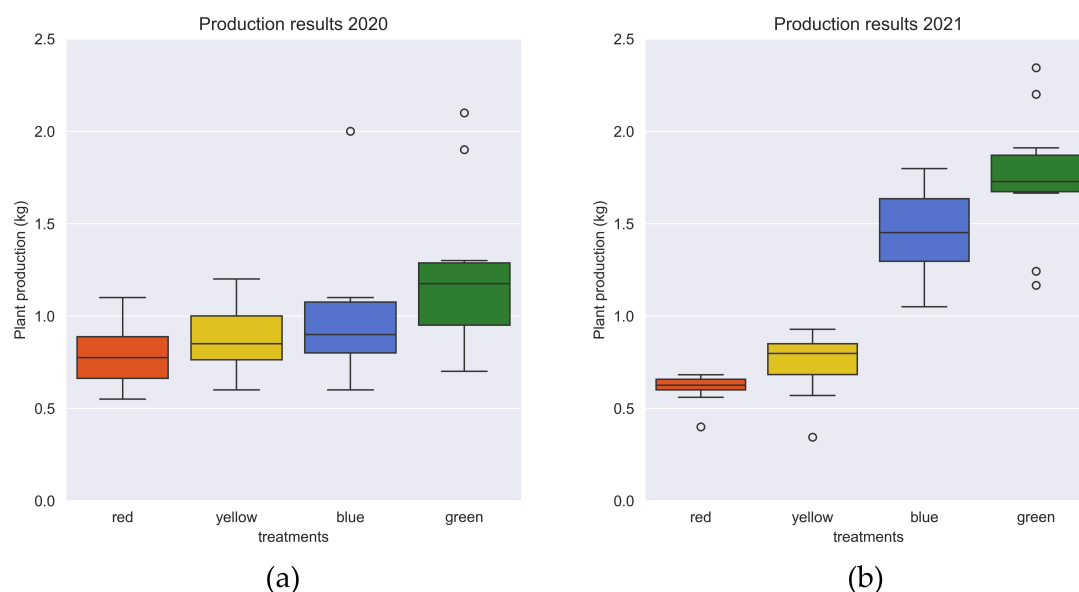
### 3. Production results

#### 3.1. Production quantitative analysis

As anticipated, the production outcomes in 2020 (Figure 8a) were closely aligned with the fertilizer supply patterns described in Table 1.

The green treatment yielded the highest grape production, averaging 1.25 kg of grapes per plant, followed in descending order by the blue, yellow, and red treatments. In the second

year (Figure 8b), a more pronounced divergence in production was observed, with the green and blue treatments yielding approximately 1.75 and 1.5 kg of grapes per plant, respectively, outperforming the other treatments.



**Figure 8.** Production results in 2020 (a, c) and 2021 (b, d).

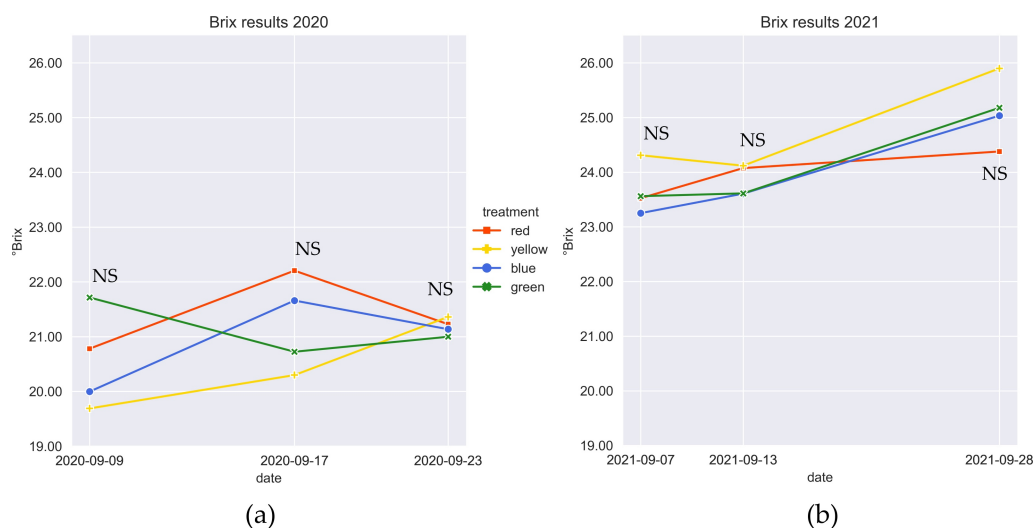
This trend underscores the potential influence of biostimulant products on fruit production, which appeared to have a greater impact on yield than on vegetative vigor alone, or the well-known seasonal variability, influenced by the nutrient availability and meteorological conditions relationship (Figure A1 and Figure A2).

It is important to note that the production data represents a single measurement taken before harvest and therefore does not capture the dynamic progression of grape growth throughout the season.

A key observation in the second year is that all treatments, except for the blue treatment, exhibited reduced variability in yield (excluding outliers), signifying an overall improvement in plant condition compared to 2020. This reduction in variability suggests that, over time, the application of biostimulants may contribute to a more consistent and stable production outcome across the different treatments.

### 3.1.1. Grapes quality assessment

Brix results exhibited a fluctuating pattern in the first year (Figure 9a), with no significant differences between treatments and no notable improvements throughout the growing season. The initial Brix values for 2020 ranged between 20° and 22° Brix, and by the final survey date, the treatments displayed homogeneous sugar content.



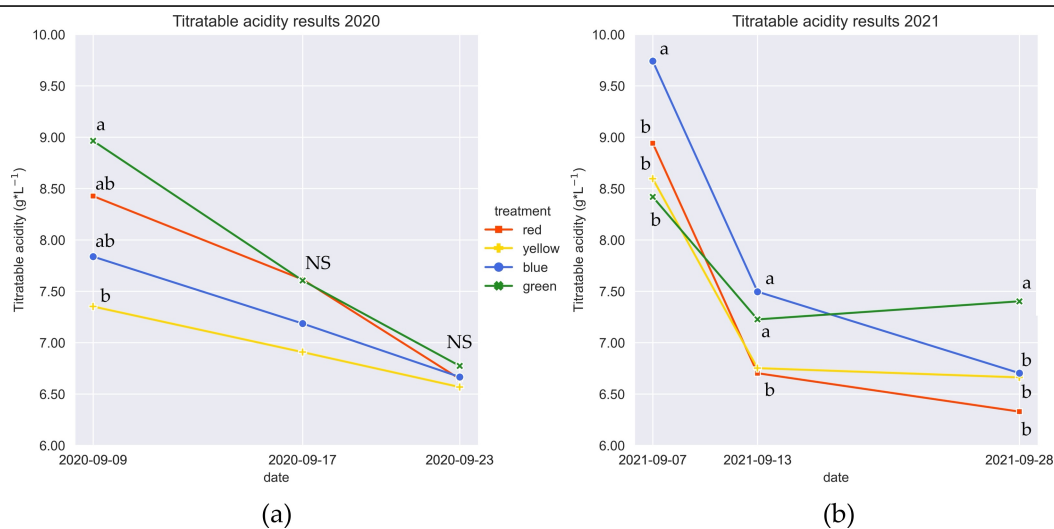
**Figure 9.** Brix results in 2020 (a) and 2021 (b).

However, the second year (Figure 9b) revealed a substantial upward trend in Brix levels throughout the season, again with no significant differences between treatments.

This improvement in sugar content may be more closely linked to the critical temperature changes observed in 2021 [36], rather than the effects of biostimulant products.

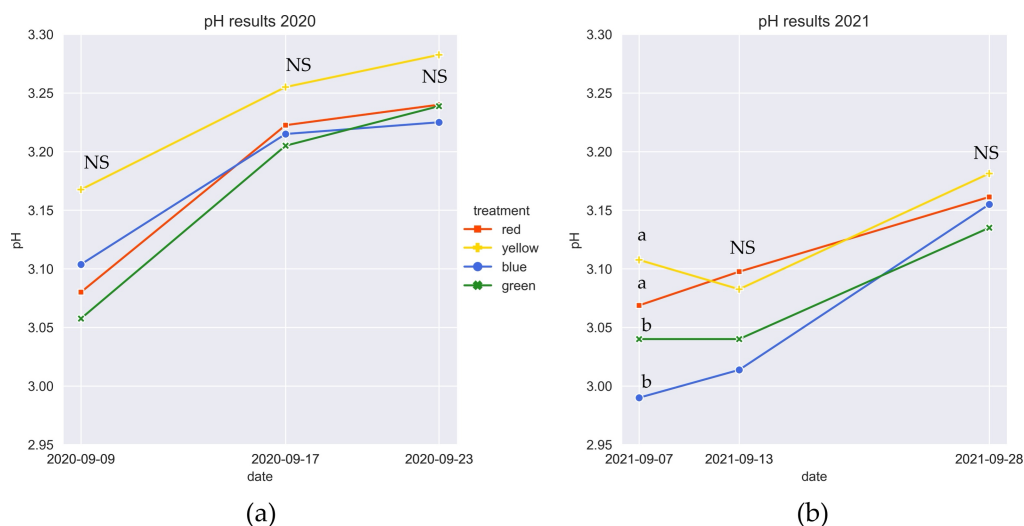
The titratable acidity measurements showed a comparable decreasing trend in both growing seasons. Early assessments in 2020 (Figure 10a) revealed substantial differences between treatments, but these differences diminished over time, with all treatments converging to a similar acidity level of 6.75 at the end of the season. In 2021 (Figure 10b), the treatments started under more favorable conditions, with the blue treatment unexpectedly achieving the highest acidity levels initially.

However, as the season progressed, trends shifted, and by the final assessment, the green treatment emerged as the best-performing treatment, paralleling the results observed in grape production.



**Figure 10.** Titratable acidity results in 2020 (a) and 2021 (b).

The pH readings exhibited a rising trend in both seasons, with the first season yielding the most favorable results for the evaluated cultivar. The 2020 results (Figure 11a) indicated no significant differences between treatments, except for the yellow treatment. In 2021 (Figure 11b), the treatments demonstrated higher, but still acceptable, acidity levels compared to the previous year, with the blue treatment initially attaining the highest acidity levels, before eventually reaching levels comparable to the other treatments.

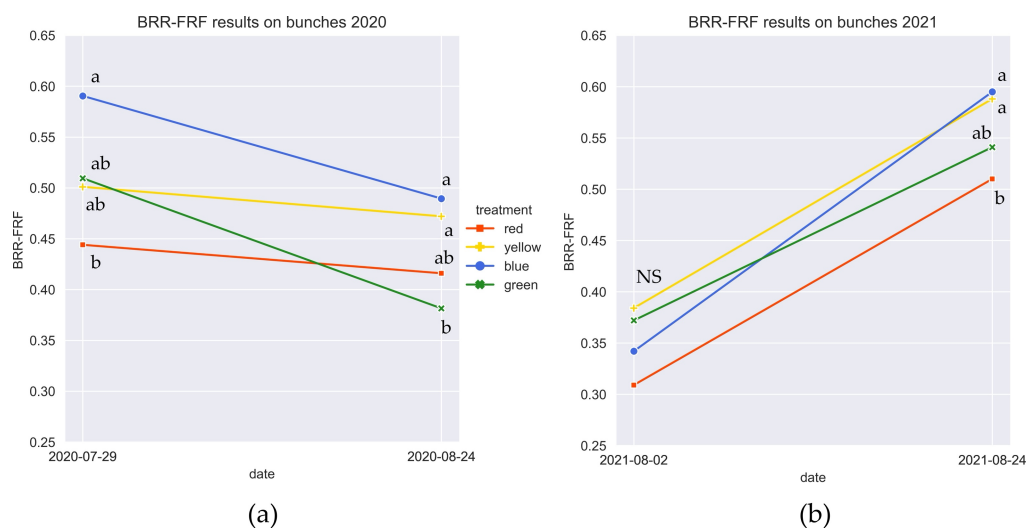


**Figure 11.** pH results in 2020 (a) and 2021 (b).

### 3.1.2. MFA grapes analysis

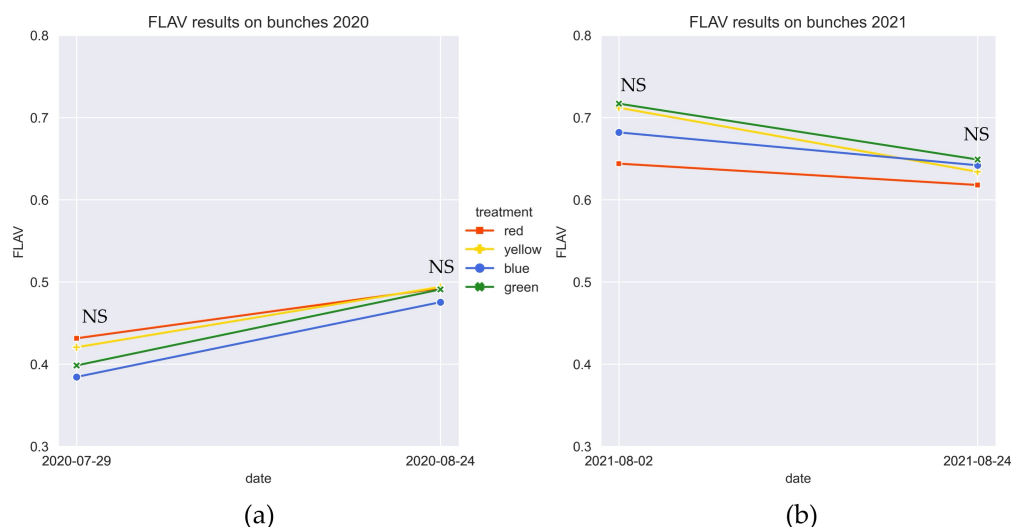
The BRR-FRF index (Figure 12), measured on the grape bunches, displayed a gradual decline in the first year and a significant increase in the second year. This shift suggests that grape bunches experienced higher levels of environmental stress in 2021, confirming similar findings reported for leaf stress on August 2, 2021 (Figure 4b). When comparing the two growing seasons, the BRR-FRF values on the first monitoring date in 2021 were lower than

the corresponding values from July 29, 2020, indicating reduced stress early in the season. However, by the final monitoring date, the BRR-FRF values had increased sharply, reflecting a sudden and unfavorable change in environmental conditions at the experimental site.



**Figure 12.** BRR-FRF results for 2020 (a) and 2021 seasons (b).

In contrast, the FLAV index (Figure 13) exhibited an opposite trend to the BRR-FRF index, with FLAV values generally increasing as BRR-FRF values decreased. This inverse relationship remained consistent across all four treatments, indicating that the biostimulant application had little influence on the variability detected by the FLAV index. In 2021, the FLAV index showed a slightly decreasing but stable trend, starting from a much higher initial value than in 2020. This behavior mirrors the trends observed in leaf fluorescence indices, indicating a general improvement in grape quality during the second year.



**Figure 13.** FLAV results for 2020 (a) and 2021 seasons (b).

The difference in trends between the BRR-FRF and FLAV indices in 2021 could be attributed to the specific grape variety used in the study, typically harvested late in the season, which may have led to increased stress on the grapes due to dehydration. The relatively stable FLAV levels combined with the elevated BRR-FRF index suggest that the optimal harvest time for this grape variety, particularly for wine production, coincides with the period of higher stress and stable flavonoid content.

#### 4. Discussion

This study investigated the sensitivity of both remote and proximal sensors, alongside traditional observations, in detecting the effects of different biostimulant application rates on canopy development, water stress resistance and grape yield and quality during various vegetative and production phenological stages exploring the possibility to rely only on UAS multispectral technology to improve the sustainable management of viticultural systems. In other words, the goal was not only to explore the opportunity to evaluate the impact on vine's vigor using multiple tools, but also to understand if such discrepancy between the analyzed treatments could rely only on UAS remote analysis, more suitable on a large scale and less prone to labor fatigue.

From the agronomic point of view, the use of biostimulants, delivered foliar and/or to the soil in gradually increasing amounts, promoted, although only in the second year and probably due to the higher rainfall, greener canopy and higher productivity in treatments where the biostimulant was delivered to the soil. In contrast, parameters related to must quality at harvest, influenced by the seasonal effect, did not varied with differential use of biostimulants. Field surveys carried out showed that, at least in this experiment and with cultivar *Malvasia bianca*, the use of these specific products delivered solely by leaf route did not result in a significant improvement in the quanti-qualitative performance of the vineyard. The UAS-derived results highlighted the potential of UAS for reliable large-scale evaluations of the vegetative status of vineyards [37]. Consistent findings over two years underscore the practical utility of vegetation indices for ongoing plant monitoring and spatial variability assessment, as proved by M.V. Ferro et al. (2023) [38]. NDVI and NDRE indices effectively reported treatment trends, although differentiation between treatments was limited, except for blue treatment, which consistently underperformed over the two years, in contrast to what has been reported from the SPAD and in a more limited way by the SWP. The limited UAS ability to differentiate between treatments may derive from the lower sensitivity of NDVI and NDRE indices in subtling variations related to canopy status or the insufficient biostimulant application rates.

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Proximal hand-held monitoring devices, such as the Pressure Chamber for SWP and the MFA for biochemical indices, mirrored vegetation index trends indicating similar treatment responses in both seasons. However, it is worth noting that although the trends for the two seasons were similar, they impacted the results, especially for the SWP. In fact, due to lower winter and spring rainfall, all treatments compared in the former season exhibited stress levels already ascribable to "moderate" water stress conditions starting in early July, while in the following year, "moderate" stress levels were found at the end of the same month. However, in the period of grape ripening and up to harvest, the lack of rainfall in August and September, with a more severe thermal trend, promoted the achievement of stress levels considered "severe". Moreover, the vines managed with the highest biostimulant input delivered to the soil during the most drought periods in both years showed a better ability to convey water from the roots to the above-ground part. SWP patterns were consistent with cumulative stress observed by NDVI and NDRE, although they did not predict yield differences between treatments and evidence any difference between treatments, except for the green one, which, as previous mentioned, showed a generally slightly smaller water stress sensitivity in both seasons. It is crucial to highlight that because of the length of measurement operations and the fatigue of obtaining such stress values, SWP was derived from punctual sampling, relying only on four measurements for each treatment. The limited sample size affected the final results, reporting a less representative overall condition than the NDVI and NDRE indices averaged over all canopy pixels of each plot.

MFA analysis showed a gradual increase in flavonoids in leaves and clusters, indicative of berry ripening and leaf senescence, along with a slight decrease in the BRR-FRF index in 2020, while in 2021 adverse weather events resulted in a constant level of stress on vegetative parts, while clusters showed a greater increase in stress during ripening. The FLAV index measured in 2021 was higher and more stable in the canopy and almost double in the berries compared to 2020, reflecting delayed ripening as observed by increased titratable acidity. The homogeneous behavior of fluorimetric indices (BRR-FRF and FLAV) on leaves and bunches across all treatments suggests that the MFA was unable to detect inter-plot differences, probably due to the low sample size, with 20 leaves and 20 bunches per treatment, and the limited period analyzed in both seasons. However, it is notable that the fluorimetric sensor closely tracked the physiological responses of plants to seasonal meteorological changes (Figure [A1](#) and Figure [A2](#)), similar to the NDVI and NDRE results. In contrast to vegetative indices and other portable devices, the SPAD meter detected significant differences in chlorophyll levels in the second year, probably due to its higher sensitivity and measurement methodology, demonstrating a direct correspondence with yield

results, reinforcing its role in assessing vegetative vigor related to biostimulant application in viticultural scenarios. It remains to be clarified why SPAD is more sensitive than NDVI and NDRE in detecting differences between treatments and why these indices reported an inverse situation, with the blue treatment being the only underperformer (Figure 2). As can be seen from the SPAD results (Figure 5), the green and blue treatments have significantly greater nitrogen content than the red and yellow treatments, symptoms of a broadly better condition. In general, as already mentioned for the SWP and MFA, a great deal of responsibility could be attributed to the point nature of the SPAD measurement and the consequently limited sample size (with all the limitations described above), but also to the ability of the proposed indices (NDRE and NDVI) to provide generic information related to vegetative vigor, which can detect a worse performance of the blue plots, but not sensitive enough to detect the nitrogen content estimated by the SPAD. After a literature search, the work of Antonucci et al. (2023) [18] was the only one to apply UAS-assisted remote sensing technology for detecting the effects of biostimulants. Specifically, the objective was to evaluate whether UAS imaging was able to detect biostimulant treatment effects on tomato (*Lycopersicon esculentum* L., 3406 Heinz) plants under water stress conditions to determine the critical time windows in which the effects of biostimulants were significant and to assess the impact of the treatments on yield and quality parameters at harvest. The absence of effects on yield and dry matter in their study suggests that the bottleneck may not lie in the PROSAIL inversion process used to recover biophysical traits (LAI, leaf, and canopy chlorophyll content) but in the efficacy of the biostimulant itself. It is important to note that their experiment was conducted on a different crop, using glycinebetaine-based biostimulants (30%) combined with variable water inputs, which makes a direct comparison with this study less applicable, raising the hypothesis that the effects of biostimulants in the field may be hardly detectable or inconsistent with other inputs such as fertilizers or agrochemicals. The inherent complexity in interpreting results, particularly those derived from UAS and MFA indices, prevented the research team from definitively confirming the hypothesis that UAS-derived vegetation indices could reliably detect the effects of biostimulant applications. This led to mixed and, at times, contradictory findings when compared with hand-held monitoring devices, as previously discussed. These inconsistencies may be attributed to the study's design limitations, including a limited number of replicates and the impact of topographical variations [39], both of which likely influenced the final outcomes. In particular, the results for MFA, SPAD, and SWP were skewed in areas prone to environmental stress, where differences in plant vigor and yield were more pronounced, as evidenced by the production data presented in Figure 8b. To

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enhance the robustness of future research, it is essential to address these methodological constraints. Increasing the number of replicates would reduce variability and provide a more representative dataset. Additionally, controlling for topographic influences, such as slope and soil composition, would help minimize their confounding effects on plant responses to biostimulants, allowing for more accurate treatment comparisons [18]. Furthermore, extending the study across multiple growing seasons would offer a more comprehensive understanding of the temporal dynamics of biostimulant efficacy [28,29]. Incorporating additional monitoring dates within each season would also facilitate a more detailed temporal resolution, enabling researchers to capture the nuanced, evolving effects of biostimulant applications on vineyard health over time. UAS-derived multispectral vegetation indices provide a rapid and extensive means of monitoring large vineyard areas, proving particularly valuable for identifying zones susceptible to environmental stress, which may require more detailed examination using proximal sensing tools, and prove sufficient sensitivity for nutrient assessments with more extensive study, allowing a suitable and more readily available data stream for quantifying nutrients in vineyards than hyperspectral imagery [40,41]. Despite these advantages, the current limitations of UAS in consistently distinguishing between different biostimulant treatments highlight the need for further research. Advancements in developing novel vegetation indices or refining the use of specific wavelengths could significantly enhance the precision of UAS in detecting the subtle physiological changes induced by agricultural inputs. Such improvements are critical to confirm the role of UAS as an indispensable tool in sustainable vineyard management, particularly in assessing the multi-temporal impacts of biostimulants and other inputs on crop health and productivity. In this context, the ability to monitor the effects of agricultural inputs through remote sensing represents a pivotal strategy. By facilitating the accurate evaluation of specific product benefits, growers can make more informed decisions about input application. This capability also allows for the comparison of multiple biostimulant brands or compositions under varying environmental conditions, providing a robust framework for optimizing vineyard management practices. Such a strategy is not only essential for improving agricultural efficiency and sustainability but also for enhancing growers' awareness of product efficacy. In turn, this heightened awareness can accelerate the adoption of innovative technologies and practices, fostering a more data-driven approach to viticulture. Additionally, it supports research efforts by offering a scalable and efficient method for evaluating agricultural treatments, ultimately contributing to more rapid advancements in the field.

## 5. Conclusions

The combined use of UAS imagery, MFA, Scholander pressure chamber measurements, and SPAD readings provided an effective framework for vegetative growth monitoring in the Malvasia Bianca vineyard over the 2020 and 2021 growing seasons. Results showed no significant differences between remote and proximal sensing treatments, indicating that the proposed indices were not sensitive enough to distinguish vegetative vigor or water stress responses related to different biostimulant application rates. Notably, only the SPAD meter, and to a lesser extent, the Scholander pressure chamber for Stem Water Potential (SWP), identified treatments with higher biostimulant doses and demonstrated an association with yield outcomes. The successful integration of UAS-derived multispectral indices with conventional measurement methods underscores the potential of these technologies as valuable decision-support tools in sustainable viticulture. UAS imagery facilitates efficient monitoring over large vineyard areas, enabling stress zone identification and supporting nutrient assessment across broader spatial scales. However, the current limitations of remote sensing in differentiating biostimulant treatments highlight the role of these technologies in providing a comprehensive plant status overview rather than detecting subtle treatment effects achievable with manual tools. By combining remote sensing with precise proximal measurements capable of detecting single-plant anomalies, vineyard managers can adopt a more comprehensive approach to vine status monitoring, optimizing management practices, and evaluating treatment effects on vineyard performance. With increasing environmental awareness and the need to reduce the use of chemical fertilizers, biostimulants offer a promising solution for more sustainable agriculture. Agronomic experiments are therefore required to improve knowledge of the composition and mode of action of biostimulants, seeking to increase their effectiveness by optimizing them to counter specific abiotic stresses. Future studies should consider the impact of different application rates of biostimulants, alternative formulations, and certainly longer monitoring periods to improve the sensitivity of these evaluations in capturing the influence of biostimulants on vine growth and yield.

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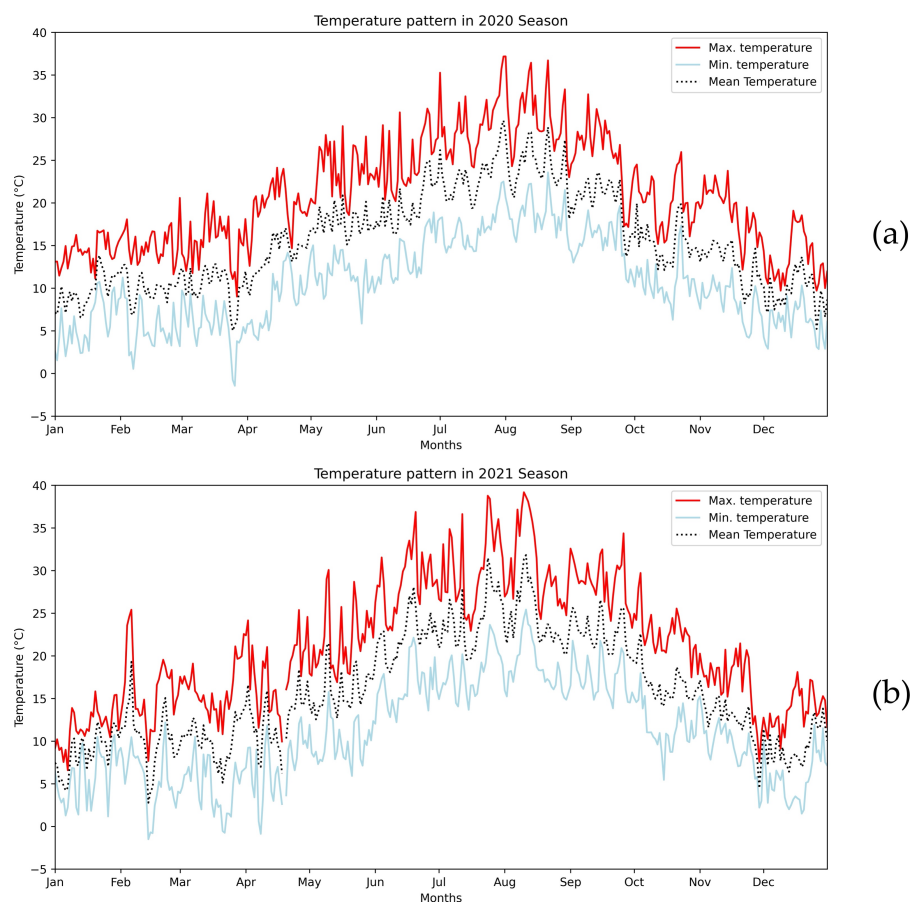
**Data Availability Statement:** The data presented in this study are available on request from the corresponding author.

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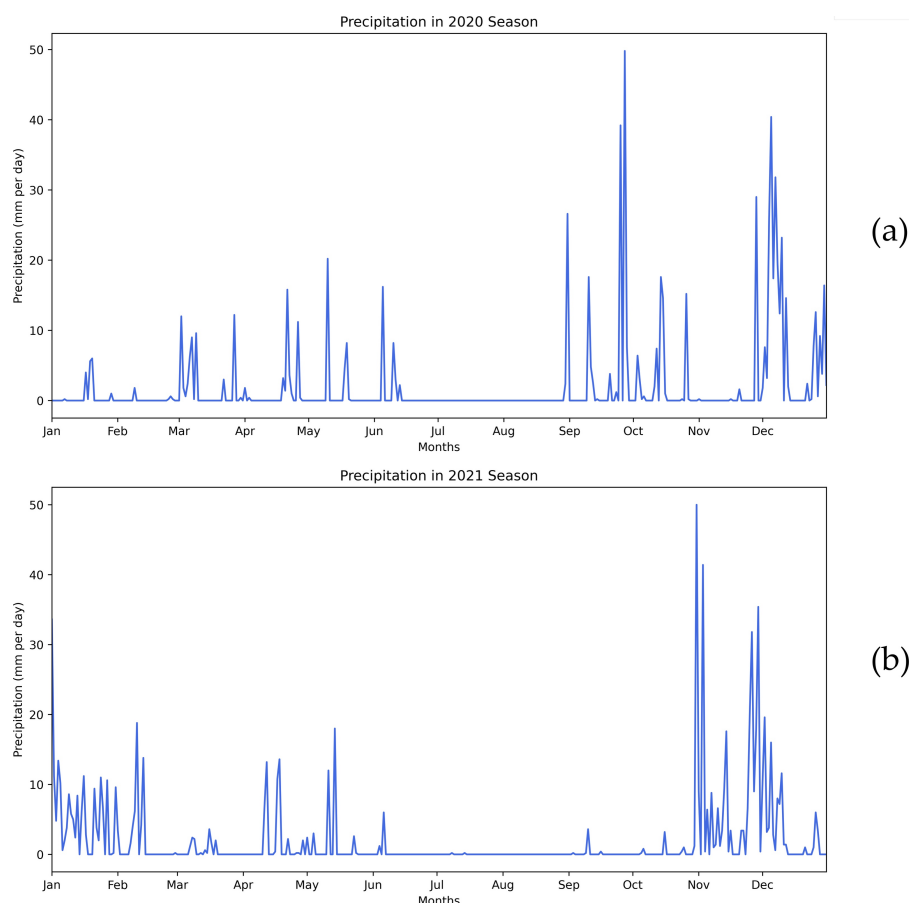
**Conflicts of Interest:** The authors declare no conflicts of interest.

## Appendix A.

### Appendix A.1. Meteorological trend during the 2-year survey



**Figure A1.** Temperatures pattern during the 2020 (a) and 2021 (b) seasons.



**Figure A2.** Precipitations pattern during the 2020 (a) and 2021 (b) season

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## **Chapter 4. A decision-supporting system for vineyard management: a multi-temporal approach with remote and proximal sensing**

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**Abstract**

Site-specific field management operations represent one of the fundamental principles of precision viticulture. The purpose of the research is to observe and analyse the evolution of a vineyard over three consecutive years to understand which factors most significantly influence the quality of the vineyard's production. The research involved technologically advanced tools for crop monitoring, such as remote and proximal sensors for vegetation surveys. In association, grape quality analyses were performed through laboratory analysis, constructing geostatistical interpolation maps and matrix correlation tables. Both remote and proximal sensing instruments demonstrated their ability to effectively estimate the spatial distribution of vegetative and quality characteristics within the vineyard. Information obtained from GNDVI and CHM proved to be valuable and high-performance tools for assessing field variability. The differentiated plant management resulted in uniform production quality characteristics, a change evident through the monitoring techniques. The research highlights the effectiveness of using advanced technological instruments for crop monitoring and their importance in achieving uniformity in production quality characteristics through differentiated plant management. From the results obtained, it was possible to observe how differentiated plant management led to a uniformity of production quality characteristics and how the monitoring techniques can observe their evolution. This result represents a positive accomplishment in field management during the three monitoring years, responding to the principles and objectives of precision agriculture.

**Keywords:** Precision viticulture; Site-specific analysis; Remote sensing; Geostatistical analysis

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## 1. Introduction

The technological development in agriculture observed in the last decades has represented a breakthrough in the management and execution of field activities [1]. Through these years, the foundation of Precision Agriculture (PA) and its evolution and combination with new technologies and approaches has paved the way for an analysis system and management of cultivation activities [2]. The affirmation of these principles and applications has contributed to creating an information system in which the operator integrates his competencies and experience with decision-support information systems (DSS), allowing them to devise the most appropriate strategy for achieving their objectives. In agriculture, monitoring sensors play a key role in agricultural practices management, providing highly detailed data crucial for decision support to the farmer [3]. These sensors, ranging from proximal sensors to those installed aboard orbiting satellites, provide unparalleled insight into various environmental factors, assessing at a site-specific level the chemical-physical and vegetative-productive characteristics of environment supporting precision management strategies [4]. This tailored approach can optimise resource utilisation and promote crop health, reducing environmental impact. In addition, sensors mounted on Unmanned Aerial Systems (UASs) or ground platforms can monitor crop conditions, identifying early signs of disease, pest infestation and water stress [5], [6]. This early detection allows farmers to implement targeted interventions, minimising the need for wide-ranging pesticides and ensuring sustainable agricultural practices. In its essence, the integration of the sensors in agriculture increases accuracy, efficiency, and sustainability. By harnessing real-time data, farmers can make immediate decisions based on the data that precision agriculture tools can provide [7], [8].

The significant results obtained from PA techniques concerning the management and economics optimisation of field activity [9] are observable in many typologies of crops, from the herbaceous to the arboreal one. Highly prestigious perennial cultivation is represented by the grapevine, which plays a historical and culturally important role in the Mediterranean area beyond the economic one [10]. Nowadays, the cultivation of wine grapes represents excellence among crops, influenced by a high-production process in which sophisticated techniques and technologies are employed [11]. These characteristics, combined with the exigency to manifest through wine the properties characterising a specific environment (soil, climate, altitude), have led over the years to a continuous search for solutions that can optimise costs, reduce environmental impact, and enhance the uniqueness of production, the ecosystem, and the territory. In Italy, the study and application of Precision Viticulture (PV) principles have played a fundamental role in improving the management of viticultural environments [12], due to contrast the agroclimatic climate changes alterations, challenging

the winegrowers to rebuild their strategies to preserve the vineyards quality and production [13]. It is easily understandable how the site-specific approach can lead to the management activities, from the planting layout decision to the scale yield based on maturation grade. This site-specific approach under spatial variability analysis might be one of the biggest challenges to management choices, in which the software application can be a helpful hand to improve the benefits.

In agreement with the Precision Agriculture approach, the optimisation of farming processes requires knowledge of the spatial and temporal trends of the variables that influence agricultural production. Support for the study and spatial analysis of the factors of interest is provided by geostatistics, which is a valuable tool to study and analyse factors of interest. It allows to regionalise crop information in a defined area, creating a forecasting model that represents the spatial distribution of the variable [14]. This model is characterised by a spatial distribution scheme of the data, making it easier to understand and interpret. Especially, the management of spatial variability through the geostatistical approach has allowed a considerable improvement in nutritional resources and crop protection [15], enabling the various production environments to manage the physiological responses determined by the heterogeneity of the surrounding environment, analysing site-specific morphological, pedological and hydraulic components [16].

As extensively documented in the literature [17], [18], [19], SSDs for the vegetative-productive variables management in vineyards are supported by the application of stationary and remote sensors capable of highlighting site-specific variability. The research and discovery of techniques and variables capable of satisfactory results for detecting in-field variability has found in reflectance-based vegetation indices (VI) a primary tool for analysis and discrimination. Sensor implementation on Unmanned Aerial Systems (UASs) has represented an innovation in agriculture, as it allows to report and estimate canopy variability in large areas in a relatively restricted time. Therefore, VIs employment for field-detection variability is an additional tool to predict production indices and quality.

Among the numerous used indices, NDVI is one of the most employed in viticulture, especially in the preliminary canopy analysis. However, it may not always provide the most accurate representation of the field, as it is susceptible to saturation, particularly in proximity to the physiological maturity of the crop [20]. As reported by Moghimi et al. [21], the Red Edge (RE) spectrum region can detect the variability in vegetation properties instead of the Visible (Vis) region. Therefore, an alternative vegetation index related to red edge reflectance is represented by the Normalised Difference Red Edge (NDRE), providing more

advantage than the NDVI for optimising harvest times based on transitions in photosynthesis activity [22].

In association with the NDVI and NDRE, an additional index of crucial importance is the Green Normalised Difference Vegetation Index (GNDVI) [23]. Its importance derives from its ability to capture the physiological behaviour of the canopy without incurring saturation phenomena such as NDVI [24] and, according to some authors, to correlate satisfactorily with the crop's water content [25]. These characteristics qualify it as a suitable tool for the physiological canopy investigation in the agricultural sector.

However, the study and discovery of these (and other) indices by scientific research is constantly in progress, discovering new applications and developments.

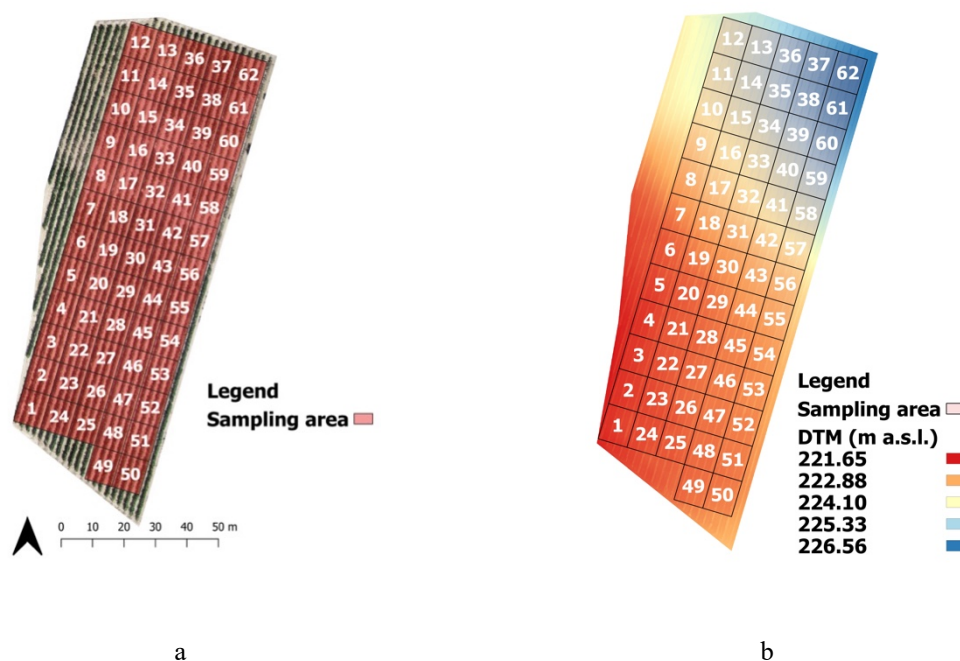
The following article can be divided into two main objectives (i) observe how the crop progresses on a three-year scale through the multiple field and laboratory employed variables and which of them are most affected by any changes.

Furthermore, (ii) estimate the correlation between field crop variables and production-quality analyses, looking at which is most highly related and better helps in quality estimation. The objectives reflect the core concepts of precision agriculture, researching site-specific crop evolution and variability for targeted management of production inputs. The objectives proposed in this paper converge on the ability of proximal and remote sensing tools to identify sub-areas of a field for tailored management of crop operations, searching through these methodologies to smooth the disparities in the field.

## **2. Materials and methods**

### **2.1 Experimental design**

The survey was conducted in a vineyard (Figure 1) of 0.8 hectares in Usini (Italy) (E 462280.302; N 4501628.588, 220 m above sea level (a.s.l.), EPSG:32632, WGS84-UTM 32N) in the 2021, 2022 and 2023 seasons. The cultivar considered in this study was a red berry variety “Cagnulari”. The rootstock adopted for the vine is the 140 Ruggeri, in a Guyot pruning system. The planting layout is 0.8 x 2.3 m, with a planting density of 5430 plants/ha. The experimental design planned for this study (Figure 1a) involved 62 sampling areas arranged in a georeferenced grid of 9 by 9 m, which was maintained constant for the three survey years. During the survey operations, the variables related to vegetation monitoring and grape production were collected within each sample area. In Figure 1b, the Digital Terrain Model (DTM) orthomosaic shows a variation in its slope, which gradually rises from the SW part to the NE side.



**Figure 1.** Experimental field characteristic. On Figure 1a, RGB vineyard orthomosaic; the experimental design involved the plant marked in purple. On Figure 1b, The Digital Terrain Model (DTM) in a pseudo-palette colour.

To provide a complete vision of the field variability, the vegetative and productive variables were observed. Specifically, the vegetative variables were analysed through a remote sensing vehicle such as an Unmanned Aerial System (UAS) and proximal monitoring system, including water potential and fluorescence analysis, which the latter was also used for the qualitative analysis of the grapes, complementing the insights gained from laboratory analyses. All surveys were conducted on bright, low-wind days so that all measurement tasks could be safely completed at the same moment, as some of the instruments used require optimal conditions for adequate inspection. The monitoring days are listed below in Table 1.

**Table 1.** Description of the survey days during the three-year experiment. From left to right: Survey Day and its respective Day of Year (DOY); BBCH phenological scale; Operation performed.

Survey Day	DOY	BBCH scale	Indices evaluated / operation executed
01 June 2021	153	69	CHM; NDVI; GNDVI; NDRE
08 July 2021	190	73	CHM; NDVI; GNDVI; NDRE; Pr. Chamber
28 July 2021	210	75	CHM; NDVI; GNDVI; NDRE; SFR_G; Pr. Chamber
26 August 2021	239	85	CHM; NDVI; GNDVI; NDRE; SFR_G
21 September 2021	265	89	Harvest

14 July 2022	196	75	CHM; NDVI; GNDVI; NDRE; Pr. Chamber
02 August 2022	215	81	CHM; NDVI; GNDVI; NDRE; SFR_G; Pr. Chamber
23 August 2022	236	85	CHM; NDVI; GNDVI; NDRE; SFR_G; Pr. Chamber
12 September 2022	256	89	Harvest
10 August 2023	223	85	CHM; NDVI; GNDVI; SFR_G;
21 August 2023	234	85	CHM; NDVI; GNDVI; NDRE; SFR_G;
01 September 2023	245	85	CHM; NDVI; GNDVI; NDRE; SFR_G;
16 September 2023	260	89	Harvest

## 2.2 Aerial surveys

The acquisition of vegetation indices was conducted with a commercial UAS. Specifically, the vehicle used for the monitoring operations was a Phantom 4 Pro-V1 (Shenzhen, China), equipped with a 1" RGB CMOS camera with 20 MegaPixels. In association with the RGB data, two Mapir Survery 3 multispectral cameras were implemented in the UAS chassis for field data acquisition. The sensors chosen for the analyses are the Red Edge (RE) and Red Green Near Infra-Red (RGN) cameras, 12 MegaPixels resolution, 41° HFOV (47 mm). The photographic sets were acquired with a 85% side overlap and 75% front overlap at 40 m height above ground level (a.g.l.). For the experiment purposes, the vegetation indices used in this experiment were:

- Normalised Difference Vegetation Index – **NDVI**

$$NDVI = \frac{(NIR - Red)}{(NIR + Red)}$$

- Green Normalized Difference Vegetation Index – **GNDVI**

$$GNDVI = \frac{(NIR - Green)}{(NIR + Green)}$$

- Normalized Difference Red Edge – **NDRE**

$$NDRE = \frac{(NIR - RE)}{(NIR + RE)}$$

The choice of these indices is due to their wide use in viticulture [x], serving as a guide for management operations [26], as they can observe not only the spatio-temporal crop variability in the field but also its productivity [22]. During the monitoring operations, NDRE index acquired by UAS did not provide a satisfactory map because of overexposure problems of multispectral RE on the first 2023 survey.

In combination with the multispectral data, volumetric canopy data were also acquired. The measurements were collected by UAS through the RGB camera, analysing the parameter using the Canopy Height Model technique [27], [28].

## 2.3 Proximal sensing for canopy monitoring

The field data acquisition involved both the analysis of vegetation characteristics and grape yield and quality; the instruments and methods used to acquire the data are detailed as follows.

### 2.3.1 Fluorimetric sensor analysis

In association with the UAS information, a multi-parametric handheld instrument was adopted to obtain information on the physiological state of the culture through the fluorescence phenomenon [29]. The sensor adopted in this experiment was a Multiplex Force-A (MFA) 3.6 (Paris, France), observable in Figure 2.



**Figure 2.** The Multiplex instrument used in the surveys. The fluorescence detectors are placed in the internal area of the instrument, and the interaction with the samples is allowed by the central opening.

Thanks to the detectors in the central part of the instrument, it was possible to monitor leaves and bunches evolution [30]. Some of the fluorimetric indices most affirmed and employed in viticultural contexts were chosen to observe the vegetative vigour and the grape qualitative characteristics [31]. The SFR\_G index was chosen to characterise the photosynthetic activity [32], whereas bunch analysis was assigned to the FERARI index, as they were the most representative candidates for qualitative characterisation [33], [34].

The index formulas [31] are shown in the following table.

**Table 2.** Fluorimetric indices employed in the experiment.

Fluorimetric indices	Destination field	Equation
SFR_G	Chlorophyll index on green ex. channel	$SFR\_G = (FRF\_G) / (RF\_G)$
FERARI	Anthocyanin content	$FERARI = \log 5000(FRF\_R)$

### 2.3.2 Pressure chamber

As described above, one of the objectives proposed in this work was to provide a valid tool for the early detection of field variability using instruments that could be considered in ordinary use and easy to employ in the agricultural sector. In commerce, among the many existing solutions, the Steam Water Potential (SWP) through the pressure chamber represents one of the main instruments, as its simplicity and low cost allowed it to be widely used in viticulture [35]. The pressure chamber sampling was conducted on the areas identified in the experimental design, opting to monitor alternating areas along the grid (Figure 3). This choice is due to the slowness of sampling all grid points, as the preparation and analysis of the SWP requires considerable processing time [36]. The sampling points were geo-referred for spatial analysis and associated with the other vegetative variables obtained from remote and proximal monitoring.



**Figure 3.** STEM Water Potential experimental grid. The block design marked in red helps to understand the areas involved in the pressure chamber analysis.

### 2.4 Production Analysis

At harvest operation and the production and quality data were collected from each area described in Figure 2 to extrapolate quantitative production and quality information on the grapes.

The fieldwork was conducted as follows:

- Calculation of the weight of plant production through measurement with a dynamometer of all bunches belonging to the plant inserted in the node;
- Counting the number of bunches on the plant;
- Random bunches (total average 1 kg) sampling per plant for successive qualitative laboratory analysis.

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First, the average weight of 20 berries per sample was analysed in the laboratory, and test samples were prepared for the qualitative analysis. After weighing and identifying the average weight of the twenty berries, the entire sample of each plant in the experimental grid was extracted and filtered from the coarse components such as skins and seeds, separating the liquid component into Falcon-type plastic test tube in the volume of 50 ml per sample. Once the separation of the solid components within the Falcon cuvette was achieved, the quanti-qualitative analysis of the liquid most was conducted using a Fourier transform mid-infrared spectrometer (WineScan™ Flex, Foss Italia srl, Italy). The instrument allowed the identification of the quality characteristics [37] that are most considered in the context of the qualitative definition of the grapes, thus defining:

- TSS Total soluble solid (°Brix)
- pH
- Total polyphenols (mg/L)
- Total anthocyanins (mg/L)

The WineScan™ software produced an electronic format spreadsheet output with the results obtained in the various years at each grid area. The linkage of the WineScan™ files on the other geo-referenced variables from proximal and remote sensing surveys enabled the spatial analysis described below.

## **2.5 Geostatistical analysis**

The structure from motion (SfM) software Agisoft Metashape® (St. Petersburg, Russia) has been used to process RGB and multispectral sensor images. Due to the information on the metadata for each image pixel, it was possible to obtain the vineyard's geometric and vegetative properties.

The orthomosaics obtained from the SfM software were implemented with the other vegetation and production indices described above, and every survey dataset was analysed by the Geographic Information System open source QGIS® to extract the vegetative indexes and production value for the 62 experimental field areas.

Successively, the geostatistical model parameters acquisition proceeded through two separate steps involving QGIS® and ArcGis. The datasets from the surveys conducted between 2021 and 2023 were analysed using QGIS® software to develop the most accurate forecast model. QGIS®, integrated with R script, was used for the exploratory data analysis and the definition of the best-fitting model, including the parameters of Lag distance, Sill, Partial Sill, and Range necessary to proceed with the geostatistical analysis.

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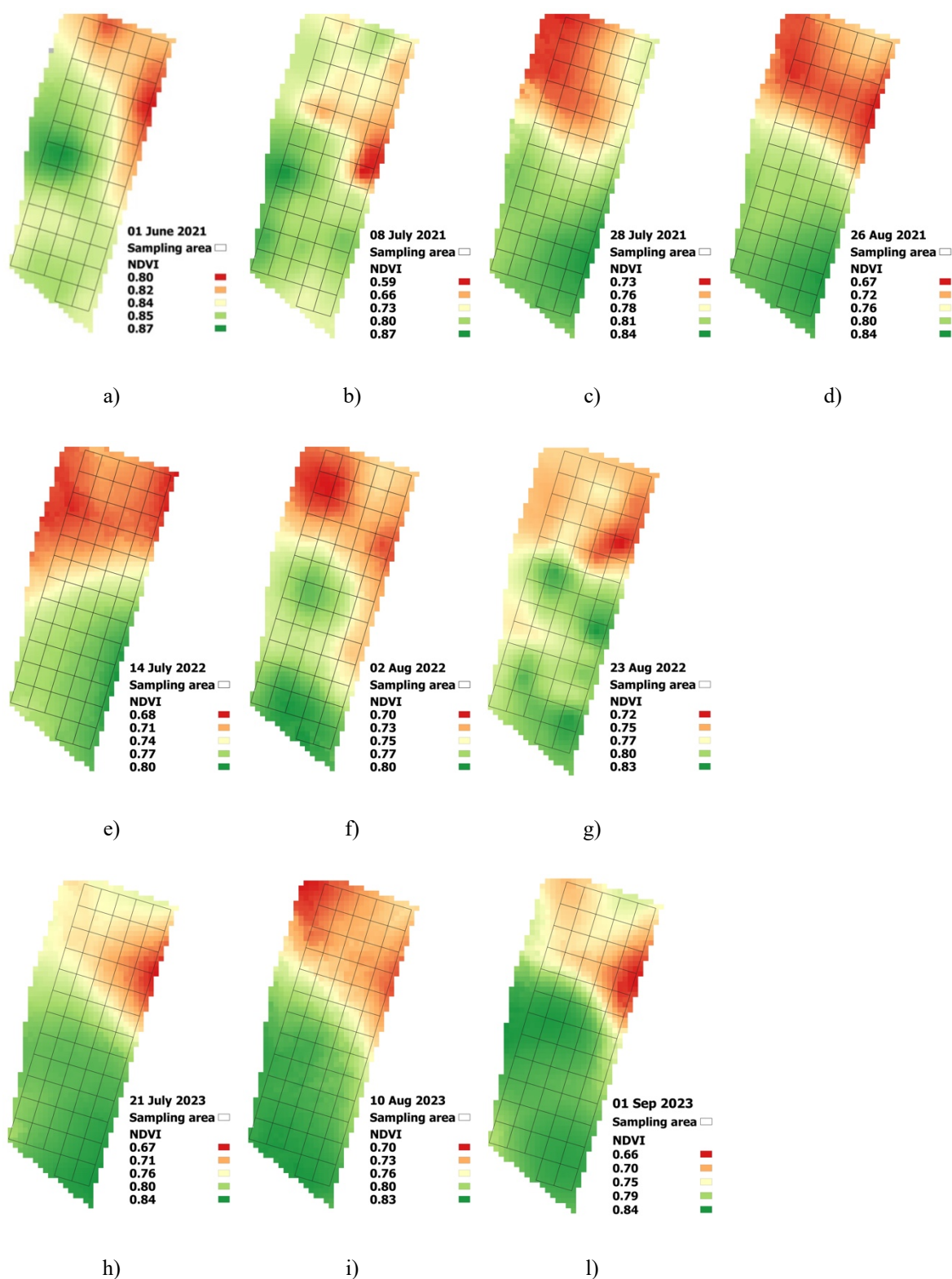
After obtaining the output parameters for each variable on QGIS, they were implemented on the ArcGis software. Specifically, the "Geostatistical Wizard" tool with the Ordinary Kriging technique was used to generate variability maps and spatial distributions of the variables within the study area. In conjunction with statistical interpolation, a correlation matrix was performed for each dataset, comparing the variables observed during the surveys. For each year, the final sampling date was associated with the harvest dataset to combine remote and proximal sensing variables with qualitative characteristics.

### **3. Results**

The following subsections outline the variables evolution during the survey years. The results are structured by dividing the canopy VIs acquired by the UAS and proximal tools as MFA and Pressure Chamber. After, quanti-qualitative analysis obtained from harvesting information and WineScan<sup>TM</sup> analysis will be discussed.

#### **3.1 Aerial measurements**

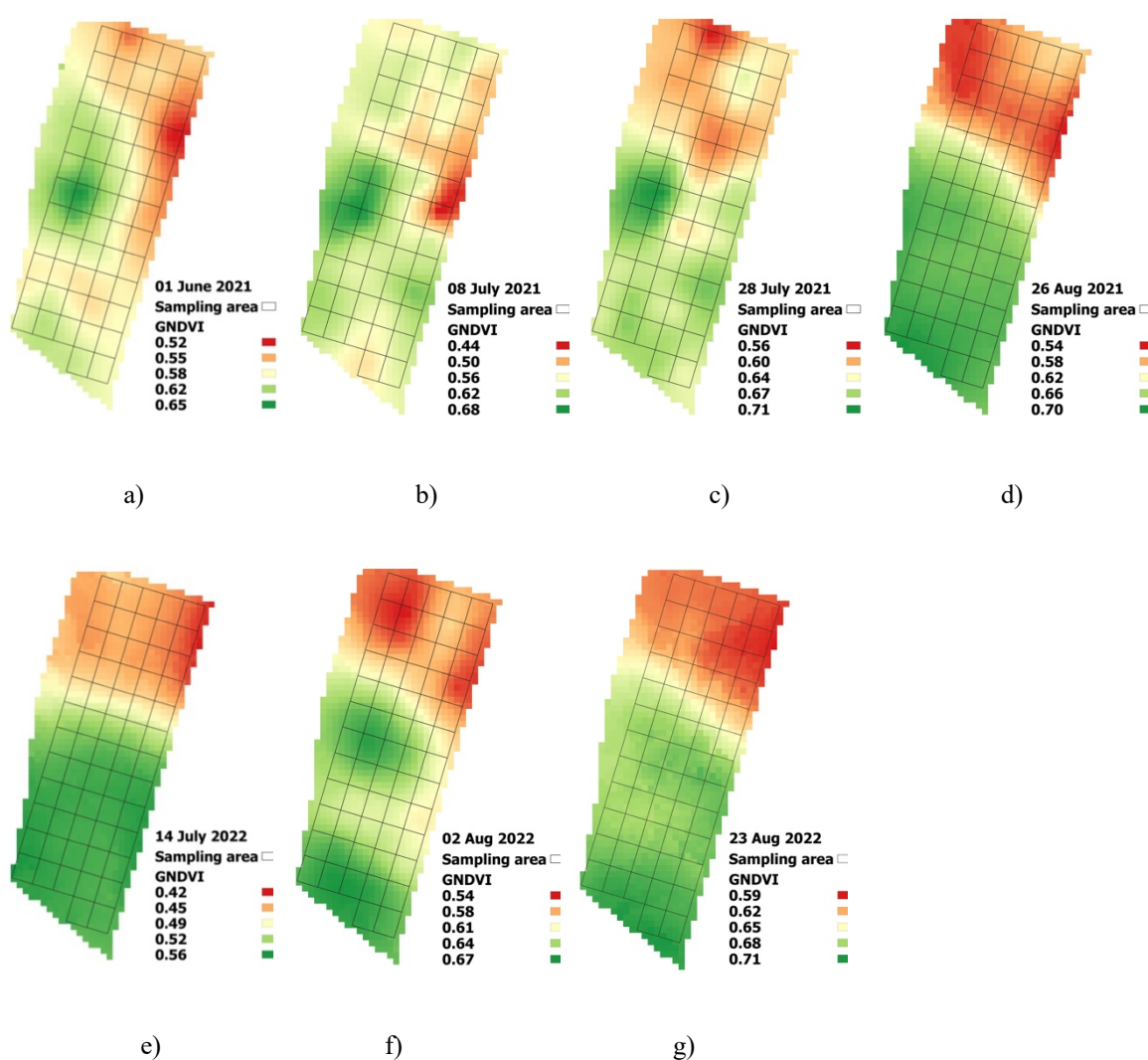
The NDVI maps derived from the geostatistical analysis reported on Figure 4 shows how the data distribution within the field split into two macro-areas with two different suggested vigour trends. Generally, most of the NDVI maps identified a constant regionalised trend over the three years, denoting two macro-areas of high and low vigour separated by a transversal septum oriented on NE-SW direction in the mid-part of the field. Furthermore, the NDVI index rapidly peaks to high-level values during the period of maximum vineyard activity (Figure 4b, 4f, 4h), achieving its maximum value at around 0.80 - 0.85, and remains stable until the end of each year, suggesting a rapid saturation and the derived low sensibility of the index during the latest growing stages. However, the NDVI index demonstrated to distinguish different areas since the first survey of each year when the stress phenomena started to manifest within the field. This index was also able to find different patterns during the early stages of the crop when these discriminations were not yet visible. The NDVI confirmatory response for the vines evolution during each survey year is considerable as an appropriate and valid tool to separate different vigour areas within the vineyard, observing how these areas may extend or enclose during the succession of the various phenological phases. Such multi-temporal scale observation, as seen in the maps (from 4a to 4j) in Figure 4, provides a valuable SSD for discrimination and subsequent vineyard management. The NDVI index maps obtained during the surveys are shown below.



**Figure 4.** NDVI maps obtained from geostatistical analysis during the three-year monitoring. The maps are ordinated in rows, following the survey year, and in columns, during the phenological phases. For each map, the 62 sampling areas within the field are marked with the black grid, as defined in Figure 1.

The variable GNDVI kept a uniform trend over the three survey years, only observing a period of readjustment in the variable's distribution in the year 2021 (Figures 5a, 5b, 5c) to display a profile similar to that evinced by the NDVI on the last date (Figure 5d). In the four

surveys conducted in 2021 (Figures 5a, 5b, 5c, 5d), GNDVI values remained overall constant, with some slight variations during the season. The following year, however, shows a significantly different trend from the previous year; as noted in Figures 5e, 5f, and 5g, the values are different during the phenological phases. In Figure 5e, the maximum value of the range corresponds to the minimum of the following survey, shown in Figure 5f, and then stabilises until the end of the year 2022 (Figure 5g). The third year proposes an intermediate situation between the trends observed in 2021 and 2022: from a numerical point of view, in fact, the range of values is similar to that observed for the year 2021, remaining constant throughout the vegetative course; from the viewpoint of data distribution, on the other hand, the resulting maps (Figures 5h, 5i, 5l) propose a trend very close to that observed in the year 2022. Figure 5 below summarises the evolution of the GNDVI index along the sampling areas during the three years of experimentation.



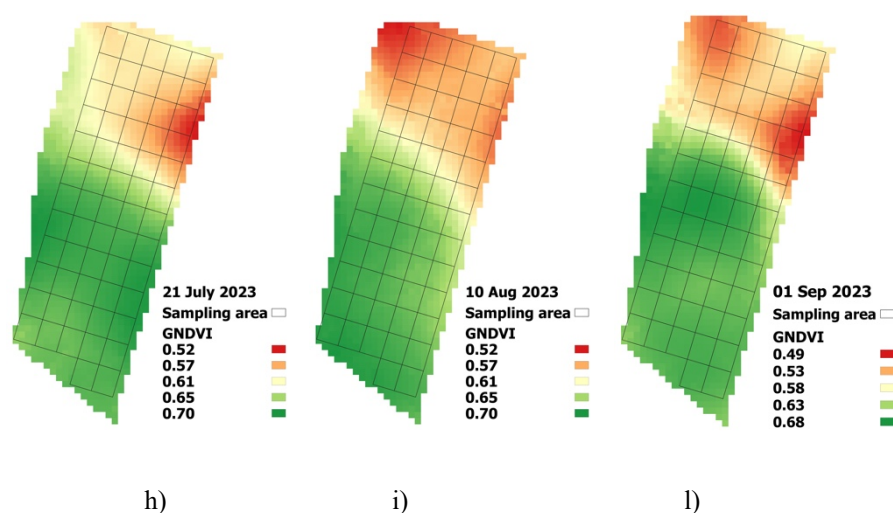


Figure 5. GNDVI outputs during the three-year monitoring. The maps are ordinated in rows, following the survey year (2021, 2022, 2023), and in columns (day of survey for the year), during the phenological phases. For each map, the 62 sampling areas within the field are marked with the black grid, as defined in Figure 1. The GNDVI data can suggest the plants evolution through the year, defining the field evolution.

Differently from the relatively homogeneous trend presented by the NDVI and GNDVI indices, the NDRE showed a different pattern. The maps in Figure 6, obtained from the geostatistical analysis, indicate a tight window of values with areas of high and low vigour spatialised differently during the three monitoring years.

Starting from the 2021 survey year (Figures 6a, 6b, 6c and 6d), the area identified with the highest NDRE values belongs to the central field area, distributing numerous hot-spot zones of low vigour at various perimeter points around the field. The trend proposed also remains unchanged for the 2022 surveys (Figures 6e, 6f and 6g), where a marked separation begins in August and September (Figures 6f and 6g). The year 2023, characterised by two surveys (Figures 6h and 6i) only, identified a critical macro-area in the north-eastern part of the vine, gradually enhanced from the initial overview in August 2023 (Figure 6h). Overall, the restricted window of values that characterised the three-year survey period for the NDRE index could be the reason for the lack of separation of high and low vigour areas, compared to what observed for the previous vegetational indices.

The maps obtained by geostatistical analysis are inserted in the Figure 6 below.

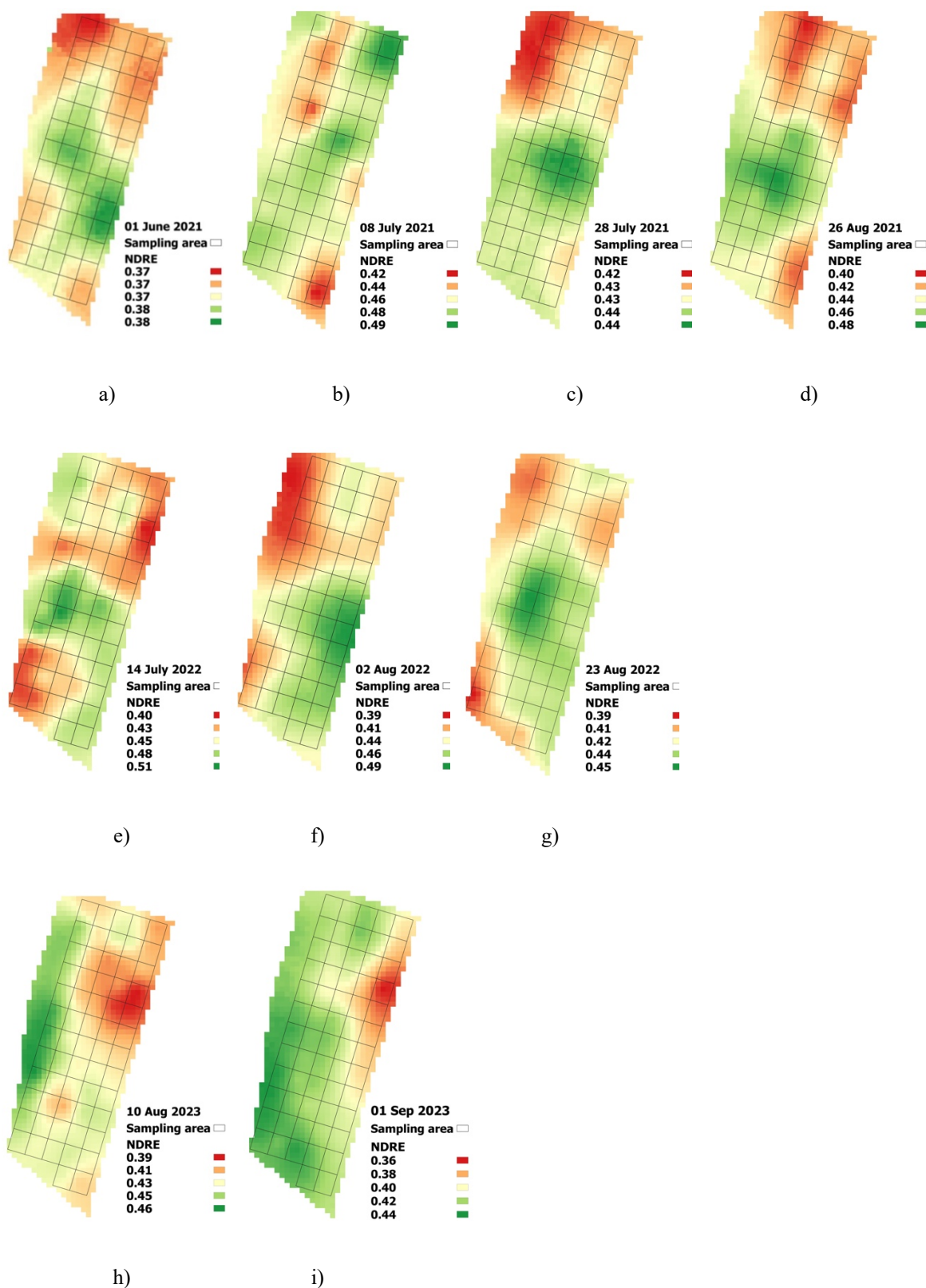
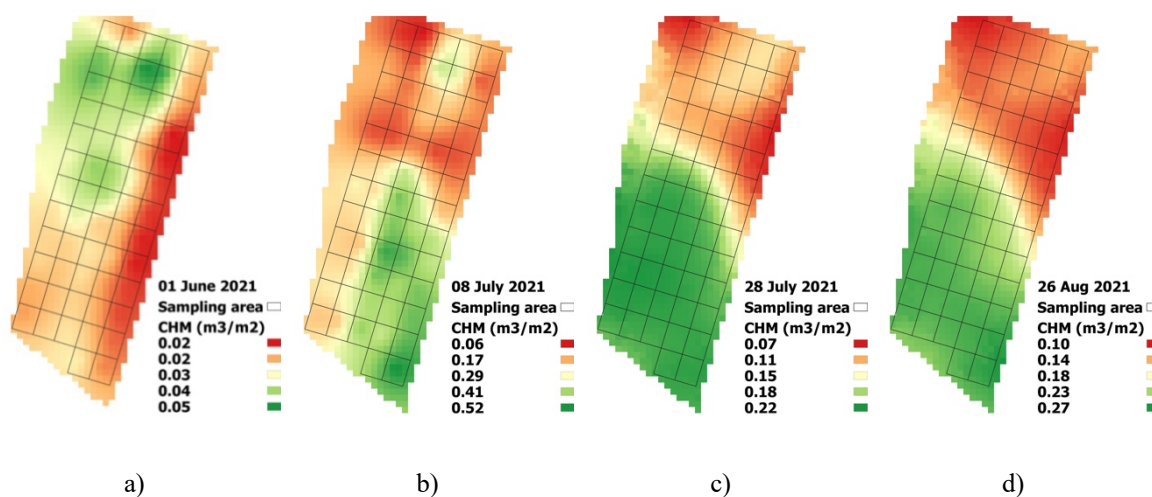


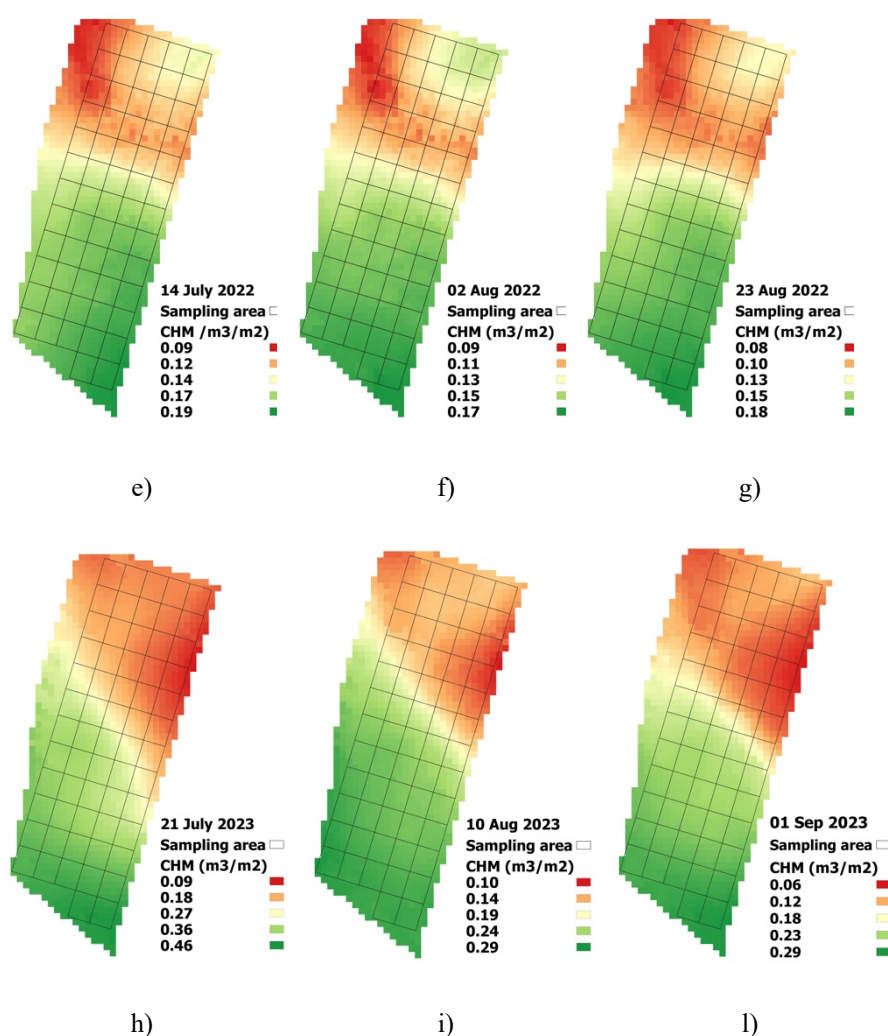
Figure 6. NDRE evolution during the three monitoring years along the 62 sampling areas marked with the black grid. The maps are ordinated in rows, following the survey year (2021, 2022, 2023), and in columns (day of survey for the year), during the phenological phases and in the panel the multitemporal index evolution. For each map, the 62 sampling areas within the field are marked with the black grid, as defined in Figure 1.

The CHM output maps in Figure 7 identify a homogeneous canopy development, suggesting what was extrapolated previously by VI NDVI, GNDVI, and SFR\_G.

The first 2021 survey, 01 June (Figure 7a), the CHM differs from  $0.02 \text{ m}^3/\text{m}^2$  to  $0.05 \text{ m}^3/\text{m}^2$ . It is an exception to the average trend observed on all the other survey dates, probably due to the absence of stress phenomena. It also proposes an opposite trend to what was observed in all other surveys, reversing the two macro-area values. The other 2021 surveys (Figures 7b, 7c, 7d) begin to widen the values window, identifying a higher index in the southern area and a gradually depressing area in the northern side. Observing Figure 7b, a lower-vigour area on the west part runs along two monitoring experimental design rows, and its unusual trend owes to the ordinary management activities of the vineyard, such as green pruning operations, operated and not completed during the survey (highlighting an high response of CHM to canopy volume modification). The pruning operation significantly decreases the plant heights and reports this alteration in the information acquired by UAS. On that date, the high-vigour data peaks at its maximum at  $0.5 \text{ m}^3/\text{m}^2$  before pruning, which vanished since the following survey (Figure 7c).

Except for the first two surveys in 2021, the other monitoring days in 2022 (Figures 7e, 7f, 7g) and 2023 (Figures 7h, 7i, 7l) replied positively to the discrimination of homogeneous areas for differentiated vineyard management. The data obtained from the processing software provided a very similar trend to that observed with the VI and MFA, observing only moderate differences in terms of windows of values during the three growing seasons. It showed that Figures 7e, 7f and 7g identify a trend of strongly reduced values concerning what was observed in 2021 and 2023, which are overall similar. As described above, during the various years, the farmer executed green pruning operations in mid-early July time, opting for different pruning intensities, as found in the relative maps in Figure 7, which were more influential in the 2022.





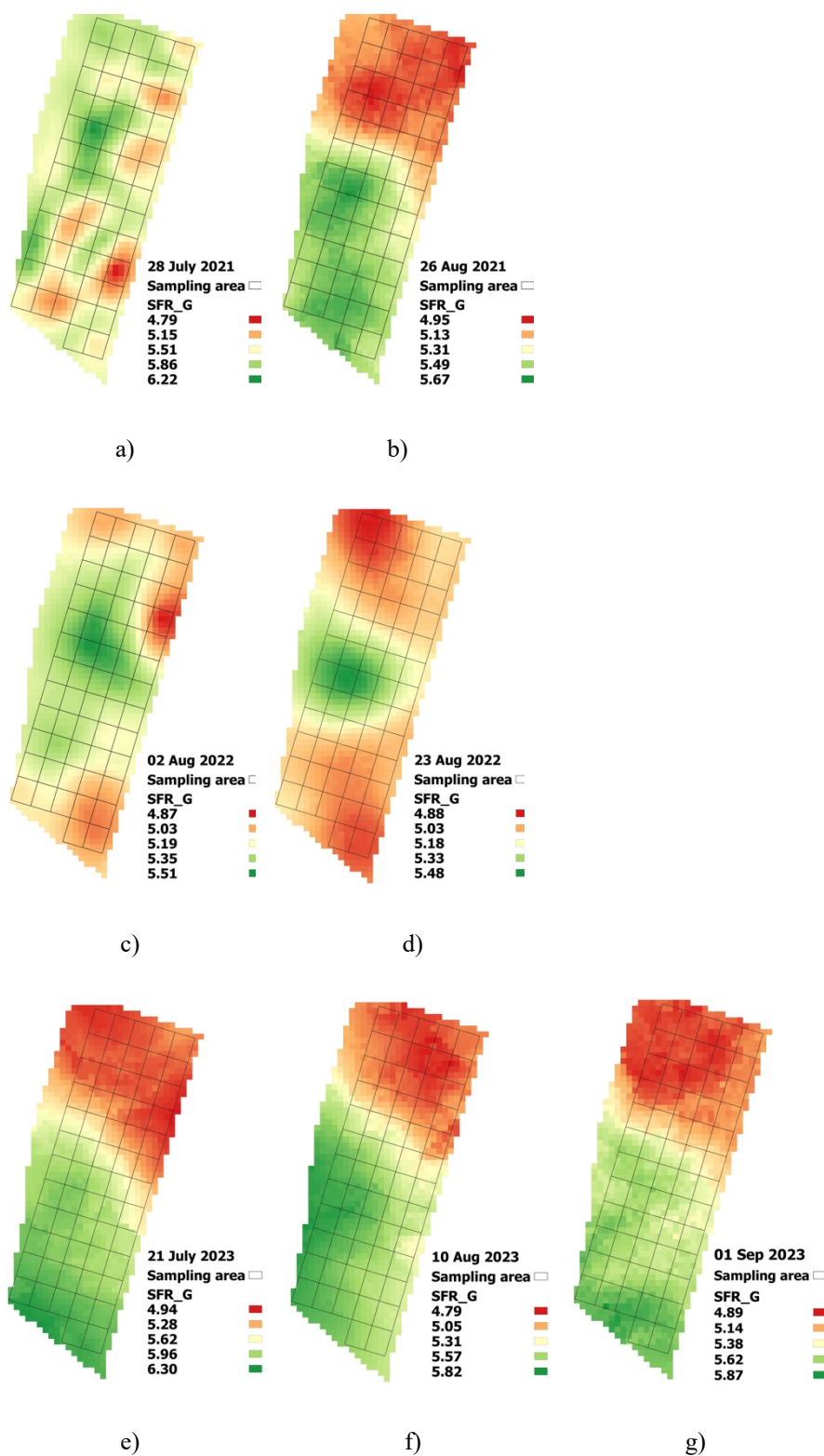
**Figure 7.** CHM evolution during the surveys along the 62 sampling areas. The constant trend observed in the 2021-2023 monitoring is altered in Figure 7a and 7b, where the early survey in Figure 7a evidenced a canopy grow anticipation in the northern area; Figure 7b results represent an alteration due to canopy pruning operation.

## 3.2 Proximal-sensing analysis

### 3.2.1 Fluorimetric index

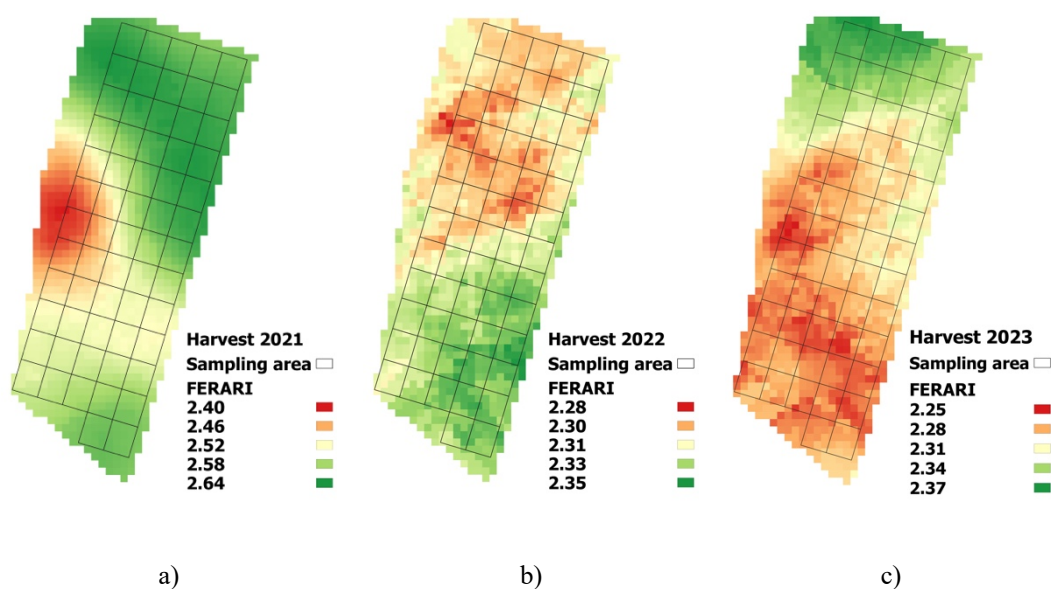
As observable in Figure 8, the leaf analysis performed by the MFA fluorimetric sensor on the 62 experimental areas provided a heterogeneous development during the three survey years. In 2021, the 28 July monitoring day (Figure 8a), did not provide a clear map for an early field subdivision, as it provided a hot-spot pattern along the experimental plot. Only on the last 2021 monitoring (Figure 8b) the map generates a suitable identification of different macro-areas. Differently from 2021, the 2022 surveys identified on both dates (Figures 8c, 8d) a single area of high photosynthetic activity located in the central part of the field. While in the first 2022 survey (Figure 8c) the low vigour areas are expanded in the extreme locations, Figure 8d identifies a notable vigour reduction around the field, reducing high activity areas to a few sample areas. The three maps obtained in 2023 (Figures 8e, 8f,

8g) recall what was observed in the NDVI and GNDVI indices and provide two different section areas as the VI suggested. SFR\_G index evolution during the survey is represented in Figure 8.



**Figure 8.** Output maps obtained from the SFR\_G MFA index along the 62 sampling areas. The hand-held sensor is susceptible to site-specific analysis, where the sampling option becomes delicate for statistical processing. It can be observed how the proposed scenario changes during the phenological phases.

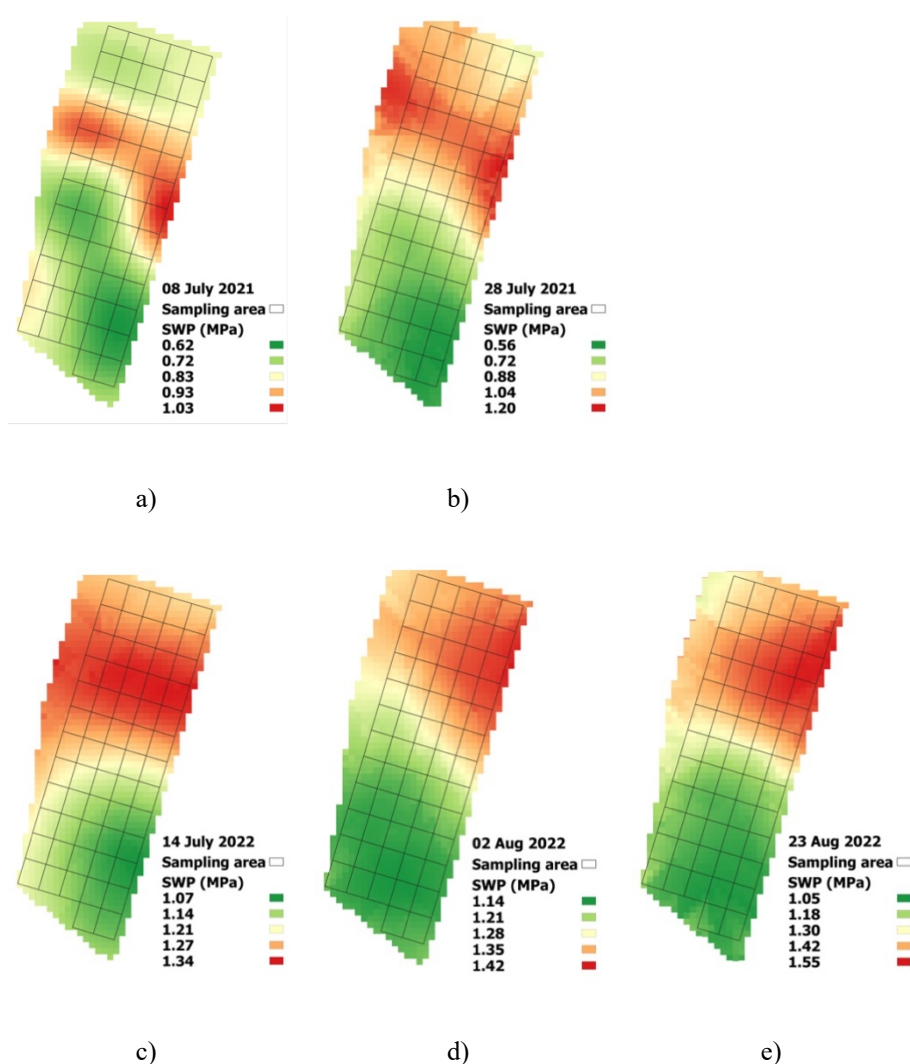
In proximity to the harvest, the MFA proximal instrument estimated the quality characteristics of the bunches. As anticipated in Chapter 2.3.1, the index selected for experimentation was the FERARI and Figure 9 shows its progress. As illustrated in Figure 9a, the first observation year provided a higher window of values than that observed in the following two years (Figures 9b and 9c), in which the maximum value suggested is lower than the minimum value observed in 2021. The FERARI maps, considering the low variability observed in the analysis, suggest a homogeneous redistribution of values within the field, in which areas of higher and lower vigour interchange according to the variability of the agricultural year. This trend suggests a reduction in spatial variability due to the gradual smoothing of the differences between minimum and maximum within the field, indicating a situation of widespread homogeneity. The FERARI index maps are resumed below.



**Figure 9.** FERARI index extracted from MFA sensor. The low FERARI variability on the 62 sampling areas perceived by the sensor explain the different vigour areas during the surveys.

### 3.2.2 STEM Water Potential

The application of traditional analysis approaches, such as the SWP, allowed the comparison of consolidated analysis methodologies with modern techniques. The field inspection by pressure chamber analysis was performed in 2021 and 2022 during the mid-summer months (Figure 10). An initial situation of a critical area localised at the maximum slope within the field (Figure 10a) followed an expansion of the highest potential area into a wider sector comparable to that proposed by the other VIs. This trend was repeated for 2022 (Figures 10a, 10b, 10c), identifying the same areas of high and low SWP and a higher level of potential, rising from about 0.6 MPa in 2021 to over 1 MPa in 2022.



**Figure 10.** Stem Water Potential during 2021 and 2022. The two vigour areas identified by the SWP technique provide a homogeneous information for different management strategy.

### 3.3 Production Analysis

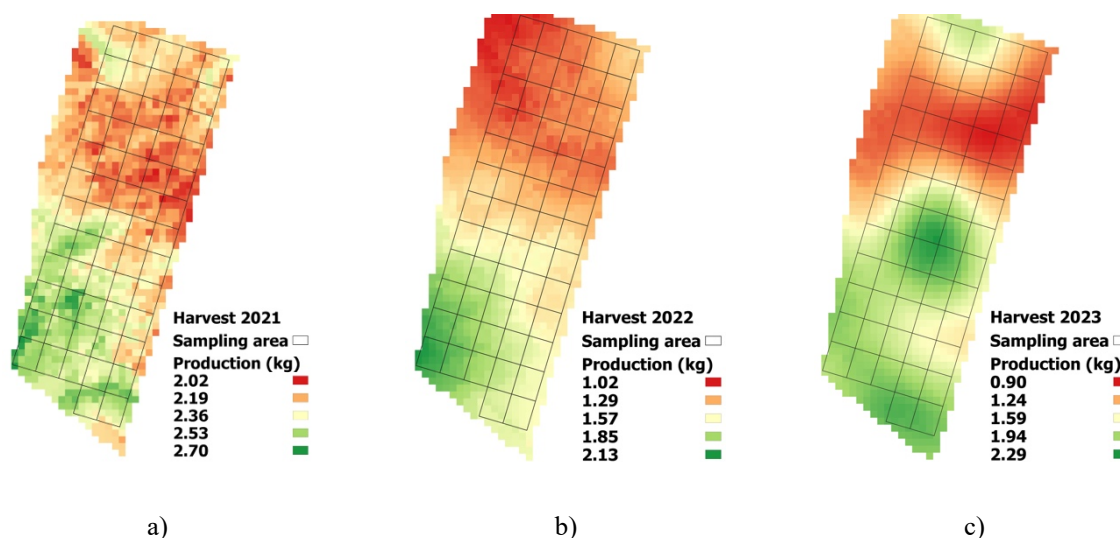
During the harvesting operations, production data were acquired for each plant in the experimental design block area, considering the average bunch and berry weight over the production. The results are described as follows.

#### 3.3.1 Plant Production

Figures 11a, 11b and 11c display the results of the three harvesting operations. The geostatistical maps are created by sampling the central plants identified in the experimental design blocks. Although the maps colour palette shows a homogeneous trend, it is necessary to make some observations. Figure 11a illustrates the production variation among the sample areas, which vary from 2 to 2.7 kg, on a gradual value increase from N-E to S-W. Figure 11b shows a similar representation to the one proposed in 2022. Despite the 2021 trend, there is an evidenced decrease in plant production, ranging from a minimum of 1 kg to a maximum of 2.1 kg. The third year (Figure 11c) identifies two homogeneous areas within the field,

finding a significant difference in values compared to the previous observations. The minimum production was 0.9 kg in the northern part of the field, while the maximum was 2.3 kg in the central part. The trend observed in the three survey years indicates that production has decreased on average. The average production in 2021 was 2.4 kg/plant, which decreased to about 1.6 kg/plant in 2022 and 2023.

As discussed later, the harvesting variable was influenced by the bunches pruning operation executed during the vineyard management, executed without any DSS described in this work and during the experiment.



**Figure 11.** Plant production along the 62 sampling areas. During the harvesting operation, the production value provided different information among years, but suggesting the same vigour trend.

### 3.3.2 Bunch weight

Comparing the Figure 11 production maps, the bunch weight output maps in Figure 12 manifested a similar trend. The observed difference between the extreme values between Figure 12a and 12c harvest are almost superimposable on the Figure 11 results. The bunch weight flow through the three monitoring years decreases from an average value of 0.320 kg in 2021 to 0.200 kg in 2022, rising to 0.270 in 2023.

The values window replicates what was observed previously in Figure 11 for the harvesting data, proposing a range in Figure 12a and Figure 12b of around 150 grams, while in the 2023 harvest, this difference increases significantly to 250 grams due to the high variability observed in the field.

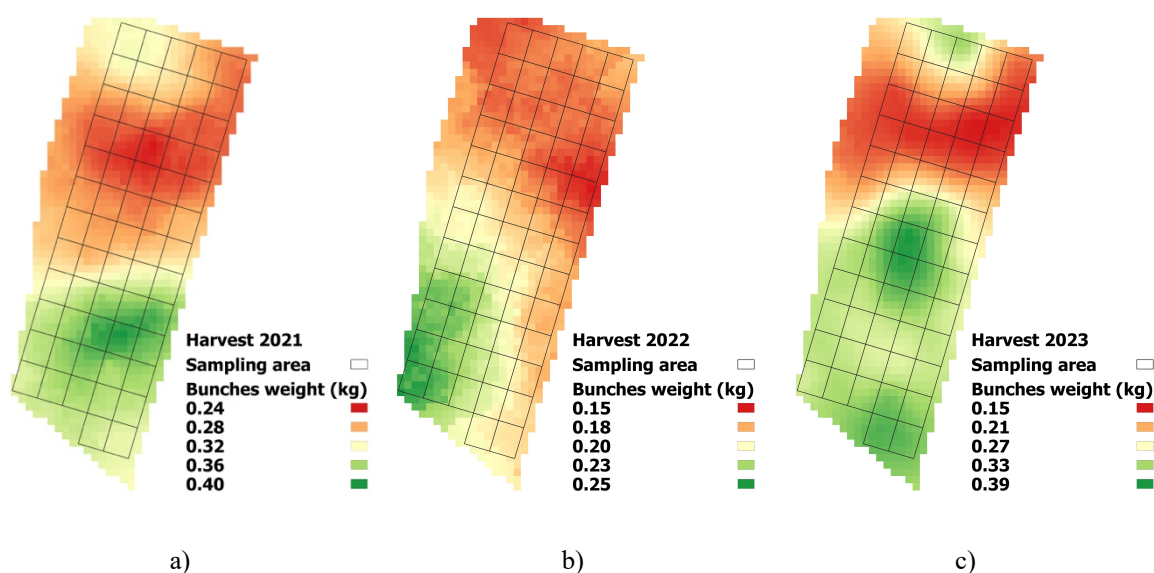


Figure 12. Bunches weight progress during the survey. The information provided along the 62 sampling areas by the analysis converge to the production variable.

### 3.3.3 Berry weight

The results obtained on Figure 13 for berry weight confirm a trend coherent with the previous variables concerning the different vigour areas identification.

If berry weight responds positively in the subdivision of two macro-areas of vigour from a general perspective, the variation in berry weight during the harvest identified a generalised inflexion during 2022 (Fig. 13b), in which a decrease in average weight is observed throughout the field.

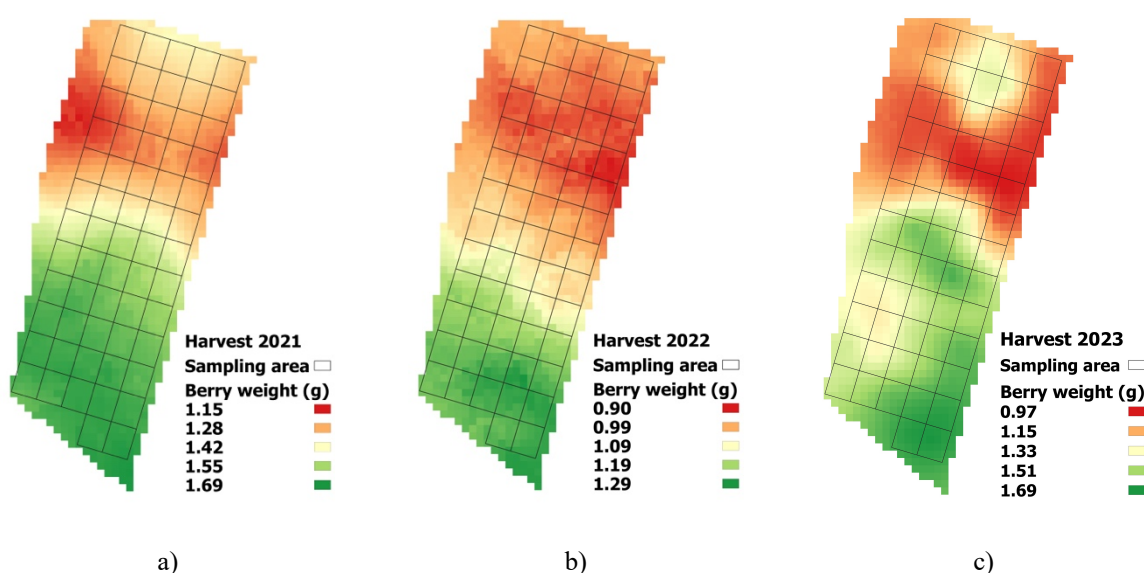
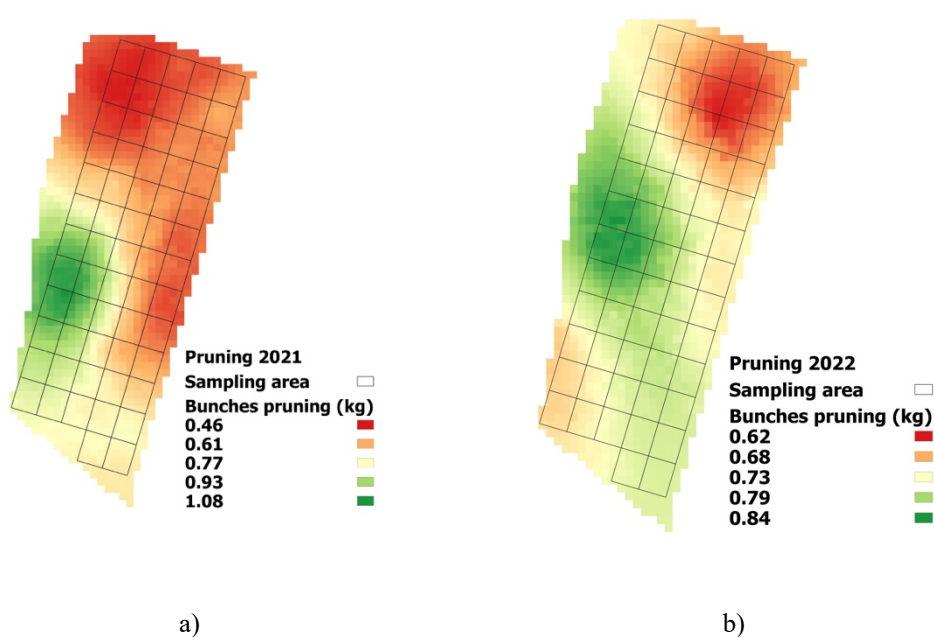


Figure 13. Berry weight distribution maps along the 62 sampling areas. The trend estimates a homogeneous variation within the field, losing the high variability values observed in Figure 11 and Figure 12.

### 3.3.4 Bunches pruning

As anticipated, during the green pruning management, the operator executed cluster thinning operations to manage the canopy in each field part. During this operation, the farmer choosed the number of bunches to prune based on his experience and the historical knowledge of the field. A significant consideration concerns the pruning entity during the first two experimental years when the pruning operation was monitored. The first year (2021, Figure 14) showed a consistent gap between the most intensively pruned areas and the most critical ones, amounting to a difference of around 0.6 kg, while in the following year, the pruning observed a considerably smaller window of values, around 0.2 kg.

As observable in Figure 14, the management of bunches pruning found similarities to what was extracted by the CHM vegetative variable used in the experiment, where the operator opted for more intense pruning in the vigorous areas compared to the more depressed areas of the field. In particular, the areas identified as "higher vigour" by the operator coherently followed what the UAS surveys suggested, including CHM and canopy vegetation indices.



**Figure 14.** Bunches pruning operation during 2021 and 2022 along the 62 sampling areas. The operation performed from the operator provide a management evolution during the years, where a wider pruning area along the field is defined (Figure 14b), respecting the single hot-spot zone in the first year (Fig 14a).

### 3.4 Must analysis

As anticipated, the instrument used for qualitative analysis is the FOSS WineScan™, capable of obtaining numerous information through the main enologically influential variables. For the qualitative analysis, Brix, pH, anthocyanins, and polyphenols variables have been analysed. All these parameters were elaborated during the three monitoring years.

### 3.4.1 Total soluble solid

The first information provided by WineScan™ analyses and described in Figure 15 regarded the data distribution of Total Soluble Solid (TSS) along the 62 sampling areas, expressed as Brix grade. During the experiment, the Brix data estimated by WineScan™ highlighted a variable trend.

In the first year, likely VIs and production data observed above, the 2021 Brix map (Figure 15a) identified two main macro-areas distinguished along the field. In 2022, the Brix analysis (Figure 15b) did not provide a suitable map to discriminate homogeneous areas due to the numerous hot-spot zones, observing a wider window of values concerning the previous year in a range of 6.4 °Brix. In the last monitoring year (2023), the Brix map (Figure 15c) represents a similar map to 2021, which becomes much higher and uniform, lowering the gap between critical and vigorous zones to around 2° Brix.

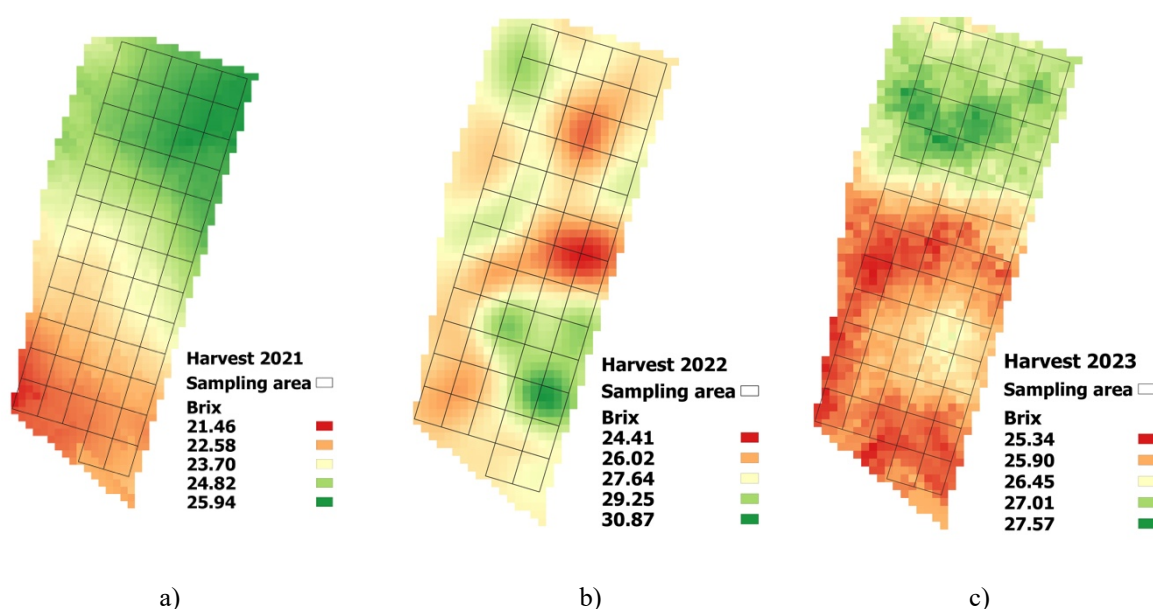
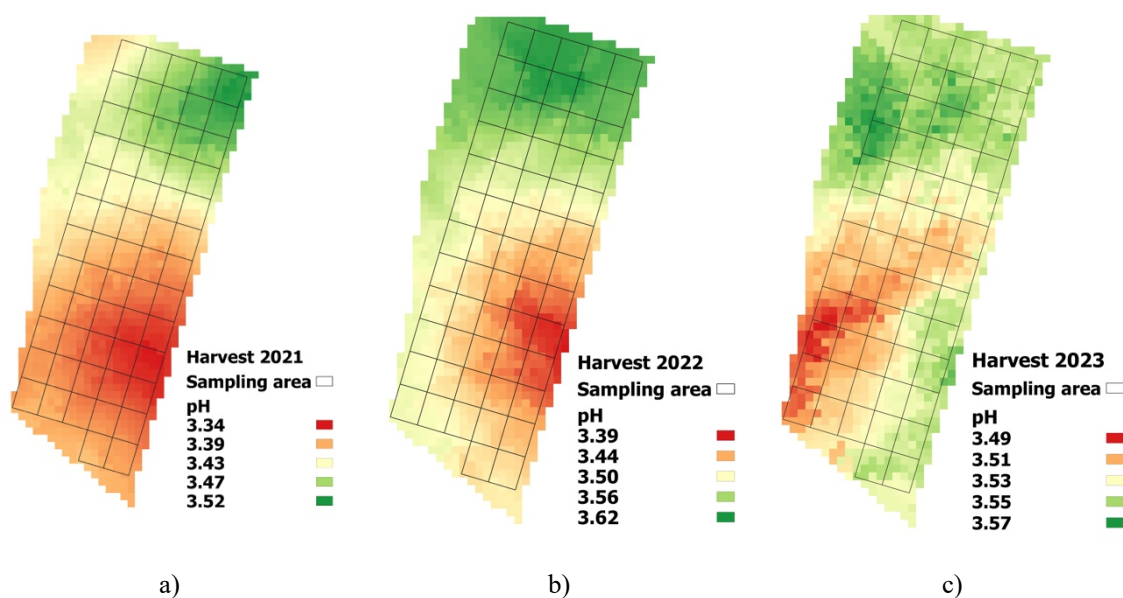


Figure 15. Brix grade analysis by WineScan™.

### 3.4.2 pH:

After conducting the Brix analysis, pH evaluation was chosen for field variability discrimination. Although pH analysis is typically used for wines and not for musts, it helped to suggest quality differences among the samples analysed. Indeed, the pH window values are limited, where the higher pH zones are at the northern part of the field, according to the higher Brix values. The different pH zones identified opposite trends compared to the Brix values areas. It suggests that chemical characteristics may vary within the field area due to irregular maturation, indicating different ripening stages. Below (Figure 16) are the three vigour maps highlighted by the pH variable. In the 2023 (Figure 16c), even though the pseudo-colour palette always tends to identify a more acid pH zone in the valley area, the

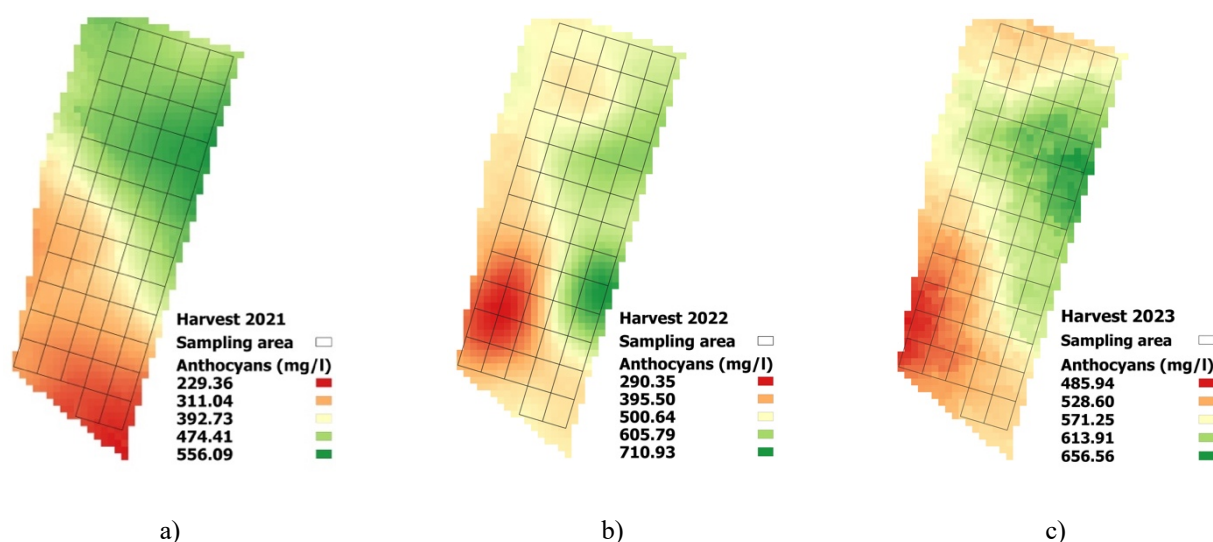
window of values suggests a variability of around 0.08, much less than the 2021 and 2022 windows (Figures 16a and 16b, respectively).



**Figure 16.** pH trend along the 62 sampling areas. The values from 2021 (16a) to 2023 (16c) identify a variability reduction along the field.

### 3.4.3 Total Anthocyanins

The analysis of the berry's anthocyanin contents showed an attractive evolution, which underlines how a restricted area such as the experimental field can lead to significant variations in the qualitative properties of the must. As for the other variables described, the spatial distribution of anthocyanins identified a division into two main macro-areas (Figure 17). As indicated in the maps, the anthocyanin data maintained a similar development between survey years. Similarly to the other WineScan<sup>TM</sup> variables observed previously (Figures 15 and 16), a well-defined initial condition of the data series (Figures 17a) was followed by an increase in both values and their range in 2022 (490 mg/L, Figures 17b). The increase in values halted in 2023 (Figure 17c), attesting to a narrow window of values (170 mg/L) and a minimum anthocyanin value significantly higher than that observed in the first year of measurements (Figure 17a).

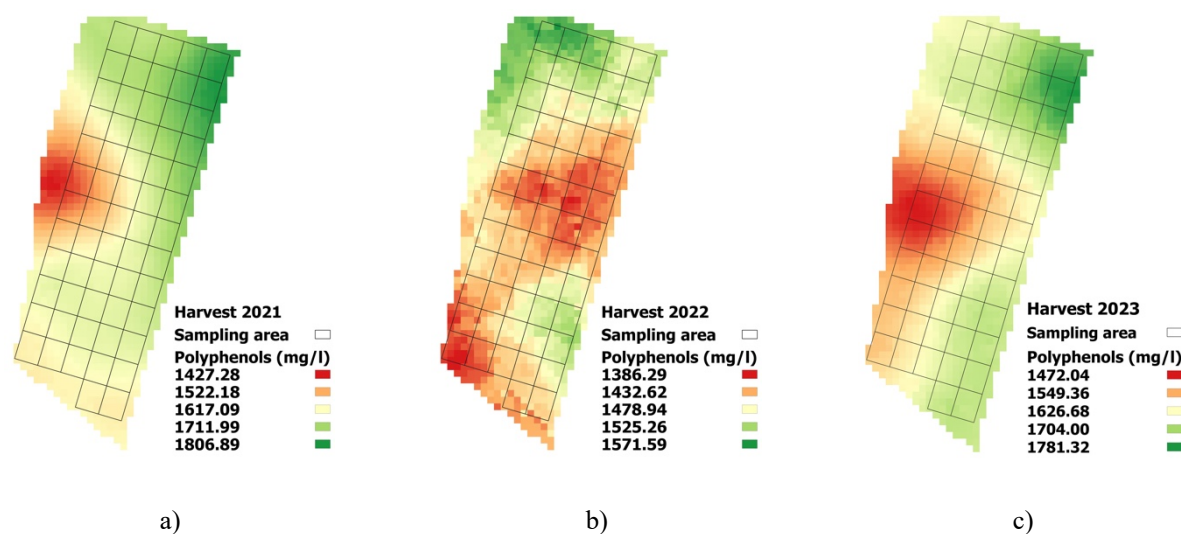


**Figure 17.** Anthocyanins content. The WineScan™ analyser provide similar trend during the three monitoring years along the 62 sampling areas, suggesting a qualitative evolution of anthocyan content from 2021 to 2023.

### 3.4.4 Total Polyphenols

As shown in Figure 18, the analysis of polyphenols demonstrated a similar trend to what was observed previously. In the case of anthocyanins, there was a general increase in the content of these macromolecules per unit volume. However, in polyphenols, there was a slight decrease in 2022 (Figure 18b) compared to the previous year (Figure 18a), returning to 2021 levels during the last harvest in 2023 (Figure 18c).

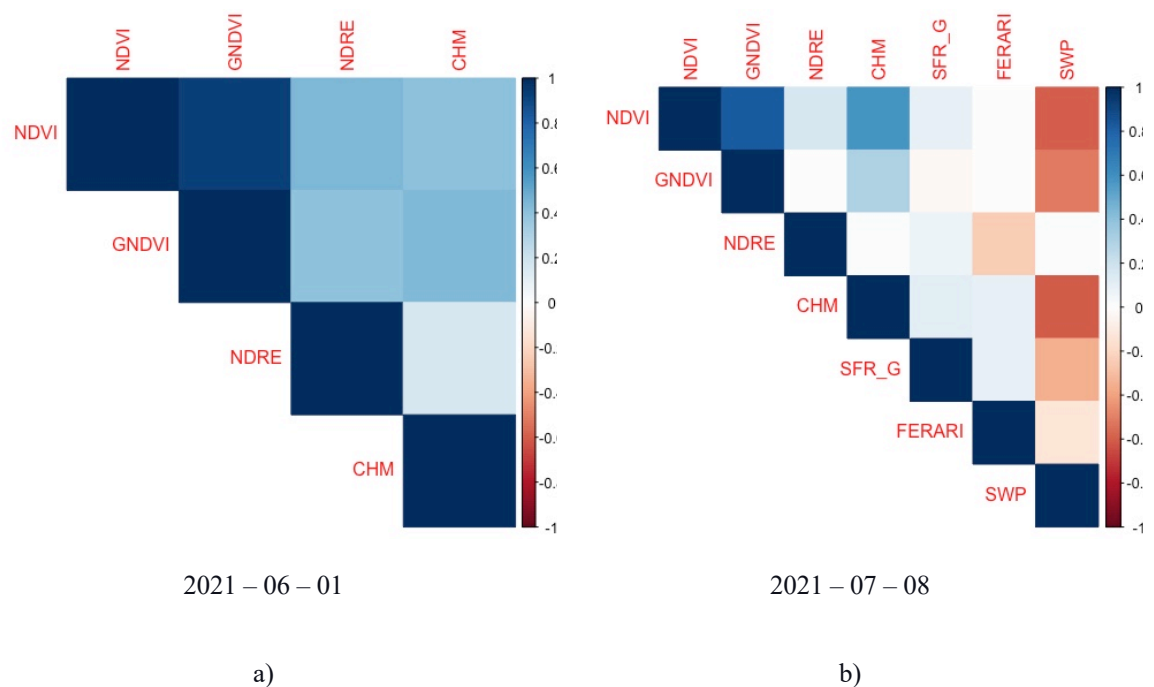
The low-vigour area identified in Figure 16a was a hotspot of a few plants located in the central nodes of the western rows. The following year, the values across the field were slightly lower and distributed over the experimental field, covering more than half of its surface. In the final survey year, the lowest area identified in 2021 reappeared in the same position, albeit distributed over an extensive portion of the field.

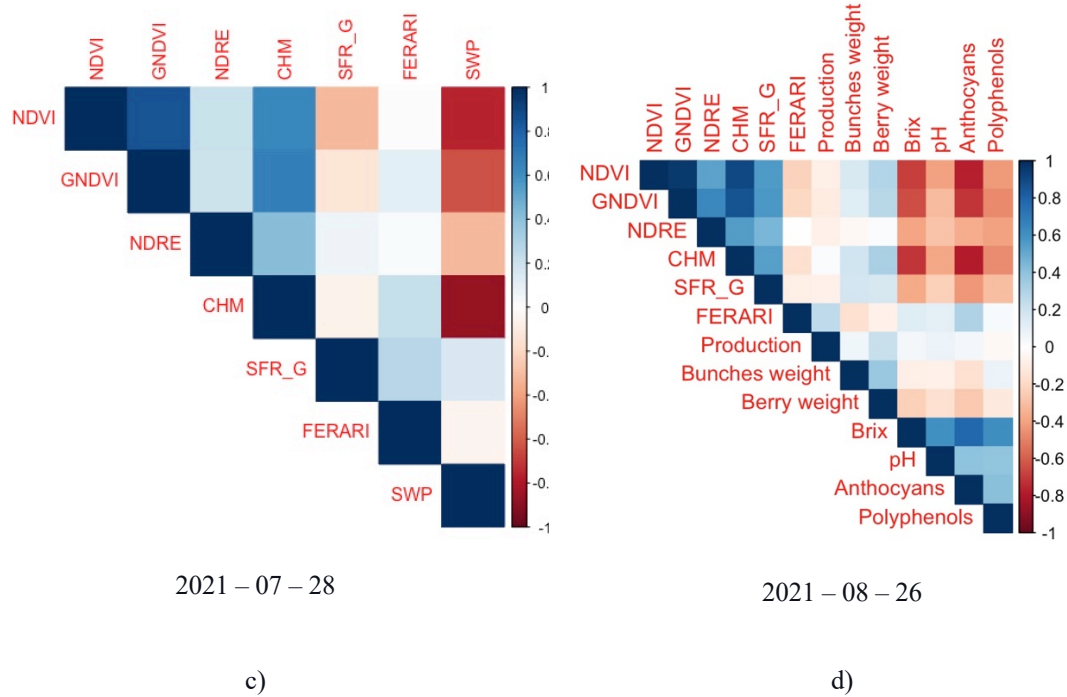


**Figure 18.** Total Polyphenols from WineScan™ analyser. The variable trend observed in 18a and 18c is interrupted by the 2022 harvest 18b, when the low-vigour area is expanded along the field.

### 3.5 Correlation Matrix

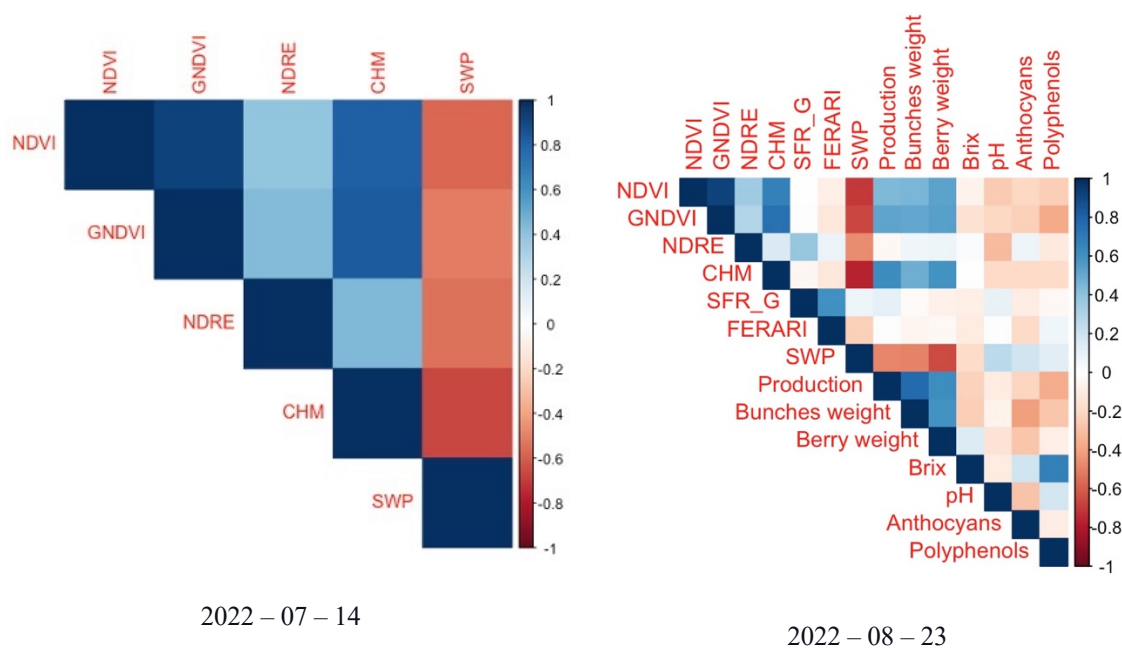
During the dataset analyses, correlation matrices were obtained to analyse the relationship between all variables. Figure 19 shows the evolution of the matrices for the year 2021, identifying through the heatmap graphs the correlation intensity and direction between the various indices. As previously observed in the geostatistical maps, the correlation between the indices is significantly high between NDVI, GNDVI, and CHM, suggesting a direct proportional relationship between the three vegetation indices on all monitoring days (Figures 19a, 19b, 19c, 19d). Except for the anomalous trend in production data per plant (*Production* variable, Figure 19d), there is a direct correlation between the VIs and the bunches and berry weights. Among the manual field survey activities, SWP well delineated the inverse proportionality concerning the main VIs obtained from UAS, observing a correlation close to 90% with the geometric variable CHM on the end of July 2021 (Figures 19c). The MFA sensor did not establish significant correlations during the monitoring surveys, except for a low direct correlation for the leaf index SFR\_G and an inverse correlation of the FERARI index (Figures 19d). This result confirms what was observed in the qualitative variables related to anthocyanins (Figure 19d), where MFA and WineScan™ present a direct relationship regarding anthocyanin content. The variables Brix, pH and Polyphenols are all negatively correlated with the VIs obtained from UAS, thus estimating high values of the qualitative variables as a function of low VIs.

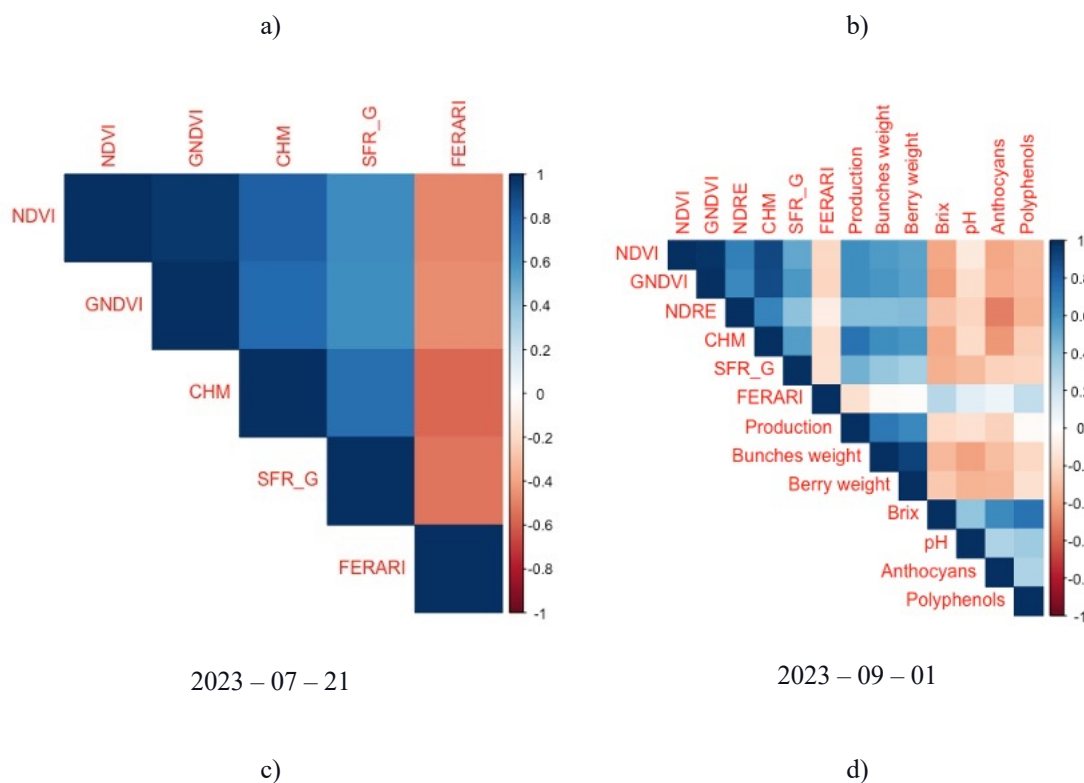




**Figure 19.** Matrix correlation about 2021 season. The correlation matrix extends to negative correlation (-1, red) to positive correlation (1, blue).

A selection of the matrices correlation results for the years 2022 and 2023 are shown in Figure 2, confirming what was observed in Figure 19. In fact, in the first monitoring of the two years (Figures 20a and 20c), the inverse proportionality trends of SWP and FERARI index with VIs obtained from UAS are confirmed. The harvest data (Figures 20b and 20d) represent and confirm what was obtained in the Kriging output described above. In fact, harvest 2022 shows milder correlations than 2021 and 2023, in association with the qualitative maps (Figures 15b, 16b, 17b, 18b) confirming the different variables trends.





**Figure 20.** Resume of correlation matrix during 2022 and 2023 surveys. Figure 20a and 20c represents the first survey of 2022 and 2023, while 20b and 20d include the last monitoring day of the two years linked to the quantitative and qualitative analysis.

#### 4. Discussion

Following the analysis and interpretation of the results, it was possible to identify areas that could be managed differently along the field due to the use of post-processing crop monitoring and production analysis systems.

One of the main results observed from the geostatistical maps and the correlation matrices is that the most common vegetation indices extracted from UAS, such as the NDVI and GNDVI, are strongly associated with the volumetric variable CHM. As described in Figures 4 and 5 of NDVI and GNDVI indexes, respectively, the VIs were able to consistently identify the ability of the canopy to respond differently according to its physiological pattern, conserving in every survey a vigorous area (in the lower part of the field) and one more susceptible to agroclimatic characteristics, located in the northern part of the field. A fundamental aspect that clarifies the observation of these trends is the altimetric variability of the field and how the observed variables are significantly associated with it. The field slope, shown in Figure 1b, is around 5 m in height difference, which, distributed over a NE-SW distance of around 120 m, had a considerable influence on the generation of two main homogeneous areas within the plot. In addition to the two VIs mentioned above, the CHM canopy volumetric analysis index well differentiated the two areas, showing very low vigour in the area located at higher altitudes. This observation merges with the physiological

characteristics that may develop in hilly contexts such as the one where the experiment took place, anticipating the vegetative awakening of the crop in the upper area (as observed in Figure 7a) compared to the remaining part of the field, and decreasing vegetative vigour in the central phases of growth and ripening in July and August.

The fluorimetric sensor suggested a similar result to UAS indexes, although not on all dates. As observable in Figure 8, the first and second monitoring years find just on the pre-harvest monitoring in 2021 a map that can be superimposable with the UAS indexes, whilst the 2023 year's SFR\_G index shows more reliable maps, where the differentiated management areas suggested converged on what the other indices suggested. Figure 8 also shows that the MFA fluorimetric sensor found some difficulties in homogeneous areas identification within the field. In 2021, the sensor produced a helpful map for the pre-harvest stages, allowing a supporting map for planning the field activities. In 2022, it identified a constant but alternative trend, different from any other VIs used. Only 2023 identifies (and confirms) what the other VIs and CHMs read during the three surveys. The maps analysis found a confirmatory response with the correlation matrix, where the MFA indices (Figure 19 and 20) shown an aleatory trend, especially for SFR\_G index in relation to UAS indices. Despite the satisfactory performance of the 2023 surveys by the MFA, who suggested on the early of July the separation of two macro-areas in the field, the inconstant trend of the readings does not make the instrument suitable for a stand-alone inspection activity, as canopy and bunches management actions take place up to a few weeks before the harvest, making the instrument unreliable for the work purpose. As discussed in the following paragraph, the evaluation of vegetative trends using the MFA follows the dynamics and limits observed for the SWP, both of environmental susceptibility (although the MFA is light-independent) and timing required to collect data over extensive areas. Excepting the vegetative analysis, the MFA could be a useful instrument as a validator of the characteristics of the bunches in the field [38] as observed in Figure 9, where the FERARI index identify a slightly but interesting trend, where the low and high vigour areas are strictly-dependent to the agricultural environment.

Over the main objective, the study observed the traditional and modern approaches in viticulture for crop management activities and how the evolution of these techniques can lead to new insights in the analysis and prediction of crop operations.

An important aspect to evaluate is how the pressure chamber instrument identified critical areas similar to what was observed by the vegetation indices and volumetric qualitative analyses. This comparison, in addition to the negative correlation with the CHM observed in Figure 19 and 20, suggests that simple and cheaper instruments can provide valid results

comparable to those obtained from more advanced sensors and analyses. As mentioned in section 2.3.2 concerning the instrument presentation, the monitoring operations with the SWP involved only a limited number of samples from the grid adopted in the experimental design due to the long sample preparation times and the physical movement of the operator in the field between the various sample points. This time dilation during the survey operation precludes the SWP from investigating large areas, as several operators would be required to operate simultaneously for the same number of points sampled per ha. Alternatively, reducing the number of samples for the same surface area might reduce the survey performance. As a further consideration, both SWP and UAS instruments are susceptible to meteorological variability, making the rapidity of monitoring operations crucial. For this reason, survey operations through UAS are preferred to SWP, as the vegetation data acquisition time takes significantly less time than necessary for the pressure chamber.

The pruning operations performed on the bunches (Fig. 14) proposed a positive development within the field, especially for the quanti-qualitative characteristics.

In particular, the combined GNDVI and CHM information represented a precise and high-performance tool to estimate the field intra-variability, as they did not demonstrate themselves to be susceptible to the phenomena of rapid saturation of values (i.e. NDVI) or altered by problems caused by variable brightness and reflectance (i.e. NDRE). The pruning activity on bunches (Fig. 14) by the operator reflected what was observed by the CHM vegetative vigour index and the qualitative analyses observed by UAS and WineScan™, respectively. The pruning activity, in fact, was intensified in the western part of the field, where the altitude a.s.l. is lower. Moreover, the quality maps relating to polyphenols can slightly overlap with bunch pruning operations. So, this analysis evidence that the operator's inspection and management work matched what was observed by the sensors and techniques used in this research, which represent valid candidates for differentiated management areas in viticulture.

Following the harvesting operations, the data obtained in the SSD maps confirmed the trend predicted and proposed by the VIs and other variables. Therefore, such information can lead the operator regarding field management decisions, allowing him to achieve precise and accurate information regarding the ripening of the grapes in the field through the equipment offered by UAS. Correlation matrix analysis was unable to clarify the correlation between VIs and quantitative production data over the three years of analyses. Only in the last two harvests the correlation matrix does provide confirmatory responses and define a correlation between canopy and harvest production per plant.

The must analysis performed by WineScan™ proposed similar trends to the vegetative variables previously observed. Comparing the qualitative results of VIs and WineScan™, the NDRE index showed a similar trend to anthocyanin concentration. Since VIs are useful in the final stages of the grape cycle as they estimate vine ripeness [39], the index could be a valuable resource for estimating the anthocyanin content on bunches. However, this trend shows some differences with the anthocyanin index, and further investigation is needed to confirm this relationship. The similarities observed in 2021 between the FERARI index and the trend in polyphenols suggest how fluorimetry can support analysis and management operations; the following years (2022 and 2023) showed not perfectly overlap trends to the initial one, but the evolution of the two variables observed in the final survey (2023) maintains a general field classification in two different areas. Regarding polyphenol content, the correlation matrix analysis estimated a negative correlation over the three years of measurement for NDRE, while the FERARI fluorimetric index observed an inconstant relationship over the three years. This information, correlated with map interpretation, demonstrated a valuable support for homogeneous area identification within the field.

However, the trend of all variables observed over the three years of the survey confirmed a division of the field into two different macro-areas. One of the most remarkable aspects of the three-year monitoring period is how different survey instruments and techniques suggest the same trends.

Overall, it can be asserted that:

The MFA and the pressure chamber proved to be valid tools for analysing the field and its characterisation into homogeneous zones, but the extended operational time for acquiring field information makes them complicated to apply. As they are two different instruments, they maintain the common characteristic of analysing the leaf (and the cluster, in the case of the MFA) on a spot-by-spot approach. These systems could consider supportive if they are included in a wide-ranging monitoring system, concentrating the application of these instruments in circumscribed areas.

The VIs obtained from UAS interpreted the characteristics of the field, distinguishing two homogeneous areas in which differentiated management can be applied; within these indices, the GNDVI and the CHM provided the most homogeneous and performing results. The chemical-physical analyses can be a confirmatory instrument to what was predicted during the VIs' multi-temporal monitoring, suggesting the trends of the quality indices under consideration in the various years. These operations, combined with the WineScan™, in a system of differentiated vineyard management can play the role of validating the operations

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carried out by the operator in the field to outline a gradual approach over time that can improve the vineyard's quanti-qualitative parameters.

## **5. Conclusion**

The research conducted during the three years 2021-2023 brought an interesting aspect of how different technologies and methods can converge in the same results, helping the reasoning and managing decisions. An important and relevant factor, such as the comparison between bunch pruning and the VI trend, can suggest a crop management method based on the intensity and distribution of the values that these variables can assume. The commonly used instruments such as UAS, fluorimetric sensor and WineScan™ highlight the practical value of instruments such as the pressure chamber and validate the use of faster and less invasive techniques for discriminating different intra-parcel areas. The distribution of data collected on a three-year scale also helped to observe the internal dynamics of the vineyard in response to different canopy management actions. Due to its ability to provide a large amount of information in a relatively short time, the adoption of rapid investigative instruments such as the UAS is preferable for obtaining data in an extensive viticultural area context. Along with these instruments, site-specific monitoring techniques are valuable to identify on-site criticalities or even minor alterations, justified by the UAS's previous surveys.

From the results of the harvest data and the qualitative attributes analysis, the vineyard evolution can be evaluated on a three-year scale. Site-specific field management and its monitoring allowed the observation of a smoothing of the qualitative characteristics of the grapes, reducing the general field variability. This result represents a valid approach for a decision-supporting system for vineyard management by multi-temporal analysis with remote and proximal sensing, following the principles and objectives of precision agriculture.

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and is the topic of Work Package 7. The specific objective of WP7 Crop management of the vineyard with the combined support of precision viticulture and Artificial Intelligence (AI) technologies is the creation of a Framework enabling the manipulation, aggregation and fusion of data from the agro-food field that allows the development from the point of view of Precision Agriculture in a European context.

### **Data availability**

The data used in this research can be made available through direct request to the corresponding author.

### **Compliance with Ethical Standards**

#### **Conflict of interest**

The authors declare no conflict of interest.

### **Author Contributions**

Conceptualization, F.G.; methodology, F.G., A.D. and A.S. ; software, A.D. and A.S.; validation, F.G., L.M., and A.S.; formal analysis, A.D., L.G., A.S. and P.F.D.; investigation, F.G.; resources, A.D., L.G. and L.M.; data curation, A.D., A.S., L.G. and L.M.; writing - original draft preparation, F.G., A.D. and A.S.; writing - review and editing, F.G., A.D., A.S., and L.G.; visualization, A.D.; supervision, F.G. and G.N.; project administration, F.G.; funding acquisition, F.G. All authors have read and agreed to the published version of the manuscript.

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## Chapter 5. General conclusion

The dissertation focused on innovative tools for proximal and remote monitoring in agricultural systems, particularly in the horticultural and viticultural sectors.

Chapter 2 focused on an explorative study of a proximal sensing application on a horticultural crop, examining the sensitivity of fluorimetry to various cultivation management strategies. The results revealed that MFA effectively tracked seasonal progression by measuring physiological differences among crop management types, with chlorophyll, stress, flavonoids, and nitrogen providing reliable indicators. Fluorimetric indices showed strong correlation with chlorophyll between ORG-I and ORG-II treatments, demonstrating consistent trends. However, NDVI results diverged, confirming that UAS and MFA capture crop traits differently. Therefore, UAS cannot replace proximal sensing in evaluating physiological differences in artichokes. Future work will calibrate MFA indices with quantitative measurements of chlorophyll, nitrogen, and flavonoids to support precision management. The DSS tools could be further enhanced by integrating thermal, hyperspectral, and LiDAR data, while long-term applications in sustainable artichoke cultivation will be validated through testing across diverse fields and climates.

Chapter 3 examined the impact of different biostimulant application rates on vineyards using various monitoring techniques that combined traditional analysis with proximal and remote sensing. The results showed that integrating UAS imagery, MFA, the Scholander pressure chamber, and SPAD readings was an effective way of monitoring *Malvasia Bianca* vineyards in 2020–21. However, no significant differences emerged between remote and proximal sensing as the proposed indices lacked the sensitivity required to distinguish between vigour or stress linked to biostimulant rates. Only SPAD, and to a lesser extent Scholander SWP, identified higher biostimulant doses and showed correlations with yield. Although UAS was efficient for large-scale monitoring, it was ineffective in detecting the effects of the applied biostimulant treatments. Combining remote and proximal methods provided a more comprehensive assessment of vine status. Future studies should refine biostimulant formulations and application rates, as well as implement long-term monitoring, to optimise sustainability and vineyard performance.

Chapter 4 of the dissertation summarised a three-year experiment designed to evaluate the capacity of different monitoring systems to detect variability in vineyard vigour and, through the application of geostatistics, provide effective decision support for targeted interventions. Results from 2021 to 2023 demonstrate how various technologies and methods can be integrated together to support decisions about vineyard management. Comparing bunch pruning and vegetation index (VI) trends suggested management strategies based on variable

intensity and distribution. Instruments such as UAS, fluorimetric sensors, WineScan™, and pressure chambers confirmed the value of rapid, less invasive monitoring in distinguishing intra-parcel variability. Multi-temporal data revealed how vineyards respond to canopy practices and showed reduced variability in grape quality. Integrating remote and proximal sensing supports precision agriculture by providing DSS tools that enhance efficiency, enable site-specific management, and strengthen the long-term monitoring of vineyard evolution.

The three chapters presented in the dissertation explore the integration and evaluation of various monitoring and analysis techniques, demonstrating the effectiveness of combining proximal and remote sensing tools to obtain a defined profile of site-specific field variability. UAS research activities have made an important contribution to the articles, confirming the functionality of these systems in agriculture for surveying crop variability. In fact, the use of these systems has accelerated the crop detection and analysis process, facilitating the research process during the various experiments.

Future experiments will aim to characterise the physiological variability of agricultural crops, with particular emphasis on horticultural species, which have shown high susceptibility to different crop management practices.

New research perspectives will focus on the use of specialised autonomous terrestrial rovers integrating advanced sensing technologies for the detection and analysis of in-field crop variability. These platforms will support the development of site-specific spraying strategies, enabling the targeted distribution of organic plant protection products according to crop status and spatial variability. This approach will provide easily accessible and intelligible information to optimise decision-making processes, improving crop protection efficiency while reducing environmental impact and input use.



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