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Research Paper

Fertigation affects photosynthesis, modulation of secondary metabolism and sensory profiles of *Vitis vinifera* cv. “Schioppettino” withered grapes and wines

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A R T I C L E I N F O

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sensory profile

A B S T R A C T

Nowadays a balance in plant production is required, especially in terms of nutrition, yield and for an optimal aroma and sensory profile of resulted wines. In this research, we compared two different methods of plant nutrition: one-single application (single-fertilization-SF) and two applications with the same amount adopting the fertigation (split fertigation-SpF), including a control not-treated, in a specific Italian region where grapevine Schiopettino cv. is being cultivated and this practice was not investigated yet. SpF promoted the photosynthesis parameters compared to a SF and to the non-treated vines (NTC). On these basis, the biological and physiological activity of the whole plant was enhanced. SpF treatment tendentially and significantly improved the qualitative, productive, physiological, and oenological parameters of “Schioppettino” wine.

1. Introduction

In several European regions, viticulture has developed into extremely specialized and intensive production systems (Mian et al., 2022a), which usually exploit the soil to the utmost (Papa et al., 2020). The loss of soil fertility can have a negative impact on yield and grape quality if not managed properly. It has been demonstrated that soil condition is one of the most important factors determining the qualitative and quantitative characteristics of the grapes and thus the organoleptic characteristics of the wines (Ferrer et al., 2020; Tomasi et al., 2022).

In this sense, soil fertilization (i.e., plant nutrition). is a crucial agricultural practice (Baldi et al., 2022) that involves the addition of nutrients to the soil to promote plant growth and yield. Different systems of soil fertilization have been developed over the years, including organic and inorganic fertilization. Organic fertilization involves the use of natural sources of nutrients such as animal manure, compost, and green manure (Mian et al., 2022b), whilst inorganic fertilization involves the use of synthetic fertilizers such as urea, ammonium nitrate, and potassium chloride (Chen et al., 2023). Both systems have their advantages and disadvantages. Organic fertilization is environmentally friendly, but it is slow-acting and may contain low concentrations of some essential nutrients, whilst inorganic fertilization, on the other hand is fast-acting, provides nutrients in readily available forms, and cost-effective (James et al., 2022).

One of the practices that has gained more ground in recent years is the fertigation, that consist in the application of the fertilizer to crops through irrigation systems, providing several benefits over traditional soil application methods. Fertigation systems allow for precise application of nutrients, reduce fertilizer waste, and improve nutrient uptake efficiency (Stefanello et al., 2020). Overall, fertigation systems have shown great potential to improve crop productivity and reduce environmental impact by reducing fertilizer runoff and leaching (Ma et al., 2020).

Additionally, fertigation systems have been shown to significantly influence plant growth, yield, photosynthesis, and secondary metabolites (Incrocci et al., 2017; James et al., 2022). Properly timed and applied fertigation can provide plants with the necessary nutrients they need for optimal growth and development, leading to increased yields. The application of nutrients through fertigation systems can also

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enhance photosynthesis and improve carbon assimilation, resulting in increased biomass accumulation and plant growth (Suárez et al., 2022). Moreover, fertigation systems have been shown to positively impact secondary metabolites, which are important for plant defence, sensory profile, and human health benefits (Sun et al., 2019). For example, fertigation with nitrogen and phosphorus has been shown to increase the concentration of secondary metabolites such as flavonoids, anthocyanins, and phenolic acids in plants (Regmi et al., 2023). Properly managed fertigation systems can help reduce water usage and improve crop water use efficiency by applying nutrients directly to the root zone of crops, which reduces nutrient loss through leaching and runoff (Chen et al., 2022). Additionally, fertigation systems can improve water use efficiency by reducing the amount of water needed for nutrient application compared to traditional methods such as broadcast fertilization (Fonteyne et al., 2021). For example, research has shown that drip fertigation systems can reduce water usage compared to traditional surface irrigation methods (Li et al., 2021).

Consequently, the extent and usefulness of the vine system depend primarily on the vigour, and performance of the canopy (Campos et al., 2021), which are related to soil, water availability, and root growth (Gatti et al., 2022). Considering that yield, the chemical composition of the must, and the sensory profile of the wine can be influenced by different types of plant nutrition and fertigation (Botelho et al., 2022) the aim of this study was to increase knowledge about the relationship between these factors, and to compare the effects of two different fertilization programs on the physiology of Schiopettino variety; namely, the yield, the chemical composition of the grape must, and the sensory profiles of the corresponding wines. This cultivar was selected because it is the only one used to produce the Schiopettino wine (in Friuli Venezia-Giulia region, Italy, NE) that undergoes withering treatment to enrich many chemical aspects (Tomasi et al., 2021), as it lacks several secondary metabolites. Withering can be carried out in planta as general practice applied by winemakers of North-East Italy, that was also performed in this study or in special withering chambers. Schiopettino was also selected for this study because it is an autochthonous red variety that is currently one of the top ten *Vitis vinifera* varieties grown in the Friuli Venezia Giulia region (Italy, NE) (repository: http://dati.istat.it), and there is not any report nor study regarding fertigation on this grapevine cv. and its effect. Thus, on this basis, our work is the first attempt to investigate several parameters in function of different plant nutrition systems yet how this translates into the wine aromatic and sensory profile. Finally, could be considered a milestone for what concerns the Schiopettino cv.

2. Materials and methods

2.1. Experimental setup

The experiments were carried out during 2017 and 2018 growing seasons. The commercial vineyard was selected in the specific area of Schioppettino (Prepotto, Gorizia (GO), Italy (46°01′38.0 ″N, 13°28′19.3 ″E). The weather conditions in the research site, reported in Supplementary Table 1, were typical for North-Eastern Italy, with warm summers and cold winters, with an average temperature of 14 °C. The total annual rainfall was 1300 and 1,800 mm in 2017 and 2018 respectively, mainly distributed in spring and autumn. The vines were planted in 2009 and trained on a Guyot trellis with a support wire at a height of 1.60 m above the ground. The vineyard density was about 4500 vines ha^{-1}, with vine rows 75 m in a south-north direction.

The studied cultivar was "Schioppettino", grafted on rootstock "Kober 5 BB" (Berlandieri × Riparia), grown mainly on clay-calcareous soils (tipical of this geographical area), homogeneous for chemical composition. The grapes were harvested when the total soluble solids content (SS) ranged from between 22 and 26 °Brix, in order to obtain a final balanced alcohol level for this type of wine (Tomasi et al., 2022). The experimental plan, set up as a randomized complete block (RCB), involved 3 treatments (Table 1), each consisting of 80 plants divided into 4 replicates of 20 plants each (total of 240 plants). The description of the treatments was described in Table 1.

The phytosanitary treatments were performed based on Integrated Pest Management (IPM), with recommended products commonly used by the local growers. Commercial products were used in accordance with label instructions and recommendations. Emergency irrigation was applied when required to restore the soil moisture to the field capacity (Tomasi et al., 2020).

To gain insights on the effect of different fertilization programs and in planta withering gas exchange were analysed in both years at the beginning of veraison (BBCH 81) (BBCH: Biologische Bundesanstalt, Bundessortenamt and CHemische Industrie). The yield and yield-components, as well as the chemical composition of the berries were analysed at harvest time, thus, after the in-planta withering. Finally, the sensory profiling of the yielded wine of each treatment was examined after vinification.

2.3. Gas exchange rate

The main objective to measure the photosynthetical activity was to investigate how different fertilization programs impact photosynthesis, the reciprocal influence between photosynthesis and primary/secondary metabolism, and to evaluate any effects on other physiological parameters.

The Gas Exchange Rate parameters were measured in accordance with reference protocols (Jiang et al., 2017; Lu et al., 2012). The net photosynthetic rate (A), stomatal conductance (*gs*), transpiration rate (E), and internal CO_2 concentration (*ci*) were measured on three replicates per treatment (8 leaves per treatment) between 8:30 – 10:30 am, in fully expanded and healthy leaves between the junctions 5 and 10 of a central grape stem. Analysis was carried out with a portable photosynthesis system (Li-6400XT, Li-Cor Inc.) at the veraision onset in both seasons using photosynthetic photon flux density (PPFD) set up at 1,200 mol m^{-2} s^{-1}, CO_2 concentration of 400 μ mol^{-1} and relative humidity (RH) setting at 65 %.

2.4. Yield and components

All vines from each treatment were individually hand-picked. Yield and yield-determining parameters data were measured at harvest, as described in a previous work (Mian et al., 2022c). The following parameters were considered: $yield.vine^{-1}$ (kg), grape average weight (g), number of clusters per vine (n°) shoots number (n°).

2.3. Quantification of sugars and organic acids profiling

The fruit composition at harvest was measured on 2.0 kg of grapes randomly collected in each treatment. The total soluble sugars (S.S.) were quantified using an ATAGO PR-32 digital refractometer (Fischer

Table 1
Description of the different fertilization programs evaluated thorough the grown season of 2017 and 2018. Prepotto, Gorizia (GO), North-East, Italy.

Treatment	Description
Non-Treated Control -NTC	Throughout the growing seasons of 2017 and 2018, no fertilization was applied as a control
Single Fertilization (SF) -T1	One-time of mineral fertilization in March of both years, with 165 $kg.ha^{-1}$ of the formulation 15–10–20 (NPK) + Mg and S.
Split Fertigation (SpF) -T2	The amount applied in both years was the same (also the equal to T1), but instead of a one-time application, it was split into three separate applications in March, April, and May. To ensure the fertilizer was delivered in the correct amount and the appropriate time, a Fertigation System was employed. Each application utilized a 55 kg ha^{-1} dosage of 10–10–20 (NPK) + Mg and S formulation.

Scientific, Milano (MI), Italy) (0–32 %) and expressed in °Brix degree. In the same samples, the organic acid profile of the berries (specifically tartaric and malic acids, expressed in g L^{-1}) was determined by high-pressure liquid chromatography (HPLC Agilent 1220 infinity, Thermo Fischer Scientific, UK). The samples for HPLC were prepared by taking 250 µL of grape must diluted 1:50 with distilled water (Tomasi et al., 2021). Samples were then filtered through a 0.2 µm cellulose filter (Merck KGaA, Darmstadt, Germany) and analysed. The grape must samples were prepared by pressing three subsamples of 250 g of berries. Lastly, pH was measured using an automatic titrator (Crison Micro TT 2022, Riera Principal, 34–36 08,328, Alella (Barcelona), Spain).

2.4. Quantification of pigments and volatile organic compounds (VOCs)

The investigated VOCs where: furans (FU), benzenoids (BZ), nor-isoprenoids (NO), monoterpens (MO), C-6-aldehydes (CA), aliphatic alcohols (AA).

The analysis was performed as described by Tomasi et al. (2021). In brief, phenolic compounds from skins were extracted with ethanol (75 %), in 50-berry per treatment, total flavonoids and anthocyanins were quantified by HPLC, expressing in mg kg^{-1} of grapes.

The determination of VOCs was carried out as described by Rosso et al., (2016). Briefly, the aglycones relased from glycoside-bound aroma precursors were analysed by gas chromatography mass spectrometry (GCMS–, EI 70 eV, Thermo Fischer Scientific, UK) after performing enzymatic hydrolysis. GC–MS analyses were performed using a 6850-gas chromatography system (Agilent Technologies, Santa Clara, CA, USA), fitted with a fused silica HP-INNOWax polyethylene glycol capillary column (30 *m* × 0.25 mm, 0.25 µm i.d.) (Agilent Technologies), coupled with a HP 5975C mass spectrometer and 7693A automatic liquid sampler injector (Agilent Technologies). Compound identification was performed using the NIST Mass Spectral Libraries Database (rev08) and the in-house database. To make a comparison amongst the samples, the contents of VOCs were expressed as µg of internal standard per kg of dried grape. Data were normalized according to the internal standard.

2.5. Must microbial ecology characterization

Since the fungi/yeasts on berry carposphere could be influenced by the fertilization (Wu et al., 2021), indigenous microbiology in the musts was evaluated (Pallmann et al., 2001). Decimal dilutions (0.1 mL) of the musts were prepared and plated onto Wallerstein Laboratory (WL) Differential Agar (Wallerstein Differential Agar; WLD Agar), to grow all non-*Saccharomyces* yeast. WL Nutrient Agar medium (Oxoid, Hampshire, UK) was used to record *Saccharomyces* yeast growth. MRS agar (deMan, Rogosa and Sharpe) with Delvocid (25 mg mL^{-1}) was used as a substrate for the growth of Lactic Acid Bacteria (LAB). Furthermore, MRS agar plus 20 % (v/v) apple juice and Delvocid (25 mg mL^{-1}), pH 4.7 (Kelly et al., 1989), was used to promote the growth of *Oenococcus* spp., which play a key role for malolactic fermentation in wine (Vendrame et al., 2013). Petri dishes were incubated at 25 and 30 °C for 48–72 h for yeast and bacteria, respectively. Final counts were daily performed on plates showing between 30 and 300 colonies, large enough to distinguish the different colony types (Colautti et al., 2023). Microbial counts were expressed as Colony Forming Units per millilitre (CFU mL^{-1}).

2.6. Wine making and sensory analysis

The winemaking was carried out at the University of Udine winery vinification centre. 110 kg of grapes were harvested yielding 65 L of wine per treatment, following a protocol of Tomasi et al. (2021), slightly modified. Hence, the vinification process started with the collection of in-planta withered grapes, that were crushed to obtain the destemmed-crushed grape. The crushed-grape was transferred to a maceration/fermentation tank with the addition of 8 g hL^{-1} of potassium metabisulfite, 0.3 g hL^{-1} of enzymes (Lysis first®, Oenofrance, Montebello Vicentino, Italy), and 4 g hL^{-1} of ascorbic acid. After this process a sample of wine-must was collected and inoculated with 20 g hL^{-1} of selected yeast (Zymaflore FX10® and F83®, Laffort, Paso Robles, CA, USA), with the addition of 2 g hL^{-1} of thiamine and vitamins (Oenofrance), 2 g hL^{-1} of yeast extracts rich in amino acids (Oenofrance) and 2 g hL^{-1} of inactivated yeast and cellulose (Oenofrance) and then re-mixed incorporating it in the fermenter.

When the first part of alcoholic fermentation reached about 13° v/v, a high-alcohol-tolerant yeast (Lalvin 2226®, Lallemand) was added at a concentration of 30 g hL^{-1}, also adding 15 g hL^{-1} of mineral nutrients and yeasts-derivate (Vivactive Performance®, Oenofrance). The use of this second yeast strain was necessary to reinforce the fermentation under high alcoholic level. At the end of this process, a soft pressing was performed and 4 g hL^{-1} of a botanic tannin (grape seeds) (Tannino Perfect®, Oenofrance), 4 g hL^{-1} of potassium metabisulfite, 12 g hL^{-1} of yeast (Vivactiv Control®, Oenofrance) and 10 g hL^{-1} of nutrients (Philya LF®, Oenofrance) were added. After wine clarification, the batonnage was performed twice per week. At the end of the batonnage, another clarification operation was performed. At the end of this period, the wine was filtered with cardboard filters (Agrieuro™), and stabilised with Cryokappa®, Oenofrance. After that, the wine was refiltered twice with 1 µm and 0.45 µm filters and then bottled. The 2017 and 2018 wines stored in stainless steel tanks and kept in a temperature ranging from 4 to 13 °C, were tasted one year following vinification.

The sensory analysis was carried out by a test panel made up of 20 formed judges plus a panel leader. To test and to confirm the reliability and accordance of the judges, a periodic training amongst the participants to the panel test was made following precise criteria reported in the literature (Ashton, 2012). For the wine making and tasting the quantitative evaluation of the intensity of attributes (olfactory, gustatory-tactile and retro-olfactory), quantitative descriptive analysis was used (Sidel and Stone, 1993) with the help of a questionary sheet, providing discrete scale responses with intervals from 1 to 8, always based on 4 replicates each one.

2.6. Statistical analysis

Statistical analyses were performed using R v4.1.2. The presence of statistically significant differences on results were assessed performing one-way and two-way ANOVA plotting the results using ggplot2, performing also Tukey post hoc test ($p<0.05$). Heatmap of correlation between variables (photosynthesis and VOCs, based on the 4 replicates) has been calculated using Pearson Correlation Coefficient.

3. Results

Data are depicted in Fig. 1 to 7. In each figure, different letters mean a statistical difference at $p<0.05$, both intra-year (letters located in upon the bar charts) and in the year × year interaction (letters located in the boxes under the TRIAL wording). Besides, due to the great importance of results got in the two-study years for this plant (Falginella et al., 2022), results will be depicted and discussed with a major focus on the year × year interaction of treatments.

3.1. Photosynthetic activity

SF and SpF increase the photosynthetical rate compared to NTC, effect being statistically higher in SpF also compared to SF (Fig. 1A). Yet, 2018 study-year showed higher statistical significance than 2017. Only the treatment SpF enhanced the stomatal conductance (*gs*), without difference between years of study (Fig. 1B).

SpF significantly increased the internal CO_2 (C_i), compared to NTC and SF, in both 2017 and 2018, and there were no significant differences between years (Fig. 1C).

Regarding the transpiration rate (Fig. 1D), both SF and SpF

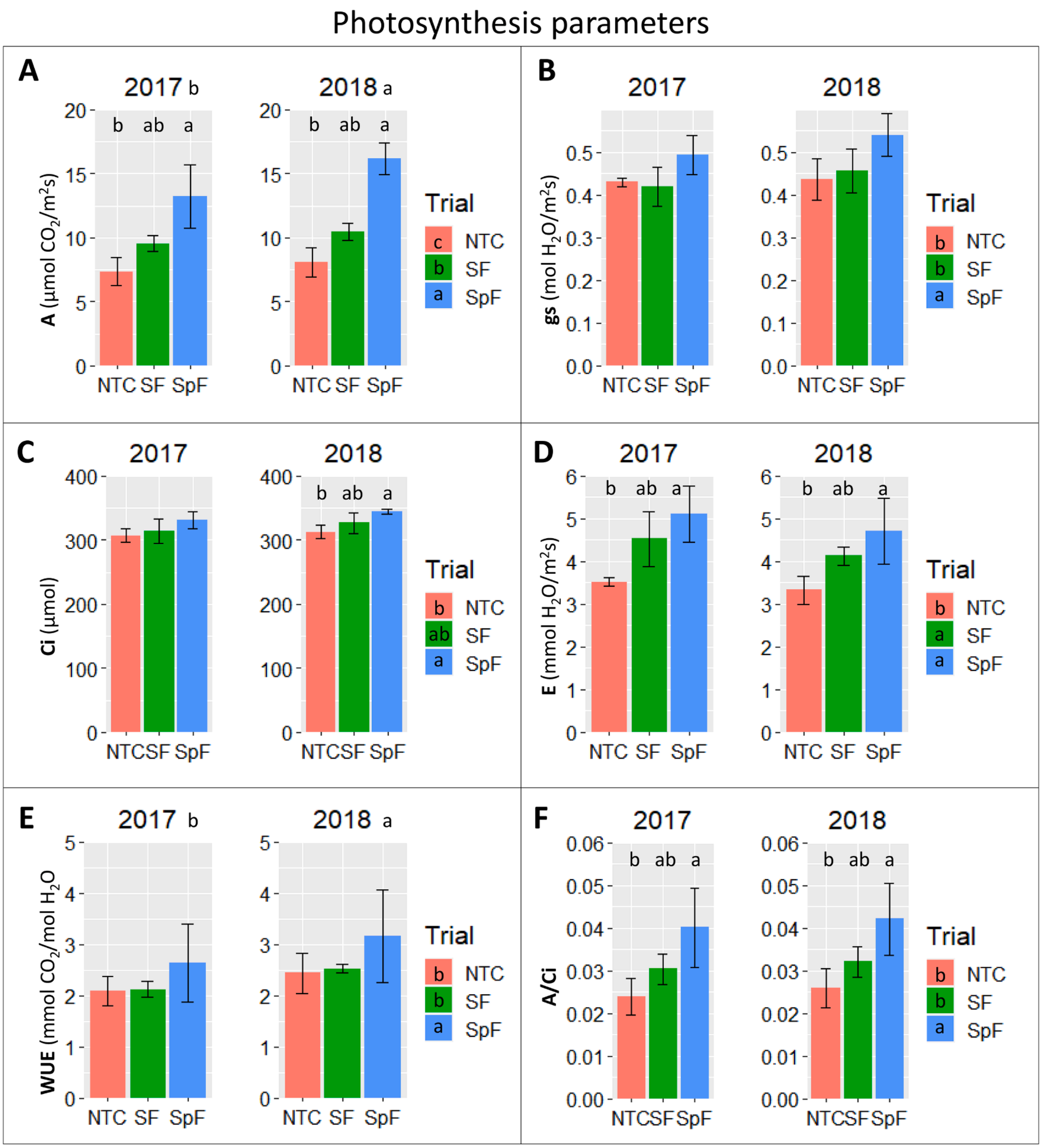

Fig. 1. Effect of fertilization on photosynthesis rate assimilation (A), stomatal conductance (B), intercellular carbon (C), transpiration rate(D), water use efficiency (E), carboxylation efficiency (F) in 2017 and 2108 and comparison between years. Bars represent the mean ± standard error. Values assigned by different letters are statistically significance at *p* value <0.05 (Tukey HSD test). Data are the mean of 4 replicates.

significatively improved this value when compared to the NTC treatment, without significant differences existing between SF and SpF, and study-years.

WUE was affected by only the SpF treatment in both study-years, yet parameters in 2018 were significantly higher than 2017, as described in Fig. 1E.

Finally, considering the carboxylation efficiency (A/C_i), in both years only SpF significantly increased this parameter, compared to the NTC and SF treatments (Fig. 1F).

3.2. Yield and quality parameters

We investigated the grape production per plant (yield/vine - kg), average grape weight (cluster weight - kg), number of shoots per planta (shoots/vine - N°), and clusters per vine (clusters/vine - N°) (all depicted in Fig. 2). The number of clusters per plant trended towards a difference (Fig. 2C), where the SpF seemed to have the higher number of grapes, however without a statistical significance. No significant differences arose between the study years, apart from shoots per vine, where 2018 had higher statistical values than 2017 (Fig. 2D).

Looking at must values (Fig. 3), polyphenols (Fig. 3A) were significantly affected only by the SpF treatment, being higher than NTC and SF. The same trend occurred for anthocyanins (Fig. 3B). In this latter case, also the year had a significant effect, with higher values in 2018 compared to 2017.

Concerning the tartaric acid, both SF and SpF showed higher statical values compared to NTC (Fig. 3C). Also in this case, 2018 data exhibited higher values than 2017. Malic acid was found to be significantly higher only in the SpF treatment and had higher concentrations in 2018 (Fig. 3D). The °Brix showed higher values in the 2018 vintage and were always significantly higher in SF and SpF treatments compared to NTC (Fig. 3E). Lastly, no significant differences arose for pH values (Fig. 3F).

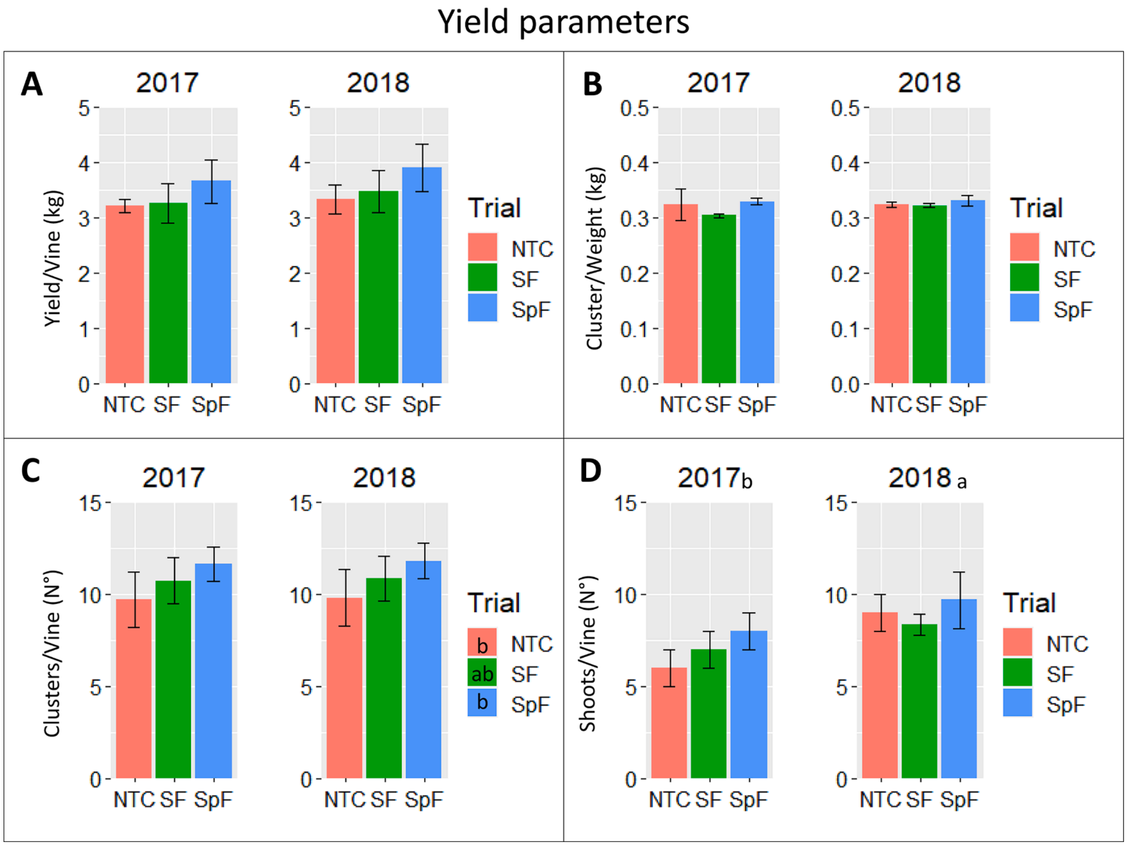

Fig. 2. Effect of different fertilization systems on yield components in the growth seasons of 2017 and 2018 and comparison between years: grape production per plant (A), average grape weight (B), clusters per vine (C), number of shoots (D). Values assigned by different letters are statistically significance at p value <0.05 (Tukey HSD test). Data are the mean of 4 replicates.

3.3. *VOCs present in the grape musts and microbiological counts*

Concerning the effect of different fertilization systems on VOCs (Fig. 4), regarding FR, NTC and SpF showed similar values, with both significantly different compared to SF (Fig. 4A), and higher values in 2018. CA showed the highest significance in SpF, with no differences between SF and NTC in both years (with higher concentrations observed in 2018) (Fig. 4B). MT were higher in SpF, followed by SF and NTC, yet, with no significant differences between study years (Fig. 4C). NO were statistically significant in SpF, compared to SF and NTC, without significant differences between them, respectively, and the years of study (Fig. 4D). BZ showed similar value between SF and SpF treatments, with both significantly different compared to NTC (no differences between years of study) (Fig. 4E). AA were significantly higher for SF and SpF treatments, compared to NTC in 2018, but with no significant differences between SF and SpF treatments and 2017 and 2018 years (Fig. 4F). Lastly, concerning the microbiological counts (Fig. 5), no difference emerged in any combination taking into account *Saccharomyces* and non- *Saccharomyces* yeasts, Lactic acid bacteria, *Oenococcus* spp., nor between years of study.

3.4. *Olfactory profile of resulted wines and correlation with photosynthetical parameters*

Going further to the sensory profile, NTC was the least ‘smooth’ in 2017. Indeed, SpF was described as more ‘pleasant’, with classic aromas for this wine highlighted: ‘red riped fruit’, ‘balance’, ‘jammy’, ‘herbaceous’, and with a higher ‘pleasantness’. Overall, SpF wine was described as more balanced. Considering the 2018 vintage, notes of ‘spicy’, ‘herbaceous’, and ‘floral’ were scored the highest in SpF, compared to the other two treatments, where again no significant differences arose (Fig. 6).

The physiological parameters (X axe) were then correlated with VOCs values (Y axe) (Fig. 7). In both years there were concordant results, with a constant relationship between all the different factors. Therefore, considering both years, CA, MO, and NO were the VOC groups most positively correlated to photosynthetic factors (values above 0.90), followed by BZs with a lower correlation coefficient (values >0.70). A lower correlation was observed for the AAs (values >0.35), while a negative correlation was identified for FU. For the latter, only a slight positive correlation was observed with E (0.60 in 2017 and 0.30 in 2018), while for Ci and A the correlation was almost absent. *gs* had a slight negative correlation with VOCs: −0.16 in 2017, and −0.08 in 2018.

4. Discussion

Despite the considerable know-how and progress made over recent decades, many aspects of vine physiology and management practices are still poorly explained or have unknown cause-effect relationships with environmental and agronomical inputs (Bowen et al., 2020; Rienth et al., 2019). This is especially true in terms of grape and special wine production, such as Schiopettino varieties that undergoes withering treatment to enrich many chemical aspects, as it lacks several secondary metabolites compared with other varieties (Slaghenaufi et al., 2020).

The biosynthesis of secondary metabolites begins with the production of primary metabolites such as sugars, amino acids, and organic acids through photosynthesis (Twaij and Hasan, 2022) which is strongly influenced by plant nutrition, also managed through fertigation, which also affects yield, canopy development (Bernardini et al., 2022). The biosynthesis of molecules such as anthocyanins represents a defence mechanism for the plant cells, and is used as strategy for reducing

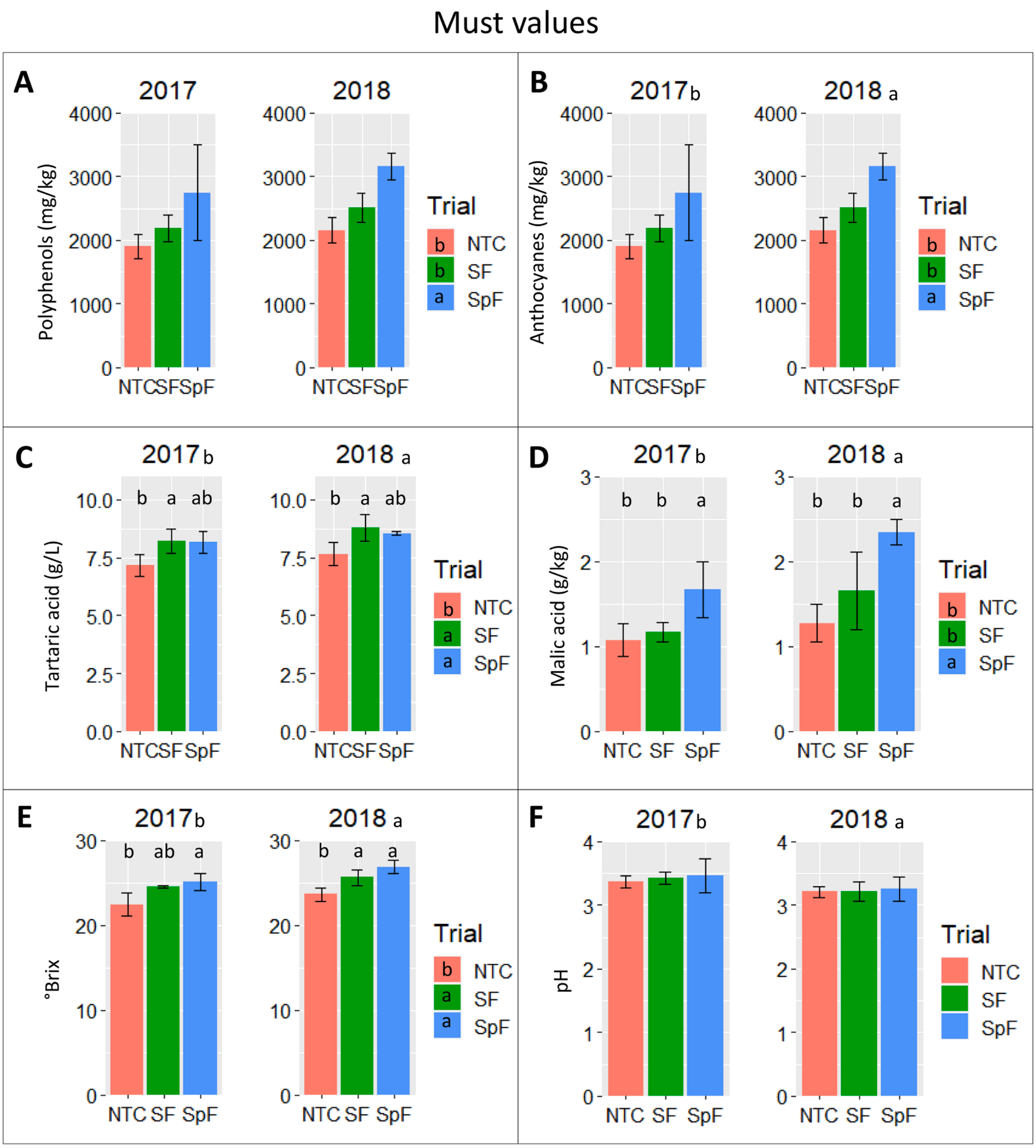

Fig. 3. Effect of different fertilization systems on polyphenols (A), anthocyanins (B), Tartaric acid (C), Malic acid (D), °Brix (E) and pH (F), in the growth seasons of 2017 and 2018 and comparison between years. Values assigned by different letters are statistically significance at *p* value <0.05 (Tukey HSD test). Data are the mean of 4 replicates.

oxidative stress and damage to cells that results from abiotic factors such as drought (Sobiecka et al., 2022). Furthermore, plant phenolics have important effects on food quality and human nutrition. Their presence in grapes and red wine may contribute to health benefits because of their anticarcinogenic activities, despite antioxidant properties may be very different from those observed in planta (Santos-Buelga et al., 2019), however, benefits for cognition (Rutledge et al., 2021).

In this regard, our study aimed to comprehend how two distinct nutritional processes could impact the physiological activity of plants (photosynthesis), metabolism (production and secondary metabolites), grape quality (sugars, acidity, malic and tartaric acids, and pH), and the sensory attributes of the wine.

Regarding the effect of fertilization management on eco-physiological parameters, a similar trend was observed in both seasons. This was an interesting result because it shows that there is a stable effect of fertilization management on carbon fixation, and subsequently effects on secondary metabolism in different environmental conditions thorough the seasons, which is a desirable way to standardize wine production.

Fertilization promotes increased photosynthetic activity in plants, thanks to an adequate supply of essential nutrients, leading to higher assimilation rates of carbon dioxide (Bravo et al., 2012), and positively impacts assimilation rates, bolstering the carbon sequestration potential of ecosystems and contributing to the mitigation of atmospheric CO_2 levels (Kovenock et al., 2018). In our study, both SF and in particular SpF treatments promoted this parameter. The split fertigation regime allows for precise and targeted delivery of nutrients (particularly N, P, and K) directly to the plant roots, ensuring efficient uptake and utilization at the right moment for plants. This leads to higher assimilation rates of carbon dioxide as plants can efficiently convert atmospheric CO_2 into organic compounds through photosynthesis.

The dual function of fertigation, in addition to enhancing the

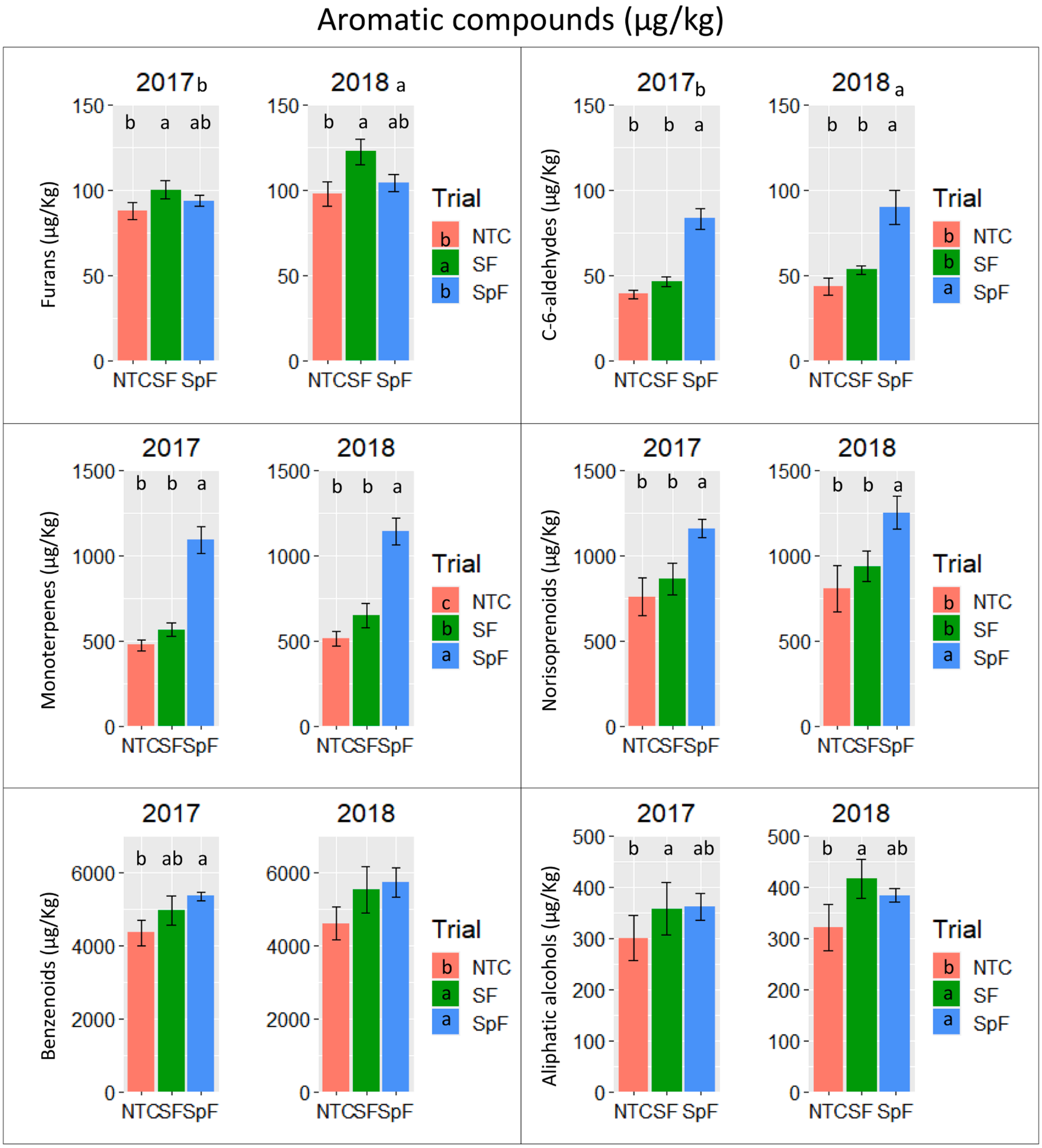

Fig. 4. Effect of different fertilization systems on furans (A), aldehydes (B), monoterpenes (C), norisoprenoids (D), benzenoids (E) and aliphatic alcohols (F), in the growth seasons of 2017 and 2018 and comparison between years. Values assigned by different letters are statistically significance at p value <0.05 (Tukey HSD test). Data are the mean of 4 replicates. Contents of compounds were calculated as µg internal standard (IS) 1-heptanol per kg of dried grape (d.g.) (µg.Kg), in the growth seasons of 2017 and 2018 and comparison between years. Values assigned by different letters are statistically significance at *p* value <0.05 (Tukey HSD test). Data are the mean of 4 replicates.

efficiency of nutrient delivery through water at specific stages of the vegetative cycle, also has a positive effect on stomatal conductance, improving gas exchange, including the absorption of CO_2 for photosynthesis and the release of oxygen. This, in turn, leads to well-developed stomata and improved stomatal conductance (Ullah et al., 2019). In our study this hypothesis can be accepted for plants grown under the SpF treatment.

By promoting optimal plant growth, fertigation contributes to a higher leaf area and chlorophyll content, both of which can result in increased internal CO_2 concentrations (Seepaul et al., 2016), as confirmed by our study, where SpF promoted this parameter in 2018.

Going further, when plants receive sufficient water and mineral nutrients through fertigation, they ensure optimal hydration/nutrition and maintain proper cell turgor pressure, especially when K is properly applied (Hasanuzzaman et al., 2018). Consequently, this encourages the stomata to remain open for longer durations, thereby increasing the transpiration rate. Regarding the transpiration, both SF and SpF treatments significatively improved E values, for SpF it was expected due its effect on *gs*.

Water use efficiency is a measure of how effectively plants utilize water for their growth and physiological processes and indicates the amount of water consumed to produce a unit of dry matter. The precise delivery of water and nutrients reduces the potential for water loss through runoff or evaporation. Moreover, the availability of nutrients through fertigation supports plant health and development, enabling plants to function efficiently with less water. Overall, fertigation enhances water use efficiency by providing the right amount of water and nutrients directly to the plants, promoting their growth and minimizing water wastage, as occurred for SpF plants compared to NTC and SF. The increase in WUE (A/E) in SpF plants occurred at increasing rates of the

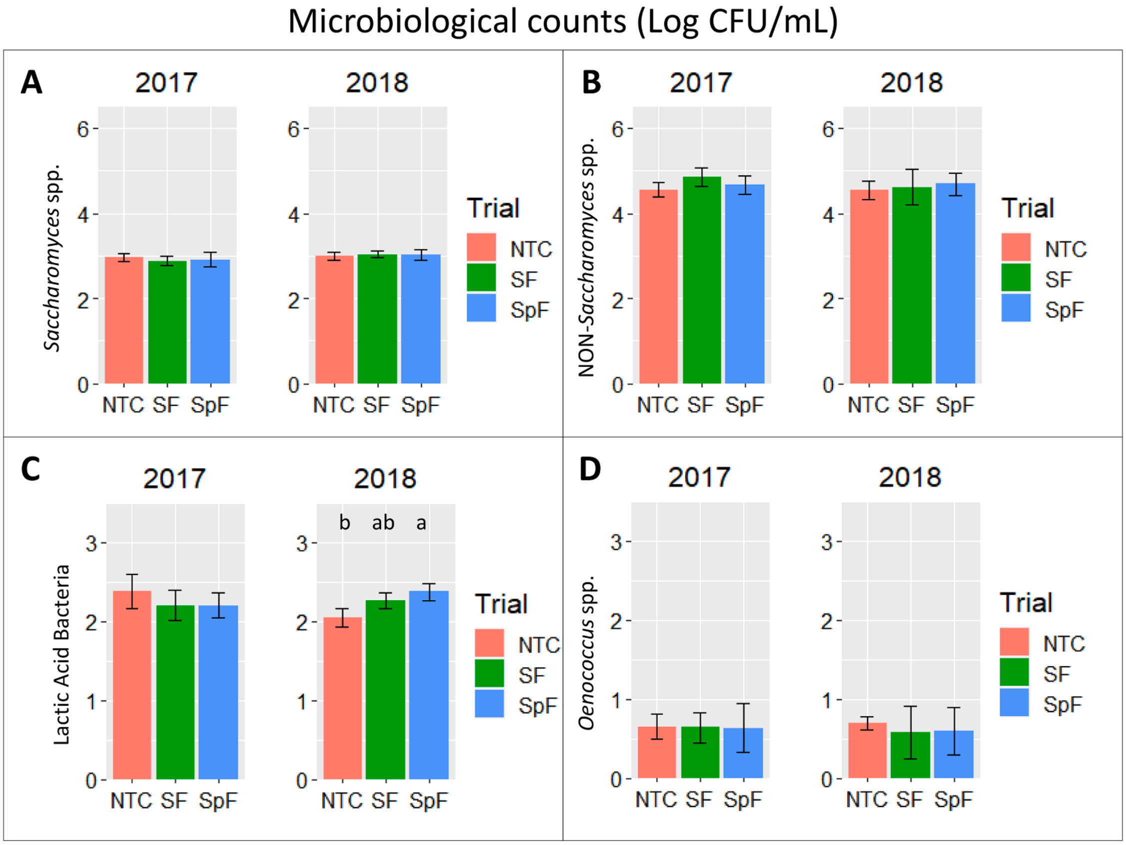

Fig. 5. Effect of different fertilization systems on Saccharomyces (A) and non- Saccharomyces yeasts (B), Lactic acid bacteria (C) and Oenococcus genus (D), in the growth seasons of 2017 and 2018 and comparison between years. Values assigned by different letters are statistically significance at *p* value <0.05 (Tukey HSD test). Data are the mean of 4 replicates.

two parameters but they were higher in percentage terms in A (about 78 %) than in E (44 %) in both years and, since A is the numerator, inevitably, the value of the ratio increases. In SF plants, in both study-years, A and E both grew by only 28 % while in 2018 A grew by 29.6 % and E by 24.2 %.

Carboxylation efficiency (A/Ci) is the ability of plants to efficiently fix carbon dioxide from the atmosphere and convert it into organic compounds through the process of photosynthesis. These, plus nutrients, support the synthesis of key enzymes involved in photosynthesis, such as Rubisco (Ribulose-1,5-bisphosphate carboxylase/oxygenase). Adequate nutrient availability through fertigation ensures that plants have optimal levels of these enzymes, enhancing their carboxylation efficiency (Kant et al., 2012). Therefore, by supplying essential nutrients and maintaining appropriate hydration levels, fertigation promotes the carboxylation efficiency in plants, enabling them to effectively utilize atmospheric CO_2 for organic compound synthesis. This was demonstrated when SpF is applied, confirming what has previously been reported.

Considering the yield and yield related parameters, no significant differences emerged. Only in 2018 the shoots number per vine were higher when compared to 2017. Thus, the higher photosynthesis in SF and SpF promoted more dry matter accumulated in the sink organs, e.g., grapes, as reported in literature (Wang et al., 2021), which might explain the higher values of solid soluble in SpF. Furthermore, apart from pH, all the other must parameters were statistically influenced by SF and SpF compared to NTC (°Brix, polyphenols, anthocyanins, tartaric and malic acid). We can conclude that the fertilization, and in particular the SpF system, not only enhanced the dry matter accumulation but also the quality parameters of must, certainly due and related to the higher photosynthetical activity of SF and SpF (Iglesias et al., 2002; Zahoor et al., 2017). It is noteworthy to emphasize the significant maintenance of acids at a good level for wines (especially in case of this cultivar), specifically malic acid, as it is the one that experiences significant decreases due to the elevated temperatures, which are a well-established consequence of climate change in many wine-producing regions. Consequently, this result underscores the potential of fertigation as a tool to ensure adequate acidity levels in the face of the consequences of climate change. Furthermore, it should be noted that high acidity is also an important factor in wine vinification and wine stability management.

The relationship between photosynthesis and indigenous microbial ecology is complex (Derilus et al., 2023; Zhang et al., 2020; Xu et al., 2002). In our study, although the different fertilization systems positively enhanced the photosynthetical activity together with yield and quality, this did not affect *Saccharomyces* and non-*Saccharomyces* yeasts, Lactic Acid Bacteria, and *Oenococcus* genus. Thus, the must indigenous microbial ecology was not promoted.

The increased photosynthetic parameters observed in SF and SpF treatments of this study are correlated with increased abundance of norisoprenoids and monoterpene VOC compounds, which is consistent with previous findings (Bureau et al., 2000; González-Barreiro et al., 2015). This highlights the importance of appropriate (and consistent; SpF) direct fertigation of plants for the improvement of Schiopettino sensory profiles. In fact, fertigation can indirectly impact the presence and concentration of these compounds in grapes, thus influencing wine quality, through: a nutrient balance, less water stress (that can lead to the accumulation of aroma precursors in grapes), and the microbial interactions that can indirectly affect soil microbial communities, which play a role in nutrient cycling and grapevine health (Colautti et al., 2023). Moreover, MO and NO correlates better with photosynthetical activity. This could be related to a better carbon allocation, and the photosynthetic activity can affect the expression of genes involved in the biosynthesis of monoterpene and norisoprenoids compounds, thereby

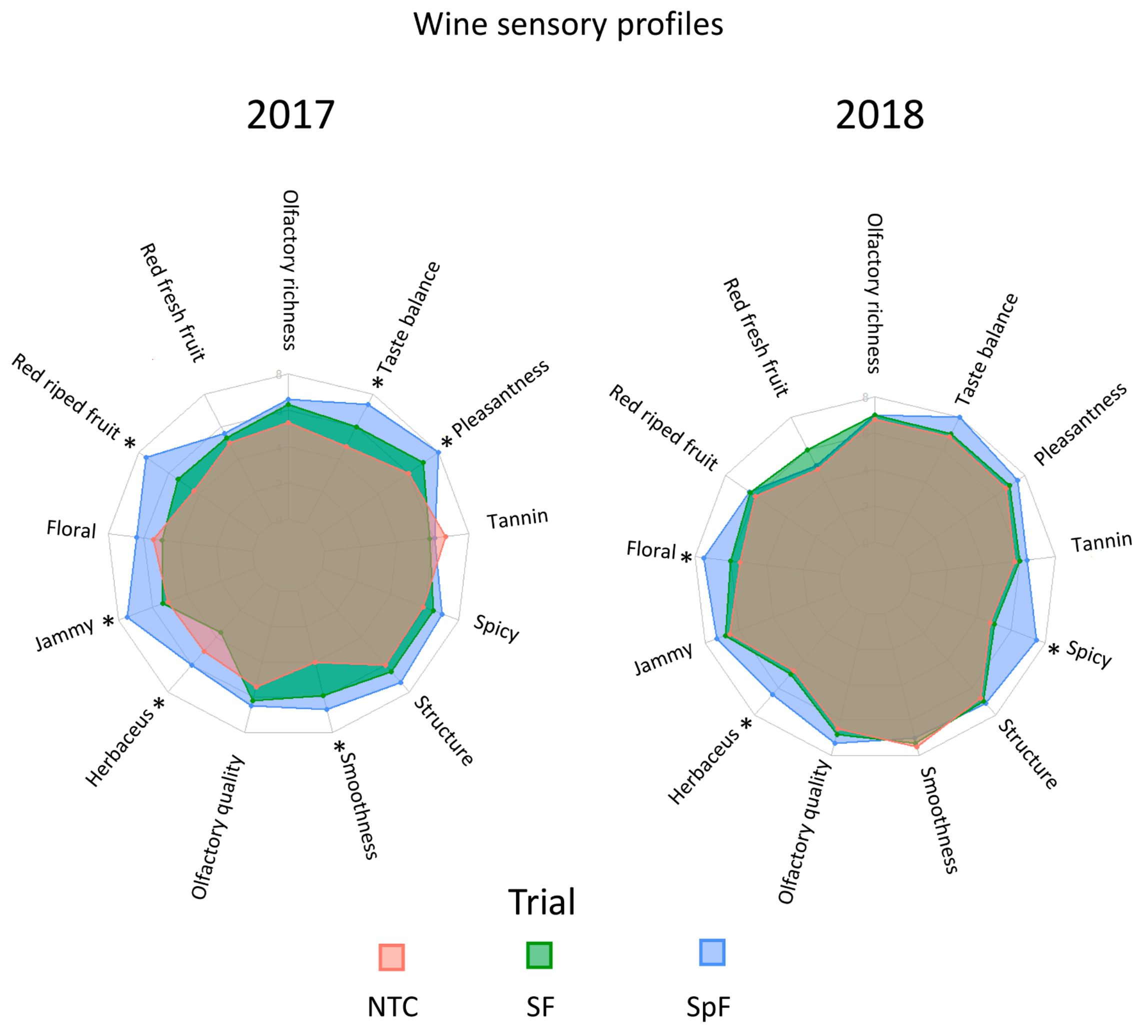

Fig. 6. Results of tasting for wines in 2017 and 2018 years. *: indicates a significant difference at p value <0.05 (Tukey HSD test). Data are the mean of 4 replicates.

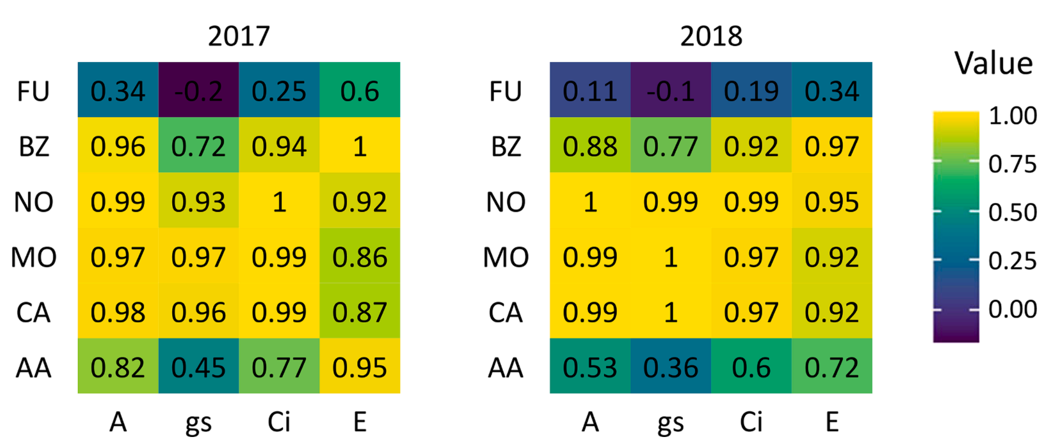

Fig. 7. Statistical correlation between photosynthetical activity and VOCs in 2017 and 2018. FU: furans, BZ: benzenoids, NO: norisoprenoids, MO: monoterpens, CA: C-6-aldehydes, AA: aliphatic alcohols.

influencing their concentrations in grapes (Cataldo et al., 2021). Lastly, is important to note that fertigation can indirectly contribute to increased resistance to mildews by promoting plant health and vigour, an important matter due to the several diseases of grapevine (Tripathi et al., 2022; Mian et al., 2023).

The synthesis and formation of VOC compounds giving rise to aroma and flavour in wines is inherently tied to soil nutritional quality and water availability (González-Barreiro et al., 2015). Sufficient nutrition allows plants to commit a greater number of photosynthetic products to primary and secondary metabolite biosynthesis (from which VOCs are derived), particularly at critical phases of development such as fruit maturation (González-Barreiro et al., 2015). Thus, adequate nutrition leads to a greater accumulation of these metabolites within the grapes, and ultimately increases the finesse and balance of the wine VOC bouquet. In this study both SF and SpF treatments saw significant increases of aliphatic alcohols, which are known to be associated with favourable palate characteristics, for example. It should be noted however, that excessive N fertilization (while improving aroma of wine) can lead to excessive vegetative growth and disease susceptibility (González-Barreiro et al., 2015). A balance is therefore required between sufficient nutrition for optimal aroma and sensory profiles and the overall health and productivity of the plant.

The photosynthetic activity of grapevines plays an important function in the development of an increased wine olfactory profile, influencing the production of aroma compounds in the grapes. Photosynthesis influences the production of precursor molecules, such as amino acids and fatty acids, which contribute to the formation of volatile compounds responsible for wine aromas (Tomasi et al., 2021). Therefore, a higher photosynthetic activity in grapevines can be correlated with a better wine olfactory profile, as it enhances the availability of sugars, precursor molecules, and secondary metabolites involved in aroma compound formation (Fregoni, 2013). In this research, regarding the olfactory sensory profile, it is clear how SpF promoted notes and scents typical of this wine variety: the judges in fact perceived the wine obtained from this treatment as being more associated with 'red ripe fruit', 'jammy', 'herbaceous', and 'spiciness', with a general higher finesse and balance. This is correlated to the higher photosynthetical activity, and related parameters: the plants produced and accumulated more secondary metabolites, which translated in a best wine sensory profile. Also, there were no statistically significant differences between treatments with regards to yield and yield-related parameters, thus, the best wine profile is probably due to higher photosynthesis rate and the fertigation treatment applied (Li et al., 2022; Lu et al., 2021).

The increased photosynthetic parameters observed in SF and SpF treatments of this study were correlated with increased abundance of norisoprenoid and monoterpene VOC compounds, which was consistent with previous findings (Bureau et al., 2000). This highlights the importance of appropriate (and consistent; SpF) direct fertigation of plants for the improvement of Schiopettino sensory profiles. The synthesis and formation of VOC compounds giving rise to aroma and flavour in wines is inherently tied to soil nutritional quality and water availability (Robinson et al., 2011). Sufficient and balanced nutrition allows plants to commit a greater amount of photosynthetic products to primary and secondary metabolites biosynthesis (from which VOCs are derived), particularly at critical phases of development such as fruit maturation (González-Barreiro et al., 2015). Thus, adequate nutrition leads to a greater accumulation of these metabolites within the berries, and ultimately increases the finesse and balance of the wine VOC bouquet. In this study both SF and SpF treatments had a significant increases of aliphatic alcohols, which are known to be associated with favourable sensorial characteristics. It should be noted however, that excessive N fertilization (while improving aroma of wine) can lead to excessive vegetative growth and disease susceptibility (González-Barreiro et al., 2015). A balance is therefore required between sufficient nutrition for optimal aroma and sensory profiles and the overall health and productivity of the plant. In general, the increase in aromatic components of the grape allows for improved aroma management during vinification, optimizing the organoleptic quality of wines by fully harnessing the oenological potential of the grape.

5. Conclusions and remarks

Fertigation (SpF) promoted the photosynthesis parameters compared to a single fertilization (SF) and to the non-treated vines (NTC). On these basis, the biological and physiological activity of the whole plant was enhanced. SpF treatment tendentially and significantly improved the qualitative, productive, physiological, and oenological parameters of Schioppettino wine.

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CRediT authorship contribution statement

Giovanni Mian: Writing – review & editing, Writing – original draft, Resources, Methodology, Formal analysis, Data curation, Conceptualization. **Andrea Colautti:** Writing – original draft, Software. **Nicola Belfiore:** Writing – original draft, Resources, Formal analysis, Data curation, Conceptualization. **Patrick Marcuzzo:** Writing – original draft, Resources, Funding acquisition. **Diego Tomasi:** Validation, Supervision. **Luke Bell:** Writing – review & editing, Writing – original draft. **Emilio Celotti:** Visualization, Validation, Supervision.

Declaration of competing interest

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

Data availability

The data that has been used is confidential.

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