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Scaling up: microbiome manipulation for climate change adaptation in large organic vineyards

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Regenerative agriculture offers important solutions to the enormous challenges that the climate crisis poses on food production. However, there are doubts about the possibility of implementing many of these solutions in a particularly important sector: the large scale. This paper addresses the issue, presenting examples of large-scale vineyard soil microbiome manipulation in Chile. The South American country has strongly faced the effects of climate change during the last decade and the organic viticulture sector is actively seeking strategies to adapt to the new climatic reality. Here the results of 4 experiments under real production conditions are shown. The experiments were designed to assess the effects of adding various microbial consortia to the soil on key agronomic parameters. Successful as well as unsuccessful cases are presented, allowing discussion of some conditions under which the microbiome manipulation can be expected to have positive effects. It was found that under good management conditions, incorporating effective microorganisms has positive effects on important production parameters (yield, root and vegetative growth). However, when fields yields are trending downward for prolonged periods, the incorporation of effective microbial consortia (e.g., antagonistic fungi, nutrient-fixing and nutrient-solubilizing bacteria) does not have a positive effect on the vineyard trend immediately. Similarly, even in favorable conditions the positive effects cannot be expected to be expressed in the short term (i.e., in just a few months). Therefore, its use should be conceived as a long-term strategy, not as an immediate solution to urgent management problems.

KEYWORDS

regenerative agriculture, ecological soil management, large-scale agriculture, plant microbiome, efficient microorganisms

1 Introduction

> *To see a world in a grain of sand and a heaven in a wild flower, hold infinity in the palm of your hand and eternity in an hour. Auguries of Innocence by* Blake W. (1988).

Extreme temperatures have become a daily occurrence, and it is not uncommon to see a new record set somewhere on the planet (Witze, 2022). Undoubtedly, we are living the beginning of a serious climate crisis at a planetary level and it is necessary to adapt to this context (Lovelock, 2007; Archer and Rahmstorf, 2010; Shen et al., 2018; Chakrabarty, 2021). For example, in Chile, a climate emergency was declared in 2021 due to the intense drought

suffered in the last decade (Aparicio, 2021). This has meant significant challenges for its viticulture sector, especially for the large-scale and export-oriented subsector (Crowley, 2000; Hadarits et al., 2010; Mills-Novoa et al., 2016; Haddad et al., 2020). Climate change poses a major threat to grapevine cultivation (Coombe, 1987; Moutinho-Pereira et al., 2004; Greer et al., 2010, 2013; Fraga et al., 2020; Jones et al., 2022) and this may have mayor economic repercussions worldwide (FAO/OIV, 2021). In the emerging organic wine sector, the situation becomes even more complex, due to restrictions impose on crop management by the different certifications and the increase in manufacturing cost, especially in systems with high dependence on external inputs (Pino, 2013; Migliorini and Wezel, 2017; Pekdemir, 2018).

Climate forecasts anticipate a global decrease in water availability in most wine-producing regions (Santillán et al., 2019). This issue has sparked significant concern within the viticulture industry, prompting a considerable number of scientific papers to delve into the subject (Fraga et al., 2012; Xu et al., 2012; Mosedale et al., 2016; Storchmann, 2016; Ollat et al., 2017; van Leeuwen et al., 2019). For instance, it is expected that the increase in aridity in the future will result in a widespread loss of suitability for viticulture in the mediterranean climate zones of southern Europe (Droulia and Charalampopoulos, 2021), region responsible for 54% of the world's wine exports (and 61% in terms of value) (Šajn, 2023). It is also important to consider that the feasibility of wine production is based both on yield and the quality of the grapes, as the latter can have a significant impact on the quality of the resulting wine and the prices consumers are willing to pay. In fact, wine prices, depending on their quality, can vary by a factor of up to 1,000, while yields usually fluctuate by a factor close to 10 (van Leeuwen et al., 2019).

The increase in temperatures and the reduction in rainfall, linked to climate change, can greatly affect the quality of the fruit and the yield of the crops in the vineyard (van Leeuwen et al., 2019). Among other aspects, climate change can impact the composition of the grape, its physiology, its phenology, and the quality of the wine. For example, high temperatures between veraison and harvest can result in an unbalanced fruit composition (due to the desynchronization in the development of sugars, acids, and other berry components) (van Leeuwen et al., 2019; Morales-Castilla et al., 2020). This can generate excessively high sugar levels, too low acidity, and an aromatic expression dominated by cooked fruit aromas, resulting in wines that lack freshness and aromatic complexity (Mira de Orduna, 2010; van Leeuwen et al., 2019; Morales-Castilla et al., 2020; Santos et al., 2020).

Among the techniques that can be used in organic agriculture to adapt agroecosystems to the new climatic context, is the manipulation of the microorganism community associated with plants, especially those present in the soil (Toro and Andrade, 2020; Chouhan et al., 2021; Antoszewski et al., 2022; Sandrini et al., 2022). This strategy can be underappreciated when the complexity of this component is underestimated (Vandermeer and Perfecto, 2018). However, soil is home to 59% of the planet's biodiversity (Anthony et al., 2023) and is a complex web of ecological interactions (Wall and Moore, 1999; Reynolds et al., 2003).

In fact, the well-being and overall health of plants is highly dependent on these ecological interactions (Barrow et al., 2008, Chouhan et al., 2021; Antoszewski et al., 2022; Sandrini et al., 2022). Particularly important are those between the microorganisms associated with them, whether inside, outside or in the immediate vicinity of their bodies (Barrow et al., 2008; Qiao et al., 2023), which can help plants withstand important stress conditions (Barrow et al., 2008; Albornoz et al., 2022). The organisms involved in these interactions are known as the plant microbiome in the scientific literature (Whipps et al., 1988; Lederberg and McCray, 2001; Marchesi and Ravel, 2015; Berg et al., 2020) and are generally referred to as efficient microorganisms in the ecological agriculture milieu (Singh et al., 2011; de Araujo Avila et al., 2021). These microorganisms are related to health, well-being and tolerance to different forms of stress in plants (Mesa-Marín et al., 2019; Redondo-Gómez et al., 2022), for example, through the production of phytohormones, such as indole acetic acid, cytokinin, abscisic acid and ethylene reduction (Martínez-Viveros et al., 2010; Basu et al., 2021; Gupta et al., 2022; Notununu et al., 2022; Carreiras et al., 2023).

The microbiome can aid in the adaptation of crops to climate change through various mechanisms. For example, one expected impact of climate change is a significant reduction in rainfall and water availability for agriculture (Malek et al., 2018; Arora, 2019; Malhi et al., 2021), a situation already present in Chilean agriculture (del Pozo et al., 2019; Fernández et al., 2019; Vicuña et al., 2021). In such circumstances, introducing efficient microorganisms into the soil can promote root development (Lareen et al., 2016; Mhlongo et al., 2018; Pascale et al., 2020; Molefe et al., 2023), enabling more thorough soil exploration for water and enhancing plant vigor under harsh conditions (Agler et al., 2016; Tao et al., 2019; Arif et al., 2020; Singh et al., 2020; Trivedi et al., 2020; Gupta et al., 2021).

To some extent, plant health can be conceived as a possible state that emerges from interactions in its microbiome. Thus, the manipulation of this community offers important opportunities for the ecological management of agroecosystem in general (Chouhan et al., 2021) and to adapt these systems to the new climatic conditions in particular (Barrow et al., 2008; Albornoz et al., 2022). In fact, efficient microorganisms can be a valuable tool to adapt vineyards to new climatic conditions (Aguilera et al., 2022; Carreiras et al., 2023). However, agroecological practices are typically associated with small-scale farming, it is therefore necessary to demonstrate that they work at larger scales (Dalgaard et al., 2003; Nicol, 2020; Petit et al., 2020; Mayer et al., 2022), as is the case of Chilean export viticulture (Crowley, 2000).

The objective of this paper is to present a set of results on the manipulation of the microbiome in large-scale viticultural systems, in the context of the climate change that Chilean agriculture is currently facing. For this, we will present: 1—Results on the effect of incorporating or not, functional microorganisms in organic vineyards, 2—An experiment where the different treatments consist in adding (at consecutive times) different functional microorganisms in organic vineyards, 3—Results, at nursery level, on the short-term effect of inoculating grapevine plants with mycorrhiza-forming fungi, and 4—An experiment in which the effect of applying effective microorganisms in conjunction with a sugar source is evaluated in organic vineyards. In summary, this research aimed to evaluate the efficacy of various microbiome manipulation strategies in real-world field conditions, in an agricultural setting that is experiencing substantial impacts from climate change (Young et al., 2010; Roco et al., 2014, 2017). Consequently, the findings of this study offer practical value to farmers engaged in organic viticulture, as they search for feasible strategies to adapt to the challenging realities imposed by climate conditions.

2 Materials and methods

This article shows data obtained under real commercial field management conditions. All trials were conducted in commercially active fields, the harvests of which were taken to vinification. For this same reason, when the response variables involve destruction or damage to plants, the sample sizes conform to the minimum suggested for conducting efficacy trials (Kalamarakis and Markellou, 2007; EPPO, 2012a,b). The treatments used in the different experiments consist of bacterial and/or fungal plant growth-promoting consortia, based on evidence showing that this strategy is superior to the use of monospecific treatments (Carreiras et al., 2023). This is because the use of a single strain of microorganism does not allow benefiting from the synergistic effects offered by consortia, thanks to the activation of different growth-promoting mechanisms and the interaction between them (Mesa-Marín et al., 2019; Redondo-Gómez et al., 2022).

For the reasons set forth above, the following criteria were used in conducting the experiments: 1—The trials were carried out in actively producing crop fields, which have similar characteristics (in terms of soil, area, planting density, and varieties used) to the production units characteristic of the regions in which the experiments were conducted. 2—The microbial consortia used are readily available in commercial formulations, and the species included are of recognized utility in organic farming. 3—The response variables evaluated in the experiments are of easy agronomic interpretation and are routinely measured and used in commercial vineyards to make management decisions. These variables were measured using the techniques routinely used in the vineyards. This set of criteria aims to encourage the use of the results of this work by farmers.

The characteristics of four experiments are presented below. In all cases the microorganisms were used under the hypothesis that their incorporation into the system would improve the performance of plants under climatic stress conditions (Barrow et al., 2008; Aguilera et al., 2022; Carreiras et al., 2023), due to high solar radiation, high temperatures, low relative humidity and reduced rainfall levels. The microorganisms used in the experiments are part of commercial formulations available in Chile, approved for use in organic agriculture according to the USDA-NOP and EU-Chilean 20.089 standards. In all cases, the extra ingredients found in the treatments are either part of the commercial formulations used in the experiments (i.e., co-formulants, humic acid and seaweed extracts) or are incorporated to evaluate their effect in conjunction with the microorganisms (i.e., natural nanoparticles, composted hyacinth extract and sugar). In all cases, multiple modeling techniques were used to analyze the data (conventional analysis of variance, non-parametric analysis by ranks, generalized linear models, and permutation analysis of variance). The details of the analyses used in each case are presented after the description of the treatments for each experiment.

2.1 Experiment 1

This experiment was conducted during the 2020–2021 season in Santa María commune (Valparaiso Region, Chile) on a total area of 8 ha planted with Cabernet Sauvignon variety (established in 2012). The vineyard has a planting distance of 2.2 m between-rows by 1.2 m in-rows, using a simple trellis system, under a controlled drip irrigation system. The site has a loam soil, with 1.8% of organic matter and pH 7.5. Daily values of air temperature (average), accumulated precipitation, and solar radiation during the experiment are shown in the Supplementary Table S1. A completely randomized one-way classification design was used (Montgomery, 2004), to evaluate the effect of incorporating into the soil (via fertigation) efficient microorganisms, in contrast to a control. Treatments are identified as: T1—Control, T2—Incorporation of effective microorganisms. Fifteen replicates of each treatment were carried out. Each experimental unit consisted of 18.180 linear meters. In total, each treatment occupied an area of 4 ha. In treatment 2, microorganisms were applied on two dates (see details in Table 1).

The variables evaluated are associated with root development (root weight) and vigor expression (pruning weight). Both are important parameters in commercial wine production. To calculate the weight of the roots, trial pits were made using the modified monolith method (Böhm, 1979). For this purpose, a block of soil (60 cm wide x 60 cm deep x 240 cm long) was extracted from the west side of the plants and the roots were obtained from it. The roots were washed and weighed in the laboratory. To determine the pruning weight, the commercial pruning of a portion of the vineyard called "claro" or "entreposte" (a 6-meter portion of a row with 5 vine plants) was carried out. In other words, after winter pruning of vineyard, the weight of plant material removed was quantified.

The following analyses were performed on the data obtained (Montgomery, 2004): 1—Kolmogorov–Smirnov normality test,

TABLE 1 Timeline of applications and microorganisms used in experiment 1.

Applications	First date (consortium 1)	Second date (consortium 2)
Treatment 1	–	–
Treatment 2	*Trichoderma rifai* (strain AMTtr02)[1] *Trichoderma harzianum* (strain AMTtr03)[1] *Trichoderma virens* (strain AMTtr12)[1] *Bacillus amyloliquefaciens* (strain AMTba21)[2] *Bacillus subtilis* (strain AMTbsR06)[2] *Lysinebacillus* spp.[3] *Bacillus* spp.[3]	*Trichoderma rifai* (strain AMTtr02)[1] *Trichoderma harzianum* (strain AMTtr03)[1] *Trichoderma virens* (strain AMTtr12)[1] *Bacillus amyloliquefaciens* (strain AMTba21)[2] *Bacillus subtilis* (strain AMTbsR06)[2] *Penicillium smithii* (strain AMTps01)[4] *Penicillium bilaie* (strain AMTpb01)[4] *Penicillium cellulolyticus* (strain AMTpc01)[4] *Bacillus megaterium* (strain AMTbm01)[2] *Bacillus aryabhattai* (strain AMT bar01)[2]

[1]Minimum concentration 3×10^8 cfu/g of *Trichoderma* spp. in total.
[2]Minimum concentration 5×10^9 cfu/g of *Bacillus* spp. in total.
[3]Minimum concentration 1×10^{10} cfu/g of bacteria in total.
[4]Minimum concentration 1×10^9 cfu/g of *Penicillium* spp. in total.
Doses applied on the first and second date are 0.5 Kg/ha.
cfu, colony forming units.

2—Levene's test for homogeneity of variances. Where these assumptions were met, the variables were evaluated using T-tests. In cases where non-normality and/or heteroscedasticity deviations occurred, which could not be corrected using Box-Cox transformations (Dag and Ilk, 2017), variables were also evaluated using the nonparametric Mann–Whitney rank test (Montgomery, 2004). Generalized linear models and permutation analysis of variance (Permanova) were also performed for each response variable (Anderson, 2001; Bolker et al., 2009). All analyses were performed in the R programming environment (R Core Team, 2021), according to the protocols outlined in Bates et al. (2015), Lawson (2015) and Oksanen et al. (2020) for the indicated analyses.

2.2 Experiment 2

The experiment was conducted during the 2020–2021 season in Chimbarongo Commune (Libertador General Bernardo O'Higgins Region, Chile) on a total area of 10 ha planted with Cabernet Sauvignon variety (established in 2005). The vineyard has a planting distance of 1.8 m between-rows by 1 m in-rows, using a simple trellis system, under a controlled drip irrigation system. The site has a loam soil, with 2.4% of organic matter and pH 6.1. Daily values of air temperature (average), accumulated precipitation, and solar radiation during the experiment are shown in the Supplementary Table S2. A completely randomized one-way classification design was used (Montgomery, 2004), to evaluate the effect of incorporating into the soil (via fertigation) different combinations and application times of efficient microorganisms, plus a control. The different treatments were (see details in Table 2): T1—Control, T2—Application (at a single point in time) of a set of microorganisms, T3—Sequential application of different sets of microorganisms and T4—Sequential application of different sets of microorganisms (same as those used in T3) plus natural nanoparticles. Treatments 2, 3 and 4 were established by crop experts and bioinput suppliers' recommendations. There were 10 replicates of each treatment, each experimental unit consisted of 95 rows. The same variables already described in Experiment 1 were evaluated (using the same methodologies).

The following analyses were performed on the data obtained (Montgomery, 2004): 1—Kolmogorov–Smirnov normality test, 2—Levene's test for homogeneity of variances. Where these assumptions were met, the variables were evaluated using ANOVAs. In cases where non-normality and/or heteroscedasticity deviations occurred, which could not be corrected using Box-Cox transformations (Dag and Ilk, 2017), variables were also evaluated using the Kruskal–Wallis rank tests (Montgomery, 2004). Generalized linear models and permutation analysis of variance (Permanova) were also performed for each response variable (Anderson, 2001, Bolker et al., 2009). All analyses were performed in the R programming environment (R Core Team, 2021), according to the protocols outlined in Bates et al. (2015), Lawson (2015) and Oksanen et al. (2020) for the indicated analyses.

2.3 Experiment 3

The experiment was conducted during the 2021–2022 season in Chimbarongo Commune (Libertador General Bernardo O'Higgins Region, Chile), in a nursery (use to obtain plants for replanting). A completely randomized one-way classification design (Montgomery, 2004) was used to evaluate the effect, in Sauvignon Blanc grapevines planted in nursery, of different forms of mycorrhiza-forming fungi application, plus a control. Specifically, three treatments were evaluated (see details in Table 3): T1—Control, T2—Application via drenching of 2 g of mycorrhizae in a 250 mL solution with non-chlorinated water, applied with a pitcher on the substrate in which the vines were planted (i.e., post-planting) and T3—Immersion of roots for 10 min in a 200 lt solution containing mycorrhizae (1 g of mycorrhizae: 125 mL of non-chlorinated water) (i.e., prior to planting).

In all cases the plants were planted on standard substrate (see Table 4) of grapevine nursery, in plastic bags (5 L). Daily values of air temperature (average), accumulated precipitation, and solar radiation during the experiment are shown in the Supplementary Table S3. The effect of treatments on root weight was evaluated. For this purpose, the plants were extracted from the bags and the substrate adhered to the roots was removed with a pressure washer. The roots were cut and taken to the laboratory, where they were weighed. Measurements were made at two different times: 180 days after application and 240 days after application. The two measurements were made on different plants. At each measurement time, 20 plants per treatment were evaluated. The data were analyzed using the same methodology described in experiment 2.

2.4 Experiment 4

The experiment was conducted during the 2022–2023 season in the Santa María Commune (Valparaiso Region, Chile) on a total area of 6 ha planted with Cabernet Sauvignon variety (established in 2010). The vineyard has a planting distance of 2.2 m between-rows by 1.2 m in-rows, using a simple trellis system, under a drip irrigation system. The site has a loam soil, with 1.8% of organic matter and pH 7.5. Daily values of air temperature (average), accumulated precipitation, and solar radiation during the experiment are shown in the Supplementary Table S4. A completely randomized two-way classification design (Montgomery, 2004) was used to evaluate the effect of two factors (each with two levels). The factors evaluated were: Factor 1—incorporation or not of efficient microorganisms to the soil, and Factor 2—application or not of a sugar source to the crop (jointly to leaves and soil). A full factorial design (Montgomery, 2004) was used to evaluate the effect of the different combinations of factor levels in the bunch weight at harvest. A total of 810 bunch measurements were made in each of the combinations of the two factors (called treatments and named: T1, T2, T3 and T4).

The sugar source used in this trial was organic cane sugar (28% total sugars, 46% organic matter), in conjunction with a composted hyacinth plant extract (enriched with willow bark). The efficient microorganisms used were: consortium 3, consortium 4 and *Trichoderma harzianum* (i.e., the microorganisms used in the treatment 3 of experiment 2). The characteristics of the different combinations of inputs evaluated in each treatment are described below:

T1—Control. Neither sugars nor efficient microorganisms were applied.

TABLE 2 Timeline of applications and microorganisms used in experiment 2.

Application dates (Phenology)	Treatment 1	Treatment 2	Treatment 3	Treatment 4
October 2nd (Beginning of budburst)	–	–	Consortium 3 Dosage: 3 kg/ha	Consortium 3 Dosage: 3 kg/ha + Nano Particles[a] Dosage: 3 kg/ha
October 16th (2 weeks after budburst)	–	–	*Trichoderma harzianum* Dosage: 2 kg/ha	*Trichoderma harzianum* Dosage: 2 kg/ha + Nano Particles[a] Dosage: 3 kg/ha
October 30 (Radical flash start)	–	Consortium 4 Dosage: 2 kg/ha	Consortium 4 Dosage: 2 kg/ha	Consortium 4 Dosis: 2 kg/ha + Nano Particles[a] Dosage: 3 kg/ha
January 20 (Pre-veraison)	–	–	Consortium 5 Dosage: 1 L/ha	Consortium 5 Dosage: 1 L/ha + Nano Particles[a] Dosage: 3 kg/ha

Consortium 3: *Bacillus thuringiensis* strain Anemophila 8 g/kg; *Bacillus cereus* strain Bromelia 8 g/kg; *Bacillus cereus* strain Peumo 8 g/kg. In the concentration of strains 1 × 10 cfu/g.
Consortium 4: *Trichoderma virens* strain Luito, *Bacillus subtilis* strain N5 7×10^7 cfu/g. Coformulants 3×10^7 cfu/g 96.4% w/w (964 g/kg).
Consortium 5: *Trichoderma* spp. 3,651% w/v, Concentrated Suspension 1×10^9 conidia/mL.
cfu: colony forming units. [a]Natural nano particles 98% w/w particle size 230 mesh.

TABLE 3 Treatments used in experiment 3.

Treatments	Application mode
T1	None
T2	Consortium 6 Via drenching (dosage: 250 mL/plant)
T3	Consortium 6 Via immersion (10 min in mycorrhizae solution)

Consortium 6 (active ingredients at 0.1%): *Glomus intraradices* (225 viable propagules/gram), *Glomus aggregatum* (225 viable propagules/gram), *Glomus mosseae* (225 viable propagules/gram), *Glomus etunicatum* (225 viable propagules/gram), humic acid (powder) approx. 49.95%, seaweed extract (powder) approx. 49.95%.
Immersion was performed by immersing plants in a solution with consortium 6 in a bucket for 10 min. Drenching was performed by applying the solution with the consortium 6 with a back pump to the bagged plant substrate.

T2—A sugar source (plus hyacinth) was applied to the foliage and soil as described in Tables 5, 6.

T3—Different efficient microorganisms were applied to the soil as described in Table 7.

T4—A sugar source (plus hyacinth) and soil efficient microorganisms were applied as described in Tables 8, 9.

The following analyses were performed on the response variable (Montgomery, 2004): 1—Kolmogórov-Smirnov normality test, 2—Levene's test for homogeneity of variances. Since the response variable presented deviations from normality and heteroscedasticity, which could not be corrected using Box-Cox transformations (Dag and Ilk,

TABLE 4 Composition of the substrate used in experiment 3.

pH	6.1	**Total magnesium (MgO)**	0.5%
Electrical conductivity	3.7 dS/m	**Total iron (Fe)**	6,965 mg/kg
Organic matter	54.5%	**Total manganese (Mn)**	262 mg/kg
Organic carbon	30.3%	**Total boron (B)**	47 mg/kg
Total nitrogen (N)	1.15	**Total copper (Cu)**	80 mg/kg
Relation C/N	26.3	**Total zinc (Zn)**	91 mg/kg
Total phosphorus (P2O5)	2.5%	**Humidity**	35%
Total potassium (K2O)	0.58%	**Dry matter**	65%
Total calcium (CaO)	3.5%	–	–

TABLE 5 Foliar applications in treatment 2 of experiment 4.

Date	Phenological stage	Sugar Lt/ha	Hyacinth Lt/ha	Spray Lt/ha
November 15	Flowering	0	10	500
December 20	Berry growth	0	10	500
January 15	Veraison	1.5	10	500
February 10	Pre-harvest	1.5	10	500

TABLE 6 Soil applications made in treatment 2 of experiment 4.

Date	Phenological stage	Sugar Lt/ha	Hyacinth Lt/ha
October 25	Beginning of sprouting	5	10

TABLE 7 Microorganisms used in treatment 3 of experiment 4.

Date	Phenological stage	Consortium 3 kg/ha	*T. harzianum* kg/ha	Consortium 4 kg/ha
October 25	Beginning of budburst	3	–	–
November 15	Flowering	–	2	–
December 20	Berry growth	–	–	2
January 15	Veraison	–	–	1

TABLE 8 Foliar applications made in treatment 4 of experiment 4.

Date	Phenological stage	Sugar Lt/ha	Hyacinth Lt/ha	Spray Lt/ha
November 15	Flowering	0	10	500
December 20	Berry growth	0	10	500
January 15	Veraison	1.5	10	500
February 10	Pre-harvest	1.5	10	500

2017), it was evaluated by means of an ANOVA performed on the data transformed using the Aligned Rank Transform (ART) procedure (Wobbrock et al., 2011; Elkin et al., 2021). It was also analyzed using a generalized linear model with a gamma-type error distribution (Bolker et al., 2009). Finally, permutation analysis of variance (Permanova) was also performed to the response variable (Anderson, 2001). All analyses were performed in the R programming environment (R Core Team, 2021), according to the protocols outlined in Bates et al. (2015), Lawson (2015) and de Mendiburu and Yaseen (2020), Oksanen et al. (2020) and Kay et al. (2021) for the indicated analyses.

3 Results

Levins (1966) rightly stated that "*all models leave out a lot and are in that sense false, incomplete, inadequate,*" and Box (1979) in the same vein said that "*all models are wrong, some are useful.*" For this reason, caution should be exercised in interpreting their results. One way to do this, without renouncing the clear advantages of their use in the interpretation of nature, is through the use of the concept of robustness proposed by Levins (1966). According to this, if multiple representations of reality (models), operating under different assumptions coincide, we are in the presence of a robust result.

In Levins' (1966) own words: "*our truth is the intersection of independent lies*." It is for this reason that here are used multiple approaches to data modeling. In this regard, it is important to mention that the results obtained from these methodologies (conventional analysis of variance, nonparametric analysis of variance by ranks, generalized linear models and analysis of variance by permutations) in the majority of cases coincided, which is an indication of their robustness (in the sense mentioned above).

In all cases the results are summarized with boxplots. The mean value is indicated by a black cross. Multiple comparison results are shown using the compact letter display. In all cases, the multiple comparison tests were performed with an alpha value equal to 0.05 (with Bonferroni correction). In addition, the average value (for each treatment) is presented with numbers and the standard deviation in parentheses.

3.1 Results experiment 1

The root weight variable fits well to a normal distribution (Kolmogorov–Smirnov test statistic D = 0.12939, p-value = 0.2251) and has no significant heteroscedasticity issues (Levene test statistic = 3.2433, p-value = 0.0825). The variable pruning weight does not fit well to a normal distribution (Kolmogorov–Smirnov test statistic D = 0.16511, p-value = 0.03605) and has no significant heteroscedasticity issues (Levene test statistic = 3.7787, p-value = 0.06202). For the latter variable, normality issues could be solved by a Box-Cox transformation with a lambda hat value of −0.32 (Kolmogorov–Smirnov test values on the transformed data: D = 0.08486, p-value = 0.8406).

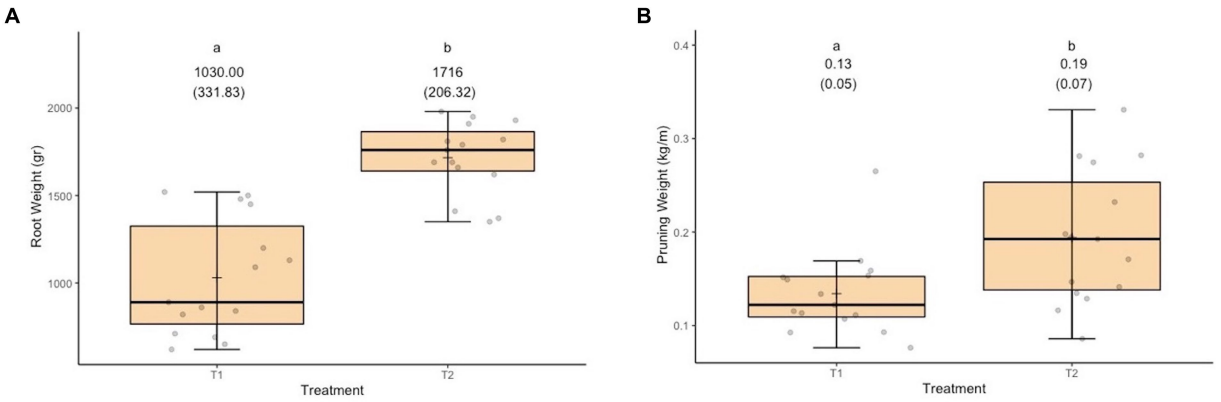
Incorporating efficient microorganisms was found to have effects (see Figure 1C) on both root growth and pruning weight. This is likely to be achieved through the interaction of microorganisms with roots, fixation atmospheric nitrogen and facilitation of phosphorus uptake, that may result in the stimulation of root and leaf growth (see Figures 1A,B). In both cases the effects are in the desired direction (considering that the plants are subjected to strong stress due to solar radiation and drought, which limits their productive potential and oenological quality). Specifically, in the treatment involving microorganisms is observed: 1—Roots have a higher development (see Figure 1A), which suggests a greater capacity of plants to take advantage of moisture and capture nutrients in the soil, as well as to increase root exploration. 2—In the case vegetative development, there is a higher pruning weight (see Figure 1B), which is associated with greater plant vigor, higher capacity to accumulate photoassimilates and therefore greater productive potential.

3.2 Results experiment 2

The variable root weight fits well to a normal distribution (Kolmogorov–Smirnov test statistic D = 0.12202, p-value = 0.14) and has no issues of heteroscedasticity (Levene test statistic = 0.55928, p-value = 0.6453). The variable pruning weight fits well to a normal distribution (Kolmogorov–Smirnov test statistic D = 0.077778, p-value = 0.7851) and has no heteroscedasticity issues (Levene test statistic = 0.81566, p-value = 0.4937). No effect of treatments was found on the response variables evaluated (see Figure 2C). In terms of the responses to the treatments, it is worth commenting that for root weight a less uniform response is observed, than what was observed for pruning weight (see Figures 2A,B). This possibly has to do with the fact that the roots are directly in the medium in which the microorganisms were incorporated, so their action could be manifested there first.

TABLE 9 Soil applications made in treatment 4 of experiment 4.

Date	Phenological stage	Sugar Lt/ha	Hyacinth Lt/ ha	Consortium 3 kg/ha	*T. harzianum* kg/ha	Consortium 4 kg/ha
October 25	Beginning of budburst	5	10	3	–	–
November 15	Flowering	–	–	–	2	–
December 20	Berry growth	–	–	–	–	2
January 15	Veraison	–	–	–	–	2



C

Roots		
Test	Statistic	p-value
t-test	t = -6.7995	2.192e-07
Wilcoxon rank	W = 12	3.35e-05
PERMANOVA	F = 46.233	0.001
GLM (gamma)	chi-sq. = 1.93	> 0.01

Pruning		
Test	Statistic	p-value
t-test	t = -2.7173	0.01116
Wilcoxon rank	W = 52	0.01126
PERMANOVA	F = 7.3881	0.012
GLM (gamma)	chi-sq. = 1.02	> 0.01

FIGURE 1
Results of experiment 1. **(A)** Results of the root weight variable. **(B)** Results of the pruning weight variable. **(C)** Values obtained in the tests performed. T1, control; T2, application of microorganisms presents in consortia 1 and 2. GLM, generalized linear model (a gamma type error distribution was used in the GLMs).

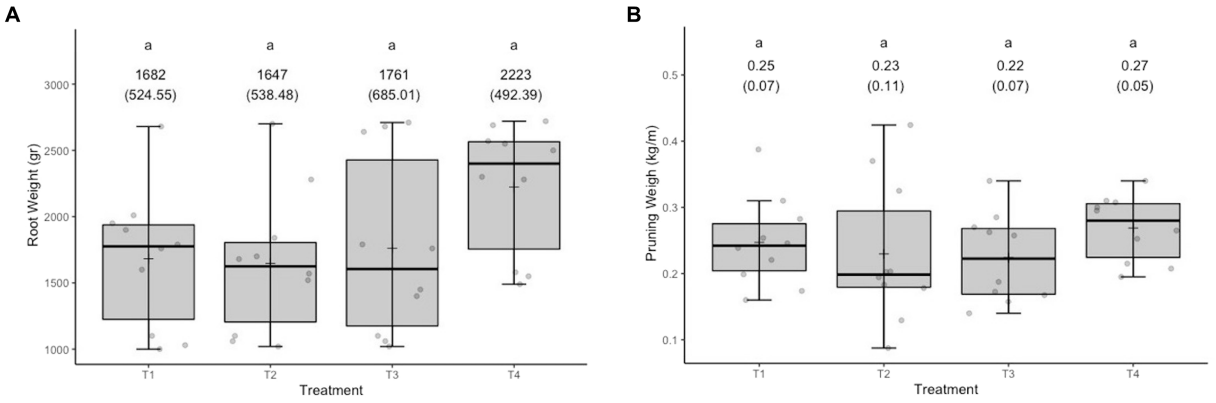
3.3 Results experiment 3

The variable root weight in the first measurement (Figure 3A) fits well to a normal distribution (Kolmogorov–Smirnov test statistic D = 0.10192, *p*-value = 0.126) and has no heteroscedasticity issues (Levene test statistic = 0.81789, *p*-value = 0.4465). The variable root weight in the second measurement (Figure 3B) fits well to a normal distribution (Kolmogorov–Smirnov test statistic D = 0.065306, *p*-value = 0.7577) and has no heteroscedasticity issues (Levene test statistic = 0.67022, *p*-value = 0.5156). The overall relationship between the different treatments is similar for the two measurement times (see Figures 3A,B). Figure 3C shows that, for both measurement moments, the *p*-values obtained are close to the historical significance threshold of 0.05. However, the *p*-values obtained on the second date are lower than those obtained on the first date. For the second date, according to the Tukey multiple comparisons test (α =0.05 and Bonferroni adjustment), only the difference between T1 and T2 presents a 95% confidence interval that does not include 0 (see Figure 3D). It is worth mentioning (in term of effect sizes) that in the treatments that involve fungi, the roots weights (in average) at least 20 gr more than the control.

3.4 Results experiment 4

The response variable of the experiment does not fit well to a normal distribution (Kolmogorov–Smirnov test statistic D = 0.12437, *p*-value < 2.2e-16) and have significant heteroscedasticity issues (Levene test statistic = 28.848, *p*-value < 2.2e-1). The variable distribution is skewed to the right and the data fit better to a model with a gamma error distribution (AIC = 32293.04) than to one with a normal distribution (AIC = 33757.9). The issues of non-normality and heteroscedasticity could not be solved by Box-Cox transformation and the data do not meet the requirements of the aligned rank transform procedure (i.e., not all column of the aligned responses sum to zero). Therefore, in this case the best alternatives are a Pernanova and a GLM with a gamma error distribution.

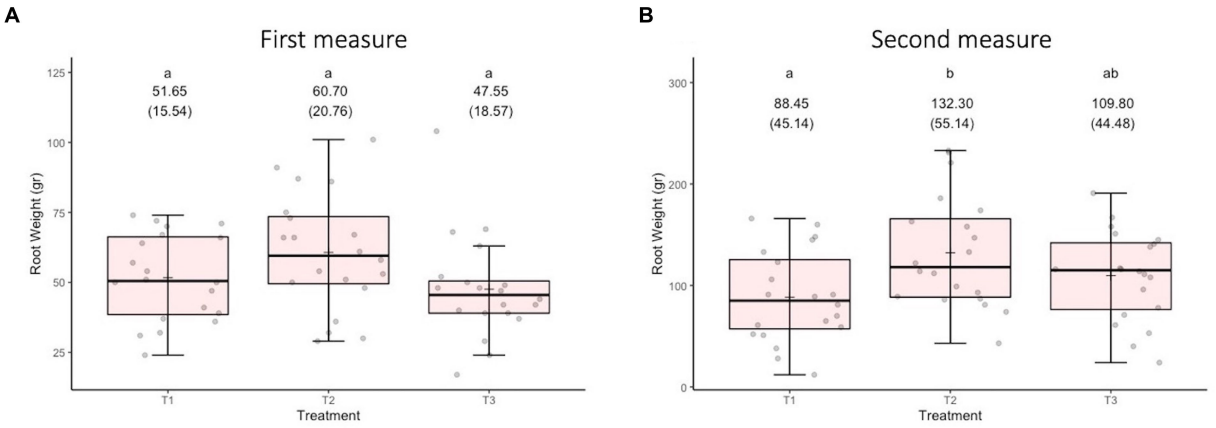
Figure 4 shows that there is a treatment effect, with the best result (higher bunch weight) obtained with treatment 4 (groups created using pairwise Permanovas, with α = 0.05 and a Bonferroni adjustment), which consists of the joint application of efficient microorganisms and a sugar source (plus hyacinth) (see Figure 4A). In fact, the joint application has a positive synergistic effect on bunch



C

Roots			Pruning		
Test	Statistic	p-value	Test	Statistic	p-value
ANOVA	F = 2.208	0.104	ANOVA	F = 0.7	0.558
Kruskal-Wallis	chi-sq. = 4.7086	0.1944	Kruskal-Wallis	chi-sq. = 3.4627	0.3256
PERMANOVA	F = 2.241	0.092	PERMANOVA	F = 0.69984	0.551
GLM (gamma)	chi-sq. = 0.12	0.6	GLM (gamma)	chi-sq. = 0.20	0.59

FIGURE 2
Results of experiment 2. **(A)** Results of the root weight variable. **(B)** Results of the pruning weight variable. **(C)** Values obtained in the tests carried out. T1, Control; T2, Application (in a single moment) of the set of organisms present in consortium 4; T3, Application in sequence of the set of microorganisms present in consortia 3, 4 and 5 (plus *Trichoderma harzianum*); T4, Application in sequence of the set of microorganisms used in T3, plus natural nanoparticles. GLM: generalized linear model (a gamma type error distribution was used in the GLMs).



C

Roots first measure			Roots second measure		
Test	Statistic	p-value	Test	Statistic	p-value
ANOVA	F = 2.67	0.0779	ANOVA	F = 4.088	0.0219
Kruskal-Wallis	chi-sq. = 5.1844	0.07486	Kruskal-Wallis	chi-sq. = 6.1369	0.04649
PERMANOVA	F = 2.6697	0.084	PERMANOVA	F = 4.0879	0.019
GLM (gamma)	chi-sq. = 0.62	0.07	GLM (gamma)	chi-sq. = 1.61	0.02

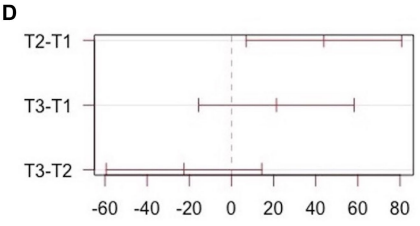
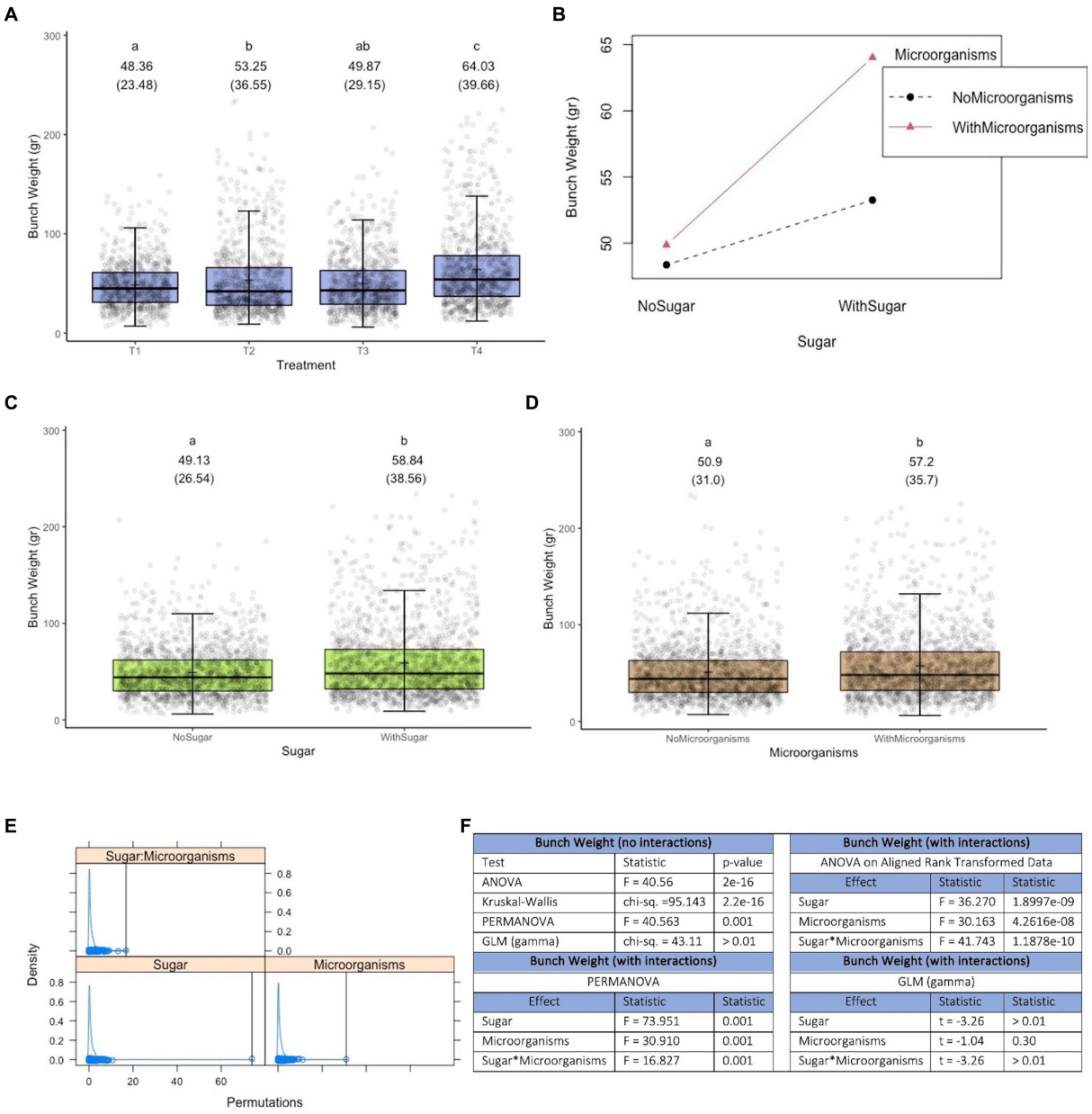


FIGURE 3
Results of experiment 3. **(A)** Results of the root weight variable in the first measurement. **(B)** Results of the root weight variable in the second measurement. **(C)** Values obtained in the tests performed. **(D)** 95% confidence interval of the Tukey multiple comparisons test for the second date. T1: Control, T2: Application of the set of organisms present in consortium 6 via drenching; T3, Application of the set of organisms present in consortium 6 via immersion; GLM, generalized linear model (a gamma type error distribution was used in the GLMs).

weight (see the different slopes in the lines of Figure 4B). It is worth noting that both, the application of microorganisms and sugar separately (i.e., main effects), have positive effects on the response variable. As can be seen in Figures 4E,F for Permanova, both the main effects and their interaction are significant at 0.05 threshold level.



Bunch Weight (no interactions)		
Test	Statistic	p-value
ANOVA	F = 40.56	2e-16
Kruskal-Wallis	chi-sq. =95.143	2.2e-16
PERMANOVA	F = 40.563	0.001
GLM (gamma)	chi-sq. = 43.11	> 0.01

Bunch Weight (with interactions)		
PERMANOVA		
Effect	Statistic	Statistic
Sugar	F = 73.951	0.001
Microorganisms	F = 30.910	0.001
Sugar*Microorganisms	F = 16.827	0.001

Bunch Weight (with interactions)		
ANOVA on Aligned Rank Transformed Data		
Effect	Statistic	Statistic
Sugar	F = 36.270	1.8997e-09
Microorganisms	F = 30.163	4.2616e-08
Sugar*Microorganisms	F = 41.743	1.1878e-10

Bunch Weight (with interactions)		
GLM (gamma)		
Effect	Statistic	Statistic
Sugar	t = -3.26	> 0.01
Microorganisms	t = -1.04	0.30
Sugar*Microorganisms	t = -3.26	> 0.01

FIGURE 4
Results of experiment 4. **(A)** Results of the variable bunch weight. **(B)** Interaction diagram between the factors microorganisms and sugar. **(C)** Main effect of the sugar factor. **(D)** Main effect of the microorganisms factor. **(E)** Density plot of the results obtained in Permanova [the black vertical lines represent the values of the pseudo-F statistic; its specific values and the associated *p*-values are presented in **(F)**]. **(F)** Values obtained in the tests performed. T1, Control; T2, Application of a sugar source; T3, Application of the set of microorganisms presented in consortia 3, 4 and 5 (plus *Trichoderma harzianum*); T4, Application of the microorganisms used in T3 and sugar; GLM, generalized linear model (a gamma type error distribution was used in the GLMs).

4 Discussion

Results of experiments 1 and 4 support published evidence on the benefits of efficient microorganisms to agroecosystems (Martínez-Viveros et al., 2010; Schütz et al., 2018; Basu et al., 2021; Ferreira et al., 2021; Antoszewski et al., 2022; Gupta et al., 2022; Notununu et al., 2022). While experiments 2 and 3 results show some elements that should be taken into account when carrying out microbiome manipulations in ecological farming.

With respect to experiment 2, in order to make a fair assessment of its results, it is good to take into account the historical behavior of the vineyard area where it was carried out. Figure 5 shows that the vineyard sector in which it was implemented, has a clear downward trend in its harvests. This area has been under organic management for more than 12 years and shows clear signs of decline. This is a completely different situation from that found in the areas where experiments 1 and 4 were conducted (data not shown).

It should be noted that, for the vineyard where experiment 2 was conducted, a harvest below 10.000 kg/ha is considered deficient (good values are between: 12.000–14.000 kg/ha). This threshold was not achieved in the 2 years prior to the development of the experiment (see Figure 5), for this reason the different treatments were elaborated

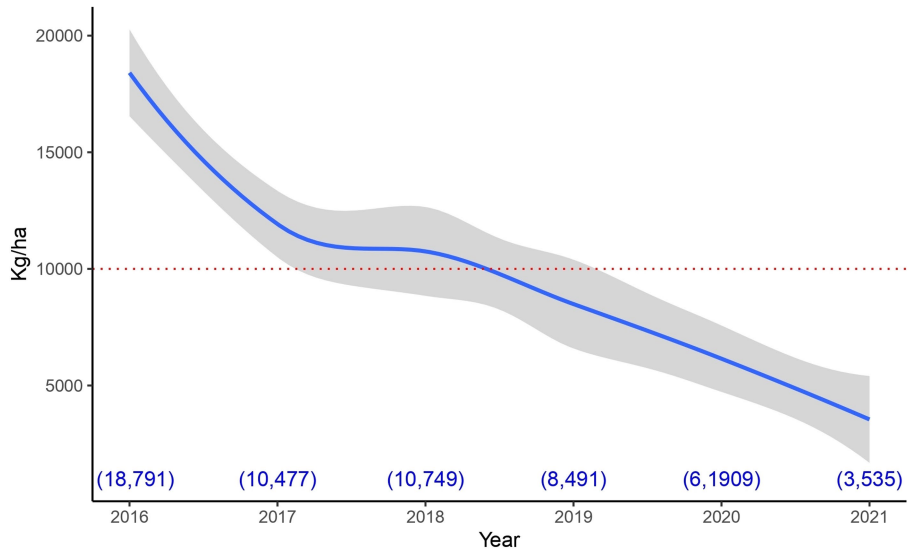


FIGURE 5
Historical production in the area where experiment 3 was carried out. The average production of the area is presented (with its 95% confidence interval). This was done using the smoothed conditional means procedure (Wickham, 2016). The red dotted line represents the minimum acceptable production according to the vineyard standards. The blue numbers in parentheses at the bottom of the graph represent the average production value per hectare for each year.

by a team of experts to try to address the production deficit. Even treatments inspired by the ecological succession process were tested, these involved the incorporation of different groups of microorganisms at different times (specifically treatments 3 and 4).

The low production associated with the sector where the experiment was carried out is possibly related to a number of causes: 1—adverse effect of weather, 2—excessive level of production before 2017 (well above the threshold of 14.000 kg/ha) and 3—mechanical damage caused (over the years) during weed control (because the distance between the rows is too short for the implement used for weeding). Thus, it is possible that the context in which efficient microorganisms were incorporated limited their effect. It should be noted that, given the historical performance of the sector, the vineyard manager decided to replant the vineyard after 2021 harvest (the year in which the experiment was completed).

It is fundamental to take into account that any manipulation of the microbiome is governed by ecological processes (at population and community levels) and that these take time. In a sense, microbiome interventions in agroecosystems are similar to augmentative approaches to biological control (Horn, 1988). In other words, the population density of certain organisms is artificially increased in order to make them perform an action desired by humans. However, changes in population densities are not immediately effective (Eisenhauer et al., 2010). In the case of soil microorganisms, it must be taken into account that in their action, important density dependent mechanisms intervene, for example, quorum sensing (Duddy and Bassler, 2021). While other mechanisms depend also on interactions between species (Qiao et al., 2023) and the nature of these interactions can change over time depending on various conditions such as, for example, the density of participating species (Bronstein, 1994; Griffon and Hernandez, 2019; Hernandez, 2021; Hanusch et al., 2023). Thus, important ecological phenomena in the soil influence the establishment and colonization of the environment by introduced microorganisms. Phenomena that, depending on different factors, may take different times, but certainly do not act immediately.

The times associated with the ecological phenomena possibly explain the results obtained in experiment 3 (particularly in the first measurement). Here it is worth commenting on the differences between the application modes of treatments 2 and 3 in experiment 3. In the case of treatment 3, the roots were in contact with the microorganism solution for 10 min, while in treatment 2 this solution was incorporated into the plant's growing substrate. Therefore, in treatment 2, a greater number of microorganisms are incorporated into the medium, which could lead to greater symbiosis with the roots. For this reason, it is likely that this method of application can achieve the population densities necessary to exert an effect on the plants in a shorter time.

The time required for the growth of microorganism populations may also be associated with the synergistic effect observed in experiment 4. Because the sugar addition to the medium can create a favorable context for the rapid growth of microorganism populations. In addition, on previous experiences we have found that sugar has a positive effect on plants under climatic stress conditions. In this sense, it is important to mention that, in experiments not presented here (not involving efficient microorganisms), only applications of sugar to soil and leaves (together) were found to have positive effects on bunch size. The physiological explanation of this result in terms of fine mechanisms is unknown to us. It is an adaptation strategy inspired by a similar practice used in the management of avocado trees under stress due to climate change in Peru. These results may indicate that the effects of sugar extend to the microbiome found in the plant shoots. It is important to note that the consortia used in this experiment are the same as those used in the treatment 3 of experiment 2, which points out the importance of incorporating efficient microorganisms in a favorable environment.

Recently, there has been renewed interest in the holobiont concept, originally proposed by Lynn Margulis (1991). It accounts for the combination of the host (in this case the plant) with its microbiome. In

other words, a holobiont is a composite entity, consisting of a host together with its microbiome (Roughgarden, 2020). It is important to mention that this controversial proposal already has an eco-evolutionary biomathematical theory that supports it (Roughgarden, 2023). In the context of ecological farming, what is really important is that selection on the holobiont, causes evolutionary changes in the traits of the holobiont itself (Roughgarden, 2020; Mesny et al., 2023; Wolfgang et al., 2023). This is particularly important for evolutionary breeding, which is a breeding strategy that really makes sense for ecological farming (Ceccarelli and Grando, 2020).

Evolutionary breeding is based on Fisher's fundamental theorem of natural selection (Fisher, 1999), which states that the action of natural selection increases the average fitness of populations (as long as they present genetic variation). This theorem can be extended to a context of species interactions (León and Charlesworth, 1978). Thus, the objective of evolutionary breeding is that the forces of evolution act on the agroecosystem as a whole (Ceccarelli et al., 2022). In this context, the co-evolution of the microbiome with the rest of the system is fundamental. Now, for this to be possible, this component must be explicitly included in the breeding programs with an evolutionary approach. However, this promising research (and field management) program should not be taken as an invitation to introduce exotic microorganisms into agroecosystem soils, as there is a long history of failed introductions with disastrous consequences (Ladau et al., 2023). On the contrary, these programs should be based on the use of indigenous organisms.

It is also important to assess, albeit on a subjective note, the impression that this set of experiments left on the people who manage these agricultural systems. In this regard, in all cases efficient microorganisms were incorporated into the vineyard management schemes. This means an area of 450 ha under regenerative soil management. It is worth noting that the current trend in ecological soil management seems to be towards a regenerative type of management, which not only involves fixing atmospheric carbon in the soil and incorporating rhizobacteria and mycorrhizal fungi, but also seeks to incorporate microorganisms such as predatory nematodes, amoebae, protozoa and aerobic fungi, thus increasing the complexity of the system (Ingham, 2000; Pane et al., 2012; St. Martin, 2014; Johns, 2017; St. Martin et al., 2020; White, 2020; Lazarova et al., 2021; Curadelli et al., 2023; Eon et al., 2023; Mishra et al., 2023). Therefore, the characterization and understanding of the ecological interaction network of soils is a promising research program, that can provide valuable results for regenerative agriculture in the near future.

It is also important to highlight that the results presented here correspond to exploratory experiments. These motivate and suggest other questions to be addressed. For example, it is interesting to evaluate if there is a threshold density at which microorganisms begin to have a positive effect on plants. Hence, a subsequent step could involve conducting experiments in which different concentrations of microorganisms are evaluated. Similarly, it is worthwhile to study if there is an optimal concentration for the applications. This last question could be explored using the response surface methodology (Montgomery, 2004). Also, it is compelling to study if there is an optimal structure (e.g., in terms of species richness) for the applied microbial consortia, this question could be explored using treatments of increasing complexity.

Finally, it is useful to draw comparisons with other studies on the subject, to highlight the particularities of our study, specifically regarding our experimental setups. For instance, Carreiras et al. (2023) elegantly demonstrated the potential of marine plant growth-promoting rhizobacteria consortia as an eco-friendly solution for mitigating heatwave stress in vineyards. Their research was conducted under greenhouse conditions, with plants potted and treated with microorganism consortia prepared and applied under controlled conditions, with light and temperature also controlled and the experiment was relatively small-scale (treatments consisted of 5 replicate plants). Such experimental setups are crucial for advancing knowledge in the field. However, they significantly differ from the conditions experienced during fieldwork on commercial farms, which can hinder their adoption by farmers. For this reason, our study was specifically designed to mirror real field management conditions, making it more relatable for farmers.

Naturally, our approach comes with certain trade-offs in accuracy (particularly in controlling sources of variation). Factors such as temperature, rainfall, and sunlight (which are beyond our control in the experiments) influence grape growth. Nevertheless, our study was conceived with the understanding that these factors represent the real-world variability that agriculture must adapt to. We believe that developing viable adaptation strategies requires both types of research (those conducted under clear-cut controlled conditions and those under real field conditions), because these approaches are complementary.

From a long-term perspective, soil microbiome manipulation can be used as an adaptation strategy to the new climatic conditions facing agriculture. Through an adequate configuration of stimuli, which could be partially incentivized by public policies, these microorganisms could be multiplied in the farms themselves. This can be achieved through simple techniques, such as the production of compost tea (Ingham, 2003), and thus help reduce the costs that affect the economic viability of the sector (Reganold and Wachter, 2016; Meemken and Qaim, 2018; Łuczka and Kalinowski, 2020).

5 Conclusion

Manipulation of the microbiome in large-scale organic farming as a climate change adaptation strategy is feasible. But in its execution, it must be taken into account that this promising management strategy is governed by ecological processes that take time. This is why these manipulations cannot be expected to have effect automatically. Similarly, in agroecosystems subjected to different forms of stress associated with poor overall condition, it is possible that the microorganisms fail to establish themselves and thus exert positive effects on the system. In short, manipulation of the microbiome is a regenerative soil management strategy that has great potential, but is by no means a panacea.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

CP: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration,

Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. DG: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2024.1285981/full#supplementary-material

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