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The Impact Of Agricultural Crops And Fertilizers Used On The Bacterial Population Of The Soil And In Particular On Nitrifying And Denitrifying Bacteria

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Abstract:
Modern agriculture appears to have a very diversified impact on the soil not only for the crops used but also for the type of fertilizer used. One of the least studied impacts is that on the soil microbiome.
Maintaining a stable soil microbial community structure is crucial in preserving soil production potential and overall soil health. Various nitrogen (N) addition strategies induced changes in soil physicochemical properties which are correlated with alterations in microbial community structure and partial microbial abundance, thereby displaying differences in carbon and nitrogen use efficiencies[1].
Therefore it becomes important to analyze how the microbiology of the soil and the effects of the fertilizers used can vary.
This study analyses the variations of microorganisms in the soil that can be linked to the nitrogen cycle based on the main cultures present in the region and on the different agronomic practices used. This case study allows us to identify how nitrifying and denitrifying bacteria react and how they differentiate, used Real Time PCR for the identification and quantification of bacterial and in particular nitrifying and denitrifying bacteria. . The analysis of these bacterial types therefore allows us to outline a global picture of possible future treatments and the different forms of nitrogen that can be used for plant health and to avoid environmental impacts.
Key Word*: soil bacteria; Nitrifying; Denitrifying; impact of crops; impact of fertilizers.*

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I. Introduction

Nitrogen is an essential element for life on Earth, it is a constituent of DNA, proteins, chlorophyll and many other biological structures. More than 99% of nitrogen is present in nature in the form of molecular nitrogen N_2, especially in the atmosphere, of which it constitutes approximately 79% [2].

However, molecular nitrogen is not directly usable by most living organisms. The conversion from this inert form into the other defined reactive ones is mainly mediated by the action of microorganisms, called nitrogen fixers. Nitrogen conversion (or fixation) is the process by which the N≡N triple covalent bond is broken and the nitrogen atom can therefore combine with other elements, forming compounds, such as NH_4^+ and NO_3^- which can be assimilated by plants and animals[3].

In addition to the action of nitrogen-fixing microorganisms, to a lesser extent the fixation process occurs through high-energy natural phenomena, such as lightning and electric discharges which provide the necessary energy (as much as 941 kJ/mol to break the triple covalent bond N≡N) to make elemental N_2 react with the oxygen or hydrogen of water molecules allowing the formation of acid or ammonia oxides which are transported to the earth's surface through precipitation[4].

The natural transformation and movement of nitrogenous compounds within the biosphere constitutes the nitrogen cycle[5]. The nitrogen cycle is regulated by a series of reactions[6].

The transition from one state to another occurs through the activity of microorganisms, which operate in conditions of extreme variability[7]. The biogeochemical nitrogen cycle is defined by the set of transformations that the various nitrogenous species undergo and includes various metabolic reactions. Nitrogen fixation (N_2 reduction process) involves entry from the reserve pool (mainly made up of the atmosphere) to the exchange pool. In the exchange pool, the re-cyclization of nitrogen is mainly due to the processes of ammonification (mineralization of the nitrogenous organic substance) and nitrification[8].

Denitrification determines the exit from the exchange pool with the production of N_2, which is released into the atmosphere[9].

In many parts of the world, the nitrate ion is among the chemical compounds that most frequently exceed the quality standards set for groundwater by national and international regulations that regulate its use[10]. The

European Union and the World Health Organization have both set a contamination threshold of 50 mg NO_3^-/l (11.29 mg N/l)[11].

Higher concentrations of nitrates can be attributed to sources of anthropogenic origin, mainly linked to the use of nitrogen fertilizers in agriculture, both natural organic (manure) and inorganic and/or chemical organic[12]. These substances are useful for plants as an essential element for vegetative development, therefore the production level of the crop is primarily conditioned by the availability of nitrogen in the soil, as it stimulates plant growth and determines an abundant presence of chlorophyll in the leaves[13].

Fertilizers can be classified, based on the presence of one or more elements essential to the mineral nutrition of plants, as simple or compound[14].

Simple nitrogen-based fertilizers can contain nitrogen in the nitric, ammonia, nitric-ammonia and organic forms. Nitric nitrogen fertilizers are directly and promptly absorbed by the plants, while ammonia nitrogen fertilizers, despite having the same physiological value, are directly absorbed more slowly, but are mostly used indirectly by undergoing the transformation into nitric nitrogen in the soil at work of nitrifying bacteria. Organic nitrogen-based fertilizers exert their action very slowly, having to undergo subsequent transformations into ammonia and then nitric nitrogen in the soil[15].

The main nitrate nitrogen fertilizers are sodium nitrate ($NaNO_3$) and calcium nitrate ($Ca(NO_3)_2$). The latter also acts as a corrective for soils lacking or poor in calcium. Nitrogen fertilizers include ammonium sulphates ($(NH_4)_2SO_4$) and ammonium carbonate ($(NH_4)_2CO_3$). A double-acting nitrate, containing equal parts of both nitric and ammoniacal nitrogen, is ammonium nitrate (NH_4NO_3)[16]. It is the most used compound in almost all fertilizer-producing countries, but it has the drawback of being highly hygroscopic which makes storage difficult. Furthermore, ammonium nitrate is susceptible to detonation due to its sensitivity to heat and shock, so it is diluted with inert substances such as calcium carbonate, diatomaceous earth or clay[17].

Organic nitrogen fertilizers are mainly urea and calcium cyanamide. Urea, $CO(NH_2)_2$, is the solid fertilizer with the highest nitrogen content, approximately 46%; its physiological-agrarian action is comparable to that of ammonium nitrogen, given that it is easily hydrolysed in the soil, transforming into ammonium carbonate. Calcium cyanamide, $CaCN_2$, as well as being a good nitrogen fertilizer (15-20% N), has a corrective action on acid soils due to the presence of lime and also acts as a herbicide and insect repellent. In the soil it behaves like ammonia fertilizers as it is transformed into urea and ammonium carbonate[18].

In addition to simple fertilizers, those classified as complex are equally widely used, meaning they contain two or three active elements in the same compound. In detail, a distinction is made between binary ones (made up of nitrogen-phosphate, nitrogen-potassium, potassium-phosphate mixtures) and ternary ones. Also in this case, the most used fertilizers are nitrogen-based mixtures[19].

The use of chemical fertilizers was created to meet the needs of plants for nutritional elements for their development. On the other hand, however, poor management in the use of fertilizers has been the cause of massive nitrate pollution in groundwater. Several studies have highlighted how fertilizers are often administered in a single solution, thus exceeding the plants' absorption capacity. The plants instead require multiple administrations during the different seasons. But above all, it was found that fertilizers are supplied in excessive quantities compared to the real needs of the crops[20]

When this excess fertilization occurs, the nitrates present in fertilizers are easily subject to leaching due to rainfall and irrigation. In this way, the formation of polluted plumes with a high concentration of nitrates can occur in the underlying aquifers[21].

Modern agriculture heavily relies on agricultural inputs rather than improvements in cropping efficiency[22]. However, over-fertilization has resulted in degradation, erosion and structural damage of agricultural soils, posing a serious threat to global food security and climate stability. With the advancement of ecology and biotechnology, soil science research has transitioned from traditional physic-chemical methods to multidimensional high-resolution histological analysis. This approach emphasizes the role of soil microorganisms in soil carbon and nitrogen cycling, nutrient turnover, and biosecurity [23].

Maintaining a stable soil microbial community structure is crucial in preserving soil production potential and overall soil health. Various nitrogen (N) addition strategies induced changes in soil physicochemical properties which are correlated with alterations in microbial community structure and partial microbial abundance, thereby displaying differences in carbon and N use efficiencies[24]. The formation of a suitable microbial community structure can enhance N fertilizer utilization and result in increased yields while reducing N fertilization[25]. However, long-term excessive and irrational application of nitrogen fertilizers can deteriorate the microbial community structure in agricultural soils, hindering the development of soil ecosystems[26]. Various microorganisms in soil interact with each other and environmental factors, forming complex microbial networks that promote or inhibit the uptake of soil nutrients by crops through multiple biochemical and metabolic pathways[27]. Therefore, investigating the response mechanism of soil microorganisms to N fertilizer application is crucial in guiding the application of N fertilizer[28].

The present study highlights how for the main agricultural crops present in the Puglia Region the different types of fertilization have a diversified effect on the microbiology of the soil and in particular on the bacteria involved in the nitrogen cycle, thus determining its functionality due to the presence of useful elements to the plants and at the same time they can be the main proponents of the production of excess nitrate which, by leaching, leads to contamination of the underlying aquifers.

II. Material And Methods

Study Area

It was been identified six monitoring areas in the Puglia region. The selected areas were previously analyzed using the existing databases in order to identify areas that have the same lithology and soil type. In each of the six areas, the uses of the land were thus identified through the use of the Corine Land Cover map in order to identify the predominant uses present. It was proceed with the identification of sub-areas, 24 sub-areas for each area, for a total of 144 sub-areas. The sub-areas were classified into: 4 sub-areas with orchards (in total 24 sub-areas), 4 sub-areas with uncultivated fields (in total 24 sub-areas), 4 sub-areas with horticulture (in total 24 sub-areas), 4 sub-areas with arable crops (in total 24 sub-areas), 4 sub-areas with olive groves (in total 24 sub-areas) and 4 sub-areas with vineyards (in total 24 sub-areas). For each sub-area, 25 sampling sites were identified and two series of sampling were carried out in the 2021 year before the start of the agronomic practices and in the 2022 at the end of all the agronomic practices.

The soil matrix sample was carried out in according to the ―Methods of Soil Microbiological Analysis‖ issued by the Ministry of Agricultural and Forestry Politics.

The basic aim of this sampling procedure is to obtain a truly representative sample of the soil under investigation.

Samples were collected from the soil at a depth between 15-30 cm using a sterile spatula and excluding the first two centimeters presenting grass. They were then put inside sterile envelopes and stored at 10°C.

DNA extraction and sequencing

DNA was extracted from soil samples using standard bead-beating protocols (NucleoSpin Soil, MACHEREY-NAGEL, Germany). Quantification of DNA was performed by Qubit™ 4 Fluorometer (Invitrogen, Thermo Fisher Scientific).

DNA was sequencing with GENE AMPLICON SEQUENCING (MiSeq, Illumina) by Environmental Microbiology and Molecular Biology laboratory, CNR-IRSA Rome. the sequences were compared with those present in GenBank database by using BlastN (http://blast.ncbi.nlm.nih.gov/Blast.cgi[29]).

Primer design, qPCR conditions and data analysis

Primer for amplification of bacteria selected were designed by using the clone manager suite ver. 6 software (Sci-Ed, Cary, NC). Primer pairs were tested for similarities with other known sequences present in GenBank database by using BlastN [29]. The primer pair specific was designed to amplify a 100/250 bp fragment specific for each 16S rRNA gene species (Table 1,)

Table no1: Primers used for Real Time PCR in this study

Aim	Target gene	Primers	Sequence 5′–3′	Bibliography
Bacteria	16S rRNA	1055F	ATG GCTGTC GTCAGCT	[30]
		1392R	ACG GGC GGTGTGTAC	
Ammonia oxidisers	Amo	amoA-1-F	GGG GTT TCTACTGGTGGT	[31]
		amoA-2R	CCC CTCKGSAAAGCC TTC TTC	
Nitrite oxidisers	Nxr	nxrA-RT-F	GTG GTC ATG CGC GTT GAG CA	[32]
		nxrA-RT-R	TCG GGA GCG CCA TCA TCC AT	
All known Planctomycetes	Hzo	hzoCl1f1	TGYAAGACYTGYCAYTGG	[33]
		hzoCl1r2	ACTCCAGAT RTG CTGACC	
Denitrifiers	NirS	nirS 1f	TAC CAC CCSGARCCG CGCGT	[34]
		nirS 3r	GCC GCC GTC RTGVAGGAA	
	NirK	nirK876	ATYGGC GGVCAYGGC GA	
		nirK1040	GCC TCGATCAGRTTRTGGTT	

Realtime PCR was performed in an StepOnePlus^{tm} Real Time PCR System (Applied Biosystems) programmed with the following temperature profile: 2 min 50°C UDG activation, 2 min 94°C denaturation, followed by 40 cycles composed by 15 sec 94°C, 30 sec 65°C, 30 sec min 72°C elongation. Fluorescence data acquisition was done during the extension step at 72°C. A final melting curve was performed to check for product specificity. Reactions were performed in 20 μl final volume containing 10 μl of Master Mix PowerUp™ SYBR™ Green (Thermo Fischer scientific) and 10 pmols of primers. Template DNA soil samples was 1 μl (40ng/ µl). All reactions were done in triplicate. Data were analysed with the software StepOnePlus^{tm} (Applied Biosystems). PCR efficiency was calculated as efficiency = $-1 + 1 \times 10^{(-1/\text{slope})}$[35].

III. Resultand Discussion

Variation insoilbacterialconcentration

The concentrations of extracted bacterial DNA verified by real time tests were analysed on all samples using primers 100F/1392R. it was possible to observe how a greater concentration was obtained from non-cultivated soils with a concentration from 400,000 to 1,400,000 ng DNA/Kg of soil. It has been noted that there is a moderate variation in bacterial concentration in soils cultivated with orchards (from 36,000 to 550,000 ng DNA/Kg of soil), horticulture (from 100,000 to 750,000 ng DNA/Kg of soil), olive groves (from 50,000 to 700,000 ng DNA/Kg of soil) and vineyard (from 10,000 to 680,000 ng DNA/Kg of soil). A very low variation, however, was found in cultivable soils with arable land (from 50,000 to 500,000 ng DNA/kg of soil) (Fig. 1).

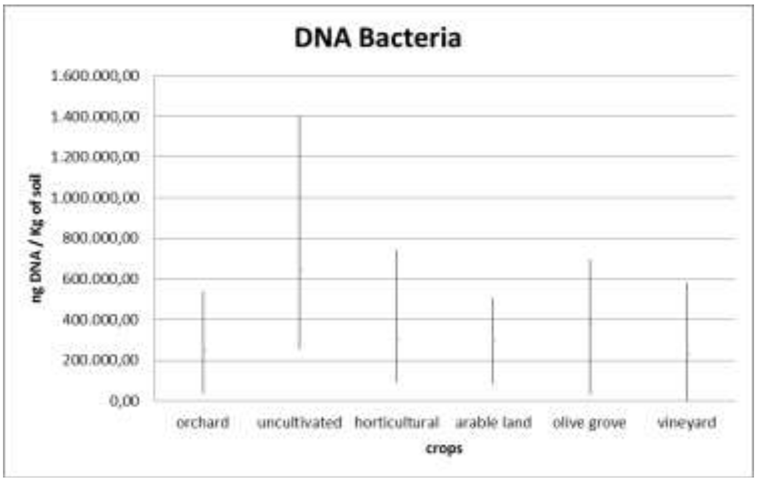

Figure no 1: concentration of bacterial DNA in soils with different crops

Following the carrying out of the agronomic cycle, an increase in the bacterial concentration was found in fields with orchards (from 470,000 ng DNA/Kg of soil pre-treatment to 537,000 ng DNA/Kg of soil post-treatment), fields with arable land (from 440,000 ng DNA/Kg of pre-treatment soil to 510,000ng DNA/Kg of post-treatment soil) and vineyards (from 500,000 ng DNA/Kg of pre-treatment soil to 580,000 ng DNA/Kg of post-treatment soil), while a decrease in bacterial concentrations in fields cultivated with vegetables (from 750,000 ng DNA/Kg of soil pre-treatment to 660,000 ng DNA/Kg of soil post-treatment) and olive groves (from 695,000 ng DNA/Kg of soil pre-treatment to 630,000 ng DNA/Kg of soil post treatment)(Fig.2).

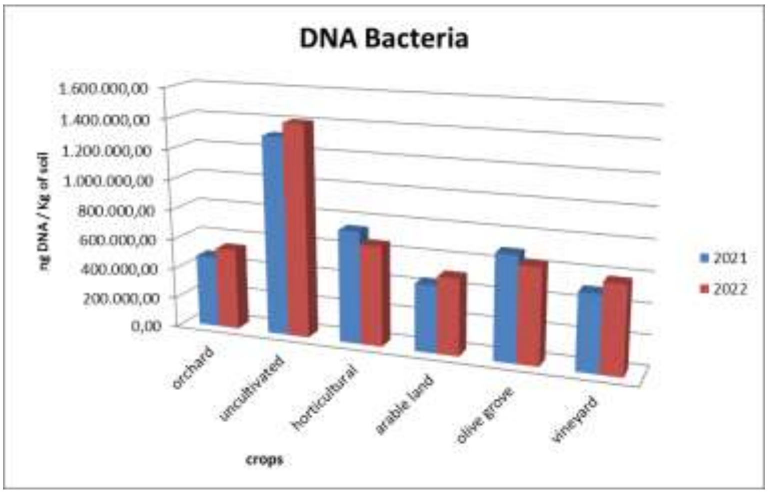

Figure no 2: concentration of bacterial DNA in soils with different crops
pre agronomic treatment 2021 and post agronomic treatment 2022

Evaluations were carried out regarding the variation in bacterial concentration for the individual crops analysed depending on the type of fertilization adopted.

With regard to orchards, the three main agronomic practices adopted in the region were analysed: chemical, integrated and organic. It can be seen that chemical fertilization has a negative impact on the microbial community of the soil, in fact the bacterial concentration goes from an average concentration of 405,000 ng DNA/Kg of soil to an average concentration of 275,000 ng DNA/Kg of soil with a decrease in all the samples analysed which varies from -29.11% to -34.63%. Relative to the other two types of fertilization, both revealed an increase in concentration. With regards to integrated fertilization, the bacterial concentration goes from an average value of 115,000 ng DNA/Kg of soil to an average value of 150,000 ng DNA/Kg of soil with an increase in all the samples analysed which varies from 17.43% to 46.65%, for as regards organic fertilization, the bacterial concentration goes from an average value of 225,000 ng DNA/Kg of soil to an average value of 300,000 ng DNA/Kg of soil with an increase in all the analysed samples which varies from 10.33% to 72.66% (Fig. 3)

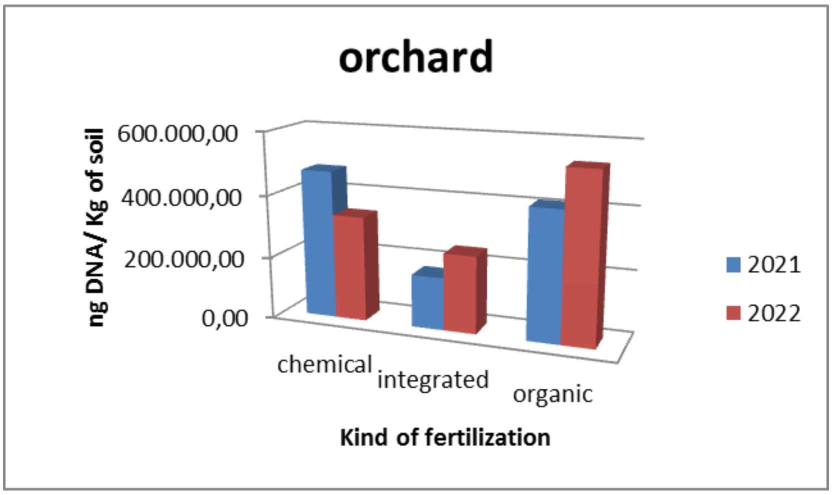

Figure no 3: Bacterial DNA concentration for orchards with different fertilization before agronomic treatment 2021 and after agronomic treatment 2022

With regards to horticulture, the two main agronomic practices adopted in the regional context were analysed: chemical and mineral. It can be seen that both have a negative impact on the microbial community of the soil, in fact the bacterial concentration passes through chemical fertilization from an average concentration of 440,000 ng DNA/Kg of soil to an average concentration of 380,000 ng DNA/Kg of soil with a decrease in all the samples analysed which varies from -10.33% to -27.69%, for the . mineral fertilization the bacterial concentration goes from an average value of 340,000 ng DNA/Kg of soil to an average value of 245,000 ng DNA/Kg of soil with a decrease in all the samples analysed which varies from 14.30% to 51.47% (Fig. 4).

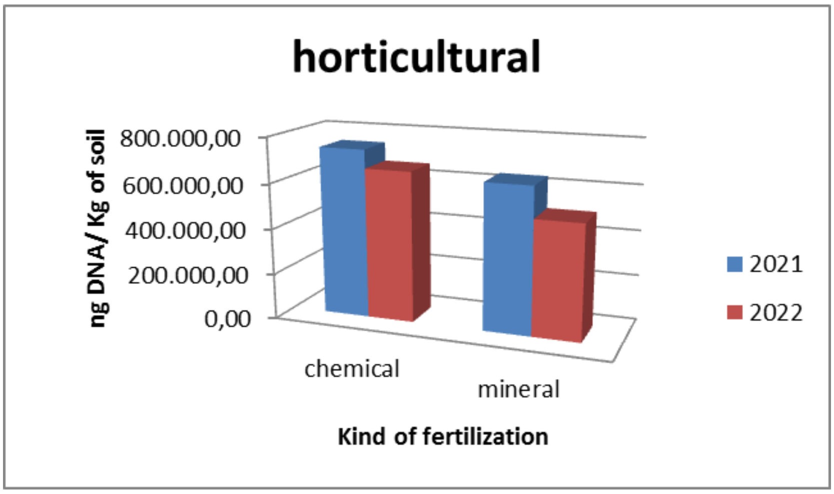

Figure no 4: Bacterial DNA concentration for horticulture with different fertilization before agronomic treatment 2021 and after agronomic treatment 2022

With regard to arable land, the two main agronomic practices adopted in the regional context were analysed: integrated and organic. It can be seen that both have an increase in bacterial concentration. In relation to integrated fertilization, the bacterial concentration goes from an average value of 280,000 ng DNA/Kg of soil to an average value of 350,000 ng DNA/Kg of soil with an increase in all the samples analysed which varies from 13.61% to 35.12%, for as regards organic fertilization, the bacterial concentration goes from an average value of 250,000 ng DNA/Kg of soil to an average value of 315,000 ng DNA/Kg of soil with an increase in all the analysed samples which varies from 17.46% to 29.14% (Fig. 5).

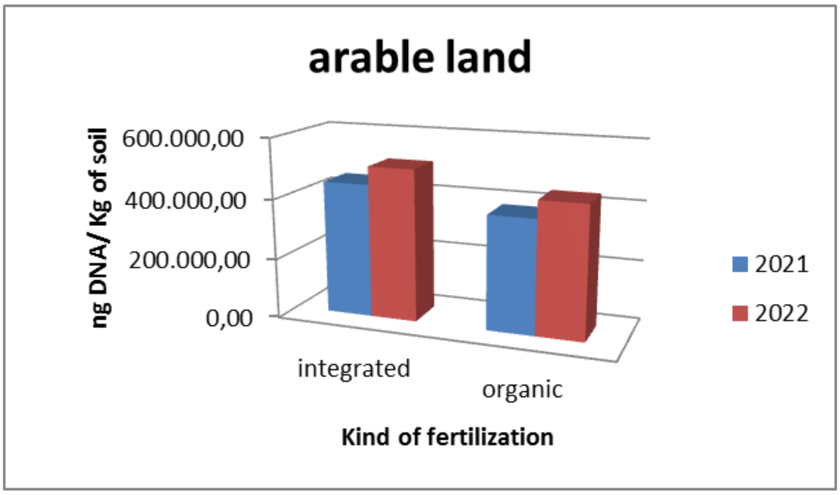

Figure no 5: Bacterial DNA concentration for arable land with different fertilization before agronomic treatment 2021 and after agronomic treatment 2022

With regards to olive groves, the three main agronomic practices adopted in the region were analysed: chemical, integrated and organic. It can be seen that chemical fertilization has a negative impact on the microbial community of the soil, in fact the bacterial concentration goes from an average concentration of 650,000 ng DNA/Kg of soil to an average concentration of 590,000 ng DNA/Kg of soil with a decrease in all the samples analysed which varies from -9.27% to -15.81%. Relative to the other two types of fertilization, both revealed an increase in concentration. With regards to integrated fertilization, the bacterial concentration goes from an average value of 180,000 ng DNA/Kg of soil to an average value of 280,000 ng DNA/Kg of soil with an increase in all the analysed samples which varies from 14.61% to 49.22%, for as regards organic fertilization, the bacterial concentration goes from an average value of 200,000 ng DNA/Kg of soil to an average value of 320,000 ng DNA/Kg of soil with an increase in all the analysed samples which varies from 41.31% to 83.72% (Fig. 6).

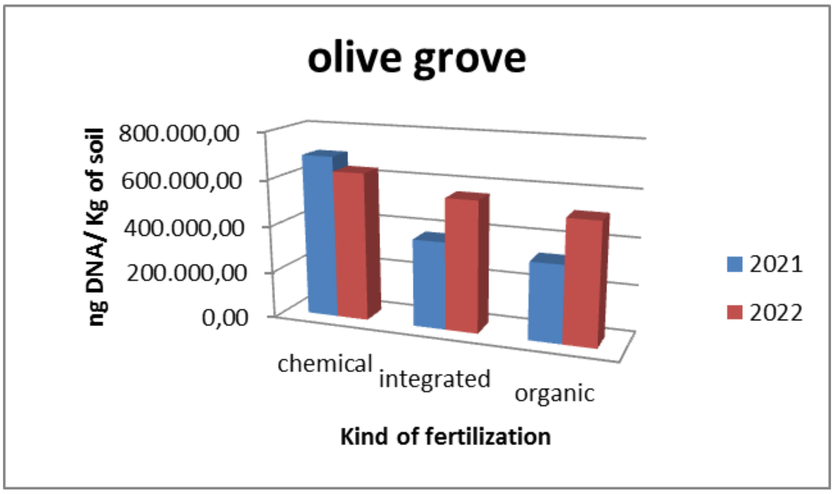

Figure no 6: Bacterial DNA concentration for olive groves with different fertilization before agronomic treatment 2021 and after agronomic treatment 2022

With regard to vineyards, the three main agronomic practices adopted in the region were analysed: chemical, integrated and organic. It can be seen that chemical fertilization has a negative impact on the microbial community of the soil, in fact the bacterial concentration goes from an average concentration of 250,000 ng DNA/Kg of soil to an average concentration of 190,000 ng DNA/Kg of soil with a decrease in all the samples analysed which varies from -10.88% to -59.42%. Relative to the other two types of fertilization, both revealed an increase in concentration. With regards to integrated fertilization, the bacterial concentration goes from an average value of 200,000 ng DNA/Kg of soil to an average value of 255,000 ng DNA/Kg of soil with an increase in all the samples analysed which varies from 12.77% to 49.95%, for as regards organic fertilization, the bacterial concentration goes from an average value of 190,000 ng DNA/Kg of soil to an average value of 260,000 ng DNA/Kg of soil with an increase in all the analysed samples which varies from 22.13% to 48.09% (Fig. 7)

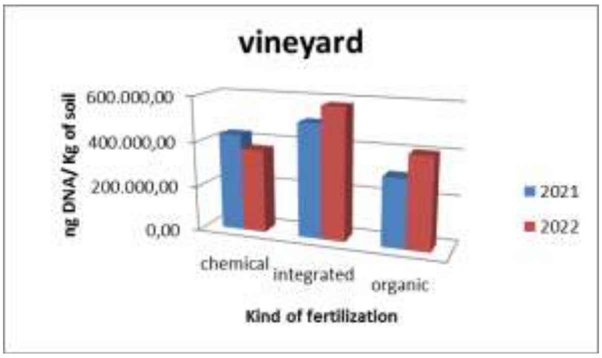

Figure no 7: Bacterial DNA concentration for vineyards with different fertilization before agronomic treatment 2021 and after agronomic treatment 2022

Variation in the concentration of nitrifying and denitrifying bacteria in the soil

The concentrations of nitrifying and denitrifying bacteria was verified by real time tests were analysed on all samples using the target gene identify.

A constant trend over time can be seen in uncultivated soils. With regards to crops, as regards orchards and vineyards, there is a decrease in nitrifying activity (Fig. 8) and an increase in denitrifying activity (Fig 9). In fact, in orchards the presence of nitrifies goes from an average presence of 9.00% pre-treatment to an average presence of 2.60% post-treatment. In the vineyards the decrease is smaller, going from an average presence of 12.00% pre-treatment to a presence of 7.80% post-treatment. As far as the presence of denitrifying bacteria is concerned, an increase in orchards is noted from an average presence of 0.30% pre-treatment to an average presence of 0.80% post-treatment, while in relation to vineyards the average presence goes from an average presence of 0.90% pre-treatment to an average presence of 1.00% post treatment.

As regards all the other crops, however, for horticultural crops there is a notable decrease in the nitrifying activity, going from an average presence of 14.40% pre-treatments to an average presence of 3.00% post-treatments and at the same time a decrease in the denitrifying activity going from an average presence of 1.00% pre-treatments to an average presence of 0.30% post treatments. As regards the fields with arable land, there was a slight decrease in the nitrifying activity, going from an average presence of 1.74% pre-treatment to an average presence of 1.60% post-treatment, and a more significant decrease in the denitrifying activity going from an average presence of 0.56% pre-treatment to an average presence of 0.30% post-treatment. Finally, as regards olive groves, also in this case there is a decrease in nitrifying activity, going from an average presence of 5.00% pre-treatments to an average presence of 1.72% post-treatments and at the same time a decrease, albeit minimal, in denitrifying activity. , going from an average presence of 0.33% pre-treatments to an average presence of 0.25% post-treatments.

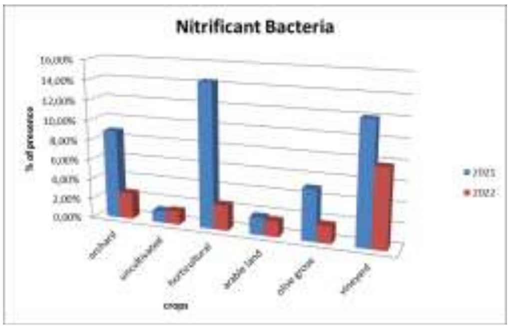

Figure no 8: % of prevalence of Nitrifying bacteria in soil pre-treatment 2021 and post-treatment 2022 in the different crops

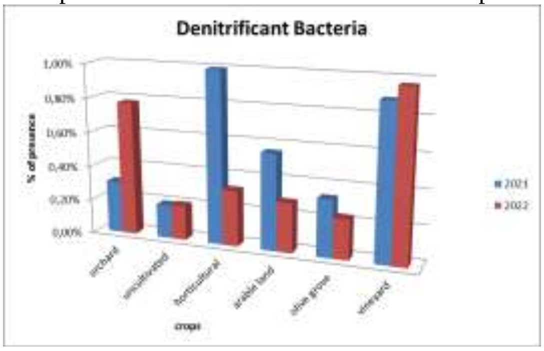

Figure no 9: % of prevalence of Denitrifying bacteria in soil pre-treatment 2021 and post-treatment 2022 in the different crops

The analysis carried out for the quantification of the presence of nitrifies and denitrifies has related how the different fertilizations adopted on the territory impact.

Regarding the orchards, three different fertilizations were analysed: chemical, integrated and organic. The analyses showed that both chemical and integral fertilizers produced a decrease in the presence of nitrifying bacteria. The chemical fertilizer reduced this presence from a concentration of 1.12% pre-treatment to a concentration of 0.27% post-treatment, while integrated fertilization from a presence of 4.21% pre-treatment to a presence of 1.95% post-treatment. Regarding the organic fertilizer, however, there is a stability of the presence of nitrifies 8.89% pre-treatment and 9.01% post-treatment (Fig.10)

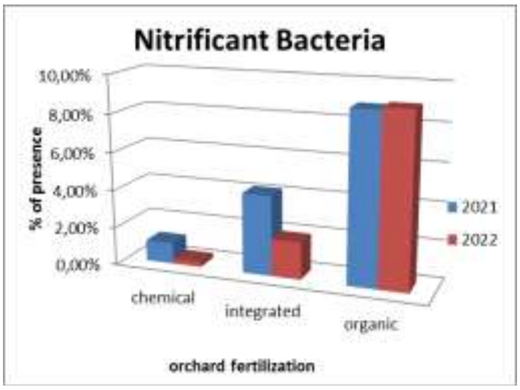

Figure no 10: % of prevalence of Nitrifying bacteria in soil pre-treatment 2021 and post-treatment 2022 in orchards**.**

Regarding the concentration of denitrifies, it can be seen that only the chemical fertilizer induces a reduction in the presence (from 0.30% pre-treatment to 0.18% post-treatment). Integrated fertilization instead induces an increase in the presence of denitrifies, going from a presence of 0.30% pre-treatment to a presence of 0.67% post-treatment. Finally, the use of organic fertilizer highlighted a stationarity in the presence of denitrifies with both a pre- and post-treatment concentration of 0.78% (Fig 11).

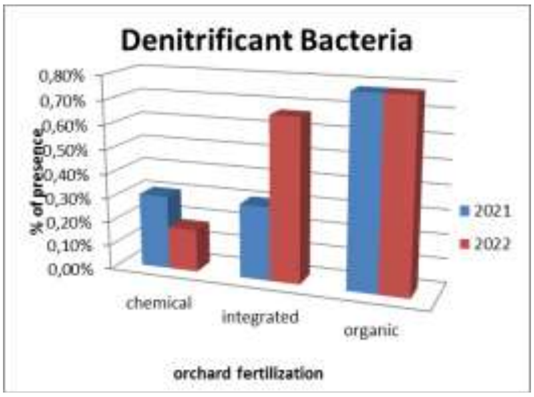

Figure no 11:% of prevalence of denitrifying bacteria in soil pre-treatment 2021
and post-treatment 2022 in orchards.

Regarding horticulture, in both fertilizations analysed, chemical and mineral, there is a decrease in the presence of nitrifies, respectively, with a concentration that goes from 1.78% pre-treatment to 0.65% post-treatment and from 14.39% pre-treatment to 2.52% post-treatment. It can be seen that the use of mineral fertilizer has a much greater impact on these bacterial species (Fig. 12).

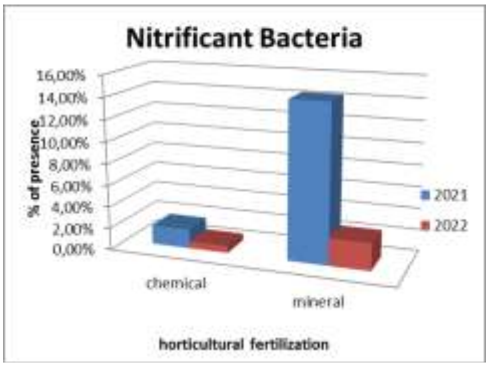

Figure no 12: % of prevalence of Nitrifying bacteria in soil pre-treatment 2021
and post treatment 2022 in horticulture.

For the same crop, the same effect of both fertilizations is also found on the population of denitrifying bacteria, with a decrease that for chemical fertilization goes from 0.34% pre-treatment to 0.21% post-treatment and for mineral fertilization it goes from 1.00 % pre-treatment to 0.32% post-treatment (Fig 13).

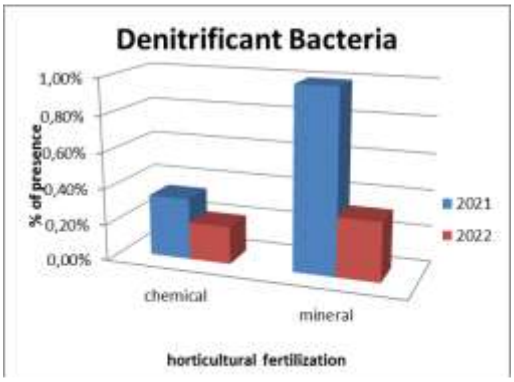

Figure no 13: % of prevalence of denitrifying bacteria in soil pre-treatment 2021
and post treatment 2022 in horticulture.

In soils where arable crops are grown, it has been noted that in relation to integrated fertilization there is a decrease in the presence of nitrifying bacteria, going from a presence of 1.04% pre-treatment to a presence of 0.34% post-treatment. Regarding fertilization with organic products, however, there is a slight increase in the presence of nitrifying bacteria which goes from 1.74% pre-treatment to 1.84% post-treatment (Fig. 14).

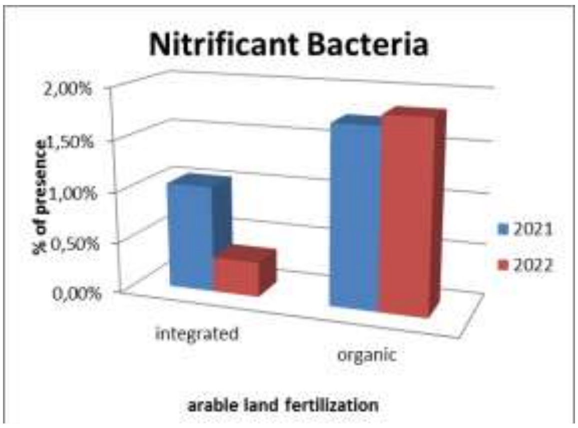

Figure no 14: % of prevalence of Nitrifying bacteria in soil pre-treatment 2021 and post-treatment 2022 in arable land.

Regarding integrated fertilization, there is an increase in the presence of denitrifying bacteria which goes from 0.18% pre-treatment to 0.29% post-treatment. Regarding organic fertilization, however, there is a very slight decrease in the presence of denitrifying bacteria with 0.56% pre-treatment and 0.52% post-treatment (Fig. 15)

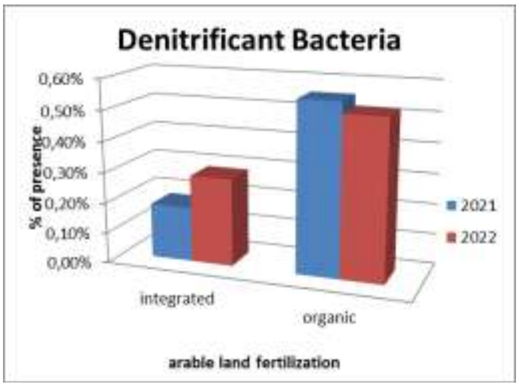

Figure no 15: % of prevalence of denitrifying bacteria in soil pre-treatment 2021 and post-treatment 2022 in arable land.

The analysis carried out on soils with olive groves shows a decrease in the concentration of nitrifying bacteria in relation to chemical and integrated fertilisation, respectively the presence goes from 3.07% pre-treatment to 0.14% post-treatment for chemical fertilisation, while for chemical fertilisation, while from 5.08% pre-treatment and 1.37% post treatment for integrated fertilization. Regarding fertilization with organic compounds, however, there is a small increase in the presence of denitrifying bacteria which goes from 3.03% pre-treatment to 3.42% post-treatment (Fig. 16).

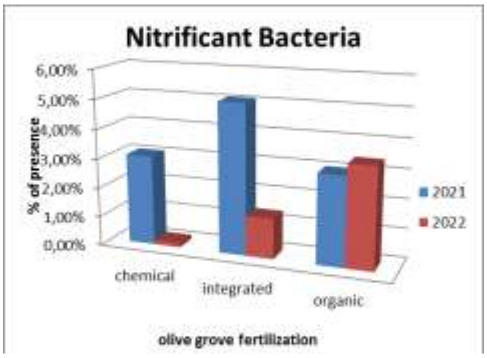

Figure no 16: % of prevalence of Nitrifying bacteria in soil pre-treatment 2021 and post-treatment 2022 olive groves.

Regarding the presence of denitrifying bacteria, there was a decrease in their presence in chemical fertilization, going from 0.60% pre-treatment to 0.35% post-treatment. However, an increase in the presence of denitrifying bacteria is found both in integrated fertilization, going from 0.56% pre-treatment to 0.97% post-treatment, and in organic fertilization, going from 0.89% pre-treatment to 0.94% post-treatment (Fig. 17)

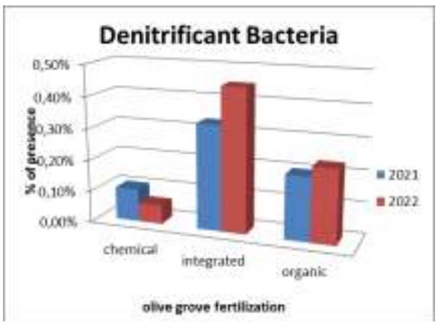

Figure no 17: % of prevalence of denitrifying bacteria in soil pre-treatment 2021 and post treatment 2022 in olive groves.

Finally, for soils with the presence of vineyards, a decrease in nitrifying bacteria was found both for chemical fertilization, which went from 3.89% pre-treatment to 1.85% post-treatment, and for integrated fertilization with a much more pronounced decrease, going from 12.04% pre-treatment to 1.83% post treatment. Regarding organic fertilization, however, there is an increase in the presence of nitrifying bacteria, going from 3.92% pre-treatment to 7.83% post-treatment (Fig. 18).

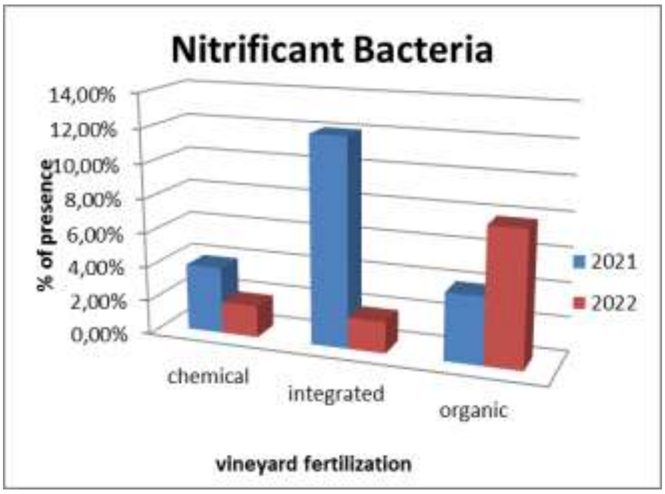

Figure no 18: % of prevalence of Nitrifying bacteria in soil pre-treatment 2021 and post treatment 2022 vineyards.

As regards the presence of denitrifying bacteria, a decrease was found in the soils in which chemical fertilization was used, going from 0.60% pre-treatment to 0.35% post-treatment, while an increase was noted both in the soils in which chemical fertilization was used. integrated, from 0.56% pre-treatment to 0.97% post-treatment, and where organic fertilization was used, from 0.89% pre-treatment to 0.94% post-treatment (Fig. 19).

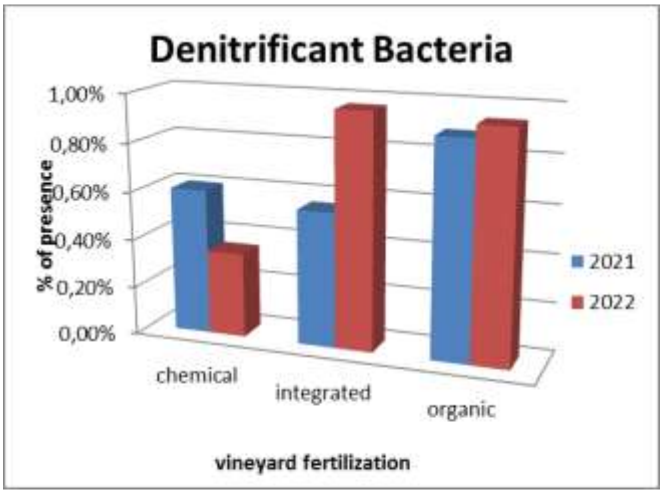

Figure no 19: % of prevalence of denitrifying bacteria in soil pre-treatment 2021 and post treatment 2022 in vineyards.

IV. Conclusion

From the analyses obtained it is possible to indicate how in non-cultivated fields there is a high variability of bacterial concentration and at the same time also a high concentration of the same which increases over time.

In relation to crops, the variability of concentration is significant and it is also of significant importance that there is an enrichment of microbial forms in fields with orchards, arable land and vineyards with a maximum increase after fertilization. Horticulture and olive groves are much more impactful from a microbiological point of view as they create an impoverishment of the microbiological concentration.

However, the results obtained on the different types of fertilization are very important. From a microbiological point of view, in fact, integrated or organic fertilizations were found to have a positive impact on microbial concentration in all crops. In fact, in all crops in which these types of fertilization are used, there is an increase in microbial concentration after the treatment. Otherwise, chemical or mineral fertilizations are harmful to microbiology, in fact in all crops there is a reduction in bacterial concentrations.

With regards to the impact on nitrifying and denitrifying bacteria, it is possible to note following the analyses carried out that the crop with the greatest impact on nitrifying and denitrifying bacterial activity are horticultural crops where mainly there is a negative impact, in fact there is a notable decrease in both activities bacterial. This decrease in nitrifying and denitrifying activity is also linked to the type of fertilization used which has a negative impact on bacterial activity.

In relation to the other crops, it can be seen that they all have a negative impact on the nitrifying bacterial activity, while for the denitrifying activity they have a negative impact on arable land and olive groves and a positive impact with an increase in the denitrifying activity in orchards and vineyards.

Regarding the types of fertilization, it is possible to conclude that chemical and integrated fertilizations have a negative impact on denitrifying bacterial activity in all crops, while they are non-impactful, maintaining the same percentage of presence, or a positive impact, in relation to the vineyards.

As regards the denitrifying activity related to fertilization, it is possible to conclude that only chemical fertilization has a negative impact with a decrease in denitrifying bacterial activity in all crops. While both integrated and organic fertilization appears to have a positive impact with an increase in denitrifying activity in all crops.

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