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Chemical and Microbiological Characterization of Soil under Different Agronomical Use and Practical: First Focus on Nitrogen Cycles

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Abstract:
The study of soil ecology and the knowledge of its chemical and biological composition is become one of the principal aim of the environmental research. Most recent studies, based on consolidated knowledge, gives a specific analytical framework that allows to start correlating the biochemical composition of the soil with its particular characteristics and uses. For a sustainable agriculture, it must be adopted alternatives to a disproportionate use of non-organic fertilizers and agro-pharmaceuticals. The practice adopted has also an important effect on the soil and its characteristics, particularly on its chemical and microbiological composition, which varies the capacity of the soil to create and provide eco-systemic activity. The chemical and microbiological composition of the soil has also effect on the quantity and quality of the agricultural product. Today we have the possibility to scientifically measure the effect of these different practices on soil composition and mainly on its biodiversity. In this study some first chemical and biome characterizations, related to the nitrogen cycle, were conducted in sites with different land use and with different agronomic practices, combining methods of chemical and metagenomics analysis. Resulted a high variability in the concentration of organic substance in the soil and no correlation of organic carbon concentration versus organic nitrogen concentration, denoting differences in the quality of the organic matter present. The soil of the ancient biodynamic vineyard shows the highest concentration of DNA found. Unexpectedly, the vegetable garden managed under biological methods shows the lowest concentration of DNA found. The composition of the biome for the bacterial species of nitrogen cycle shows a very complex picture. Some species are always absent and some always present; in a worrying case only the Neisseria species was detected. The work will continue by applying quantitative PCR techniques (Real Time), with which a complete photograph of the state of the microbiome of the analyzed soils will be possible. The applied method seems particularly suitable for bio-geo-chemical insights on the nitrogen cycle in the soil, referred to different use of the soil and in relation to the agronomic practices adopted.

Key Word*:Agronomical Use; Biomolecular analysis; Nitrogen Cycle.*

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I. Introduction

The study of soil ecology and the knowledge of its chemical and biological composition is become one of the principal aim of the environmental research. This matrix is the main non-renewable resource for humans, but it is subject to possible alterations of its natural balance, caused by anthropogenic impact and climate change [1].

Most recent studies, based on consolidated knowledge, gives a specific analytical framework that allows to start correlating the biochemical composition of the soil with its particular characteristics and uses.

The growing effort to implement and strengthen sustainable agricultural activities, alternatives to a disproportionate use of non-organic fertilizers and agro-pharmaceuticals [2,3], offers the possibility to measure scientifically the effect of these different practices on soil composition and mainly on its biodiversity, with important economic impacts.

A study on fertilizer use in agriculture carried out in 2016 found a total expenditure of $787 million [4]. This data has characterized the beginning of a turning point in agriculture; many countries have encouraged the reduction of the use of chemical fertilizers [5] and in their substitution it is growing the adoption of biodynamic "organic farming" and other natural or assisted biological pressure practices.

In some cases, for example, it has been demonstrated that the inoculation of ad hoc microorganisms involved in the degradation of certain compounds that are made available for radical assimilation has produced a 50% increase in production [6]. The impact of such uses of new agricultural systems has also increased levels of organic carbon and soil nutrients, leading to an enrichment of the soil microbiome [7].

This is starting to show not only better soil quality but, at the same time, an increase in productivity, estimated at a minimum increase of at least 20% in the cultivation of cereals, vegetables, legumes and root crops (such as potatoes) [8]. The use of biomass appears to be crucial.

The origin of biomass used in agriculture is varied: agricultural residues [9], livestock [10,11], agro-industry [12,13,14] and household waste [15]. Diversity of raw materials used is not only an ecologically sustainable solution for the management of different residues, but also represents an excellent opportunity to recycle nutrients that can enrich soils [16], especially those with low fertility.

Plant-soil interaction, in particular plant-microbial interaction, is also decisive for the achievement of a good soil status. Interactions between microbes are well known and studied in depth, but community-based associations between vegetation and microbial consortia, beyond those of the nitrogen cycle, are less understood. There is growing evidence that the structure of the plant community influences the density and composition of soil communities [17-23], and more evidence that there may be characteristic microbial communities associated with particular plant species, particularly in experimental circumstances where plants are grown in isolation [24 -28]. It is assumed that the mechanisms by which such associations are generated are related to the quality and nature of the substrate near the plants, which is known to regulate the structure of the microbial community [29]. It is assumed that there is some degree of spatial coupling between plants and microbial communities, but this has rarely been studied. The concept of "rhizosphere" essentially indicates a spatial relationship between plants and microbes focused on the interface between root and soil, and studies indicate that there may be a conditioning of microbial communities in the rhizosphere of specific plants [30,31]. It has been shown that over large spaces, where there is a rarefaction of plants, even organisms tend to distribute themselves in the soil according to a numerical gradient of individuals and species superimposed on that of plant density [32,33].

In the past, the soil was considered a black box, accessible only for the monitoring of elementary composition, gas flows or total microbial biomass and the soil microbiota was almost exclusively characterized by isolation and culture techniques. With the introduction of organic farming techniques, microorganisms present in the soil have become the key to identifying the biogeochemical processes in the soil that determine life [34]. Studies carried out have shown that the soil is one of the most diverse biomes on Earth and one of the largest reservoirs of microbial diversity [35,36,37,38,39]. Soil microorganisms are essentially "drivers" of biogeochemical processes. In particular, they play an important role in plant nutrition [40], bioremediation [41,42] and soil stress mitigation [43]. However, understanding the mechanisms underlying all these processes is not an easy task, since the vast majority of microorganisms are not cultivable in the laboratory [44]. Introduction of culture-independent methodologies has revolutionized the way to study microbial soil communities. DNA extraction and characterization has become routine in most microbial soil ecology studies [45,46,47,48,49]. Moreover, the constant improvements and accessibility of high-performance sequencing technologies have enabled researchers to characterize microbial soil communities in an unprecedented way and on ecologically relevant scales and resolutions of time, space and environmental conditions. This resolution, today, goes as far as the description of the composition of the microbial community at species or sub-species level and the evaluation of the functional potential and its expression at the level of individual genes. For example, soil metagenomics can be used to describe the use of carbon in the community [50] or the N cycle [51,52] determined by bacteria, fungi and other members of the soil biota.

Also relevant is the combination of the type of agricultural crop and the type of agricultural practice that is adopted, as well as the duration of these choices.

The practice adopted has an important effect on the soil and its characteristics, particularly on its chemical and microbiological composition, which varies the capacity of the soil to create and provide eco-systemic activity, with an effect on the quantity and quality of the agricultural product.

In this work a combined method of chemical and metagenomics analysis was applied to a first sample observation of the soil condition under different cultivation conditions and agronomic practice. The main aim is to determine the soil status and the diversification that is created when adopting different agronomic choices. This work provides the basis for further work in this way.

II. Material And Methods

Study area

Puglia is located at the south-eastern of Italy and extends for 19,350 km2 with a perimeter of 1,260 km and an overall coastal development of 784 km, the largest in mainland Italy.

Two areas of the Puglia Region have been examinedin this study: the Ionic Arc (called Area A) and the territory located between the municipality of Nardò and the municipality of Gallipoli (called Area B) (Figure no 1).

The 22 soil sample where identified using the following nomenclature: area of origin (Point A.), use the soil (Uncultivated Field=UF.; Vegetable Gardens=VG.; Grassland=G.; Orchards=O.; Olive Groves=OG.; Arable Lands= AL.; Vineyard=V.) and point of sampling in order time (1-2-3….).

All point present the same lithological and hydrogeological characteristic. There was selected n 6 Uncultivated Field; n. 3 Vegetable Gardens of which 1 with biological treatment (POINT B.VG.1); n. 2 Grassland of which 1 with biological treatment (POINT A.G.1); n. 2 Orchards; n. 4 Olive Groves of which 1 biodynamics agriculture practice (POINT A.OG.3); n. 2 Arable Lands and n 3 Vineyard of which 1 biodynamics agriculture practice (POINT A.V.3).

Sampling

The soil matrix sample was carried out in according to the "Methods of Soil Chemical Analysis" issued by the Ministry of Agricultural and Forestry Politics, approved with the Ministerial Decree of the 13th September 1999 (Uff. Journal Suppl. Ordin. n° 248, 21/10/1999).

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 10-15 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.

In the table no 1 the samples collected and their geo-localization in the two areas of study (Figure no 1, Figure no 2 and Figure no 3).

Table no 1: Sample collected

USE OF SOIL	NOMENCLATURE SAMPLE	N	E
Olive Groves	**POINT A.OG.1**	**40°29'19.8"**	**16°47'56.2"**
Orchards	**POINT A.O.1**	**40°29'18.5"**	**16°47'55.8"**
Orchards	**POINT A.O.2**	**40°31'42.9"**	**16°51'45.9"**
Olive Groves	**POINT A.OG.2**	**40°31'42.9"**	**16°51'45.9"**
Vegetables Gardens	**POINT A.VG.1**	**40°31'41.9"**	**16°51'46.5"**
Arable Lands	**POINT A.AL.1**	**40°32'36.8"**	**16°55'01.8"**
Arable Lands	**POINT A.AL.2**	**40°32'34.7"**	**16°55'02.2"**
Vineyard	**POINT A.V.1**	**40°34'30.5"**	**16°56'08.8"**
Vineyard	**POINT A.V.2**	**40°34'29.7"**	**16°56'09.5"**
Uncultivated Field	**POINT A.UF.1**	**40°34'44.0"**	**16°52'07.6"**
Biodynamics Vineyard	**POINT A.V.3**	**40°34'42.8"**	**16°52'07.0"**
Biodynamics Olive Groves	**POINT A.OG.3**	**40°36'31.1"**	**16°54'39.2"**
Biological Grassland	**POINT A.G.1**	**40°36'28.2"**	**16°54'41.5"**
Uncultivated Field	**POINT B.UF.1**	**40°12'03.2"**	**18°01'24.3"**
Grassland	**POINT B.G.1**	**40°12'03.2"**	**18°01'10.4"**
Biological Vegetables Gardens	**POINT B.VG.1**	**40°12'03.2"**	**18°01'23.8"**
Uncultivated Field	**POINT B.UF.2**	**40°04'12.6"**	**18°01'55.1"**
Uncultivated Field	**POINT B.UF.3**	**40°04'15.0"**	**18°01'55.0"**
Vegetables Gardens	**POINT B.VG.2**	**40°03'11"**	**18°01'11.02"**
Uncultivated Field	**POINT B.UF.4**	**40°03'10.0"**	**18°01'11.0"**
Olive Groves	**POINT B.OG.1**	**40°02'35.4"**	**18°01'48.2"**
Uncultivated Field	**POINT B.UF.5**	**40°02'36.1"**	**18°01'46.4"**

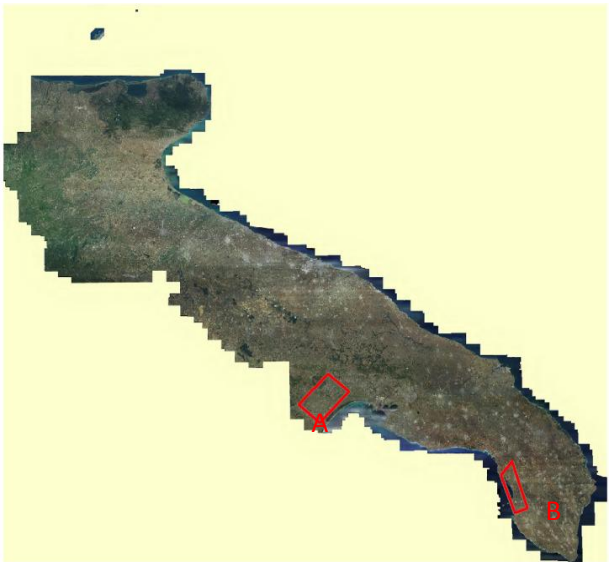

Figure no 1: *Areas of sampling: the first is the Ionic arc (called Area A) the second is located between the municipality of Nardò area and the municipality of Gallipoli (called Area B).*

Figure no 2: *Geo-localization of Samples in Area A*

Figure no 3:*Geo-localization of Samples in Area B*

Chemical analysis

The soil samples were collected in order to make a set of analyses according to the "Official Methods of Soil Chemical Analysis (MUACS), as stated by Ministerial Decree of the 13th September 1999, Ministry of Agricultural and Forestry Politics".

The sample preparation is such that the smallest weighing should be representative of the entire sample collected in the field. In particular, the following parameters were analyzed: temperature, organic carbon (Walkey-Black Method) (Method VII.3), organic matter, total nitrogen, organic nitrogen, nitrate, nitrite and ammonia.

DNA extraction

The NucleoSpinSoil kit (MACHEREY-NAGEL) was employed for the extraction of bacterial DNA from soil samples. It is designed for DNA molecules with high molecular weight of microorganisms such as positive and negative gram, archaea, fungi and algae present in soil, mud and sediment samples.

Bacterial DNA extracted from the soil was quantified using the Qubit™ 4 Fluorometer, while DNA quality was verified through electrophoretic run using the E-Gel™ Power Snap Electrophoresis System".

PCR conditions

Table no 2 lists the PCR primers and the thermal cycles conditions used in this study. PCR amplifications were performed using 50 µl total volume mixture obtained adding 4 µl HOTFIREPOOL (5x), 2 µl forward primer (10 pmol), 2µl reverse primer (10 pmol); 5 µl DNA (20 ng/ µl) in 37 µl of water.

Amplification of PCR products was confirmed by electrophoresis through "E-Gel™ Power Snap Electrophoresis System". Using 1.2% E-Gel™ agarose gel pre-stained with SYBR™.

Table no 2: *Primer*

Target Group	Primer	Primer Sequence (5'-3')	Amplicon Length (bp)	T. Annealing (°C)	Ref.
Region 16S	16SF	AGA GTT TGA TCA TGG CTC AG	1500	60	[53]
	16SR	TAC GGC TAC CTT GTT ACG ACTT			
All Bacteria	Eub338F	ACT CCT ACG GGA GGC AGC AG	200	60	[54]
	Eub518R	ATT ACC GCG GCT GCT GG			
Archea	Arch16F	CTG GTT GAT CCT GCC AG	300	58	[55,56]
	Arch344R	TTC GCG CCT GST GCR CCC CG			
Alphaproteobacteria	Alf28f	ARC GAA CGC TGG CGG CA	750	58	[57]
	Alf684r	TAC GAA TTT YAC CTC TAC A			
Betaproteobacteria	Beta359f	GGG GAA TTT TGG ACA ATG GG	450	58	[57]
	Beta682	ACG CAT TTC ACT GCT ACA CG			
Gammaproteobacteria	Gamma395f	CMA TGC CGC GTG TGT GAA	600	57	[57]
	Gamma871r	ACT CCC CAG GCG GTC DAC TTA			
Nitrospira	NSR 1113f	CCT GCT TTC AGT TGC TAC CG	151	60	[58]
	NSR 1264r	GTT TGC AGC GCT TTG TAC CG			
Bacteroidetes	798cfbF	CRA ACA GGA TTA GAT ACC CT	240	61.5	[59]
	cfb967R	GGT AAG GTT CCT CGC GTA T			

Species	Nitrifying Bacteria				
	Primer	Primer Sequence (5'-3')	Amplicon Length (bp)	T. Annealing (°C)	Ref.
Nitrosomonas spp.	NsomoF	GTG GGG AAT TTT GGA CAA TG	900	60	in this study
	NsomoR	TTA CGT GTG AAG CCC TAC CC			
Nitrosovibrio sp.	NvibrioF	GTG GGG AGC AAA CAG GAT TA	400	60	in this study
	NvibrioR	GCG CCA TTG TAT TAC GTG TG			
Nitrococcus spp.	NcoccusF	GGT CTG AGA GGA CGA TCA GC	400	60	in this study
	NcoccusR	CTA CGC ATT TCA CCG CTA CA			
Nitrobacter spp.	NitroF	TCA CTA GTG GCG CAC GTA AC	400	56	in this study
	NitroR	CTA CAA TGG CGG TGA CAA TG			
Nitrospiraceae sp.	NspiracF	ACC GGA TAT GGT GAT TTG GA	850	60	in this study
	NspiracR	TGC ATG TCA AAC CCA GGT AA			

Species	Denitrificant Bacteria				
	Primer	Primer Sequence (5'-3')	Amplicon Length (bp)	T. Annealing (°C)	Ref.
Hydrogenophilus sp.	HydroF	TGG GCT CAA CCT AGG AAT TG	600	60	in this study
	HydroR	ATG ACG TGT GAA GCC CTA CC			
Hyphomicrobium sp.	HyphoF	TGA TGA AGG CCT TAG GGT TG	800	58	in this study
	HyphoR	CAT TGT CAC CGC CAT TGT AG			
Rhodopseudomonas sp.	RhodoF	GCG GGA AGA TAA TGA CGG TA	400	60	in this study
	RhodoR	CAT TGT CAC CGC CAT TGT AG			
Pseudomonas spp.	PsF	TTA GCT CCA CCT CGC GGC	600	58	[60]
	PsR	GGT CTG AGA GGA TGA TCA GT			
Xanthomonas sp.	XantF	TGG GGA GCA AAC AGG ATT AG	500	62	in this study
	XantR	AGC CCT CTG TCC CTA CCA TT			
Kingella sp. .	KinF	CCA ATC CGA AAG ATT GGC TA	550	60	in this study
	KinR	ACG CAT TTC ACT GCT ACA CG			
Halomonas sp.	HaloF	AGA GGA TGA TCA GCC ACA CC	950	60	in this study
	HaloR	GCG ATA TTG CAA CCC TTT GT			

Species	Nitrifying / Denitrificant Bacteria				
	Primer	Primer Sequence (5'-3')	Amplicon Length (bp)	T. Annealing (°C)	Ref.
Paracoccus spp.	ParaF	TAA TAC CGT ATG CGC CCT TC	900	60	in this study
	ParaR	AAC TTC ATG GGG TCG AGT TG			
Alcaligenessp	AlcaF	AAG GCT CAC CAA GGC AAC TA	900	60	in this study
	AlcaR	GTA CAA GAC CCG GGA ACG TA			

Nucleotide sequence analysis

All the PCR products were sequenced by Mycrosint Lab (GERMANY). The sequencing analyses were conducted using the BLAST program [61] in the GenBank.

III. Result

Chemical Analysis

Nitrate concentrations in soil samples resulted in the range 10 mg N/kg - dry soil and 56 mg N/kg - dry soil, excepted the 2 sample doing in the arable land (1 mg N/kg - dry soil) and in the biological orchards (0.1 mg N/kg - dry soil) (figure 4).

Nitrites and ammonia concentrations in soil samples resulted less than 1 mg N/kg - dry soil, with the exception in POINT B.VG.1 (1.09 mg N/kg - dry soil for nitrites concentration and 18.49 mg N/kg - dry soil for ammonia concentration) and in POINT A.V.3 and POINT A.UF.1 (ammonia concentrations: 9.54 mg N/kg - dry soil and 7.67 mg N/kg - dry soil respectively) (figure 4).

The organic nitrogen concentrations in soil samples widely ranged from 100 mg N/kg - dry soil to 700 mg N/kg - dry soil with an anomalous very low concentration (96.30 mg N/kg - dry soil) in the POINT B.VG.1 (figure 4).

The organic matter in soil presents a wide range of concentrations (figure 6), with very high values (8.2% POINT B.UF.1 and 8.7% POINT B.UF.4) and some cases of very low concentrations (0.6% POINT B.VG.1 and 0.2% POINT A.AL.2), with a correlation index (0.72) (figure 7) between organic matter and organic nitrogen, suggesting the presence in the soil of more homogenous organic compounds.

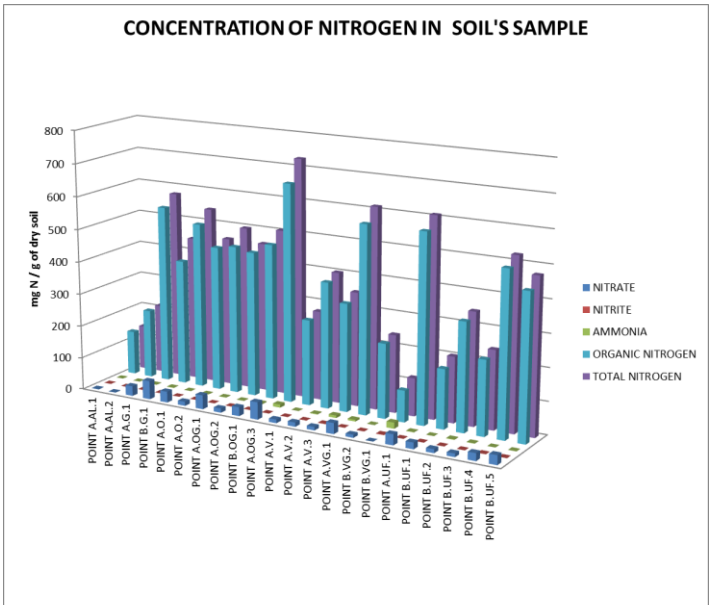

Figure no 4. *Concentration of nitrogen compounds in soil's sample*.

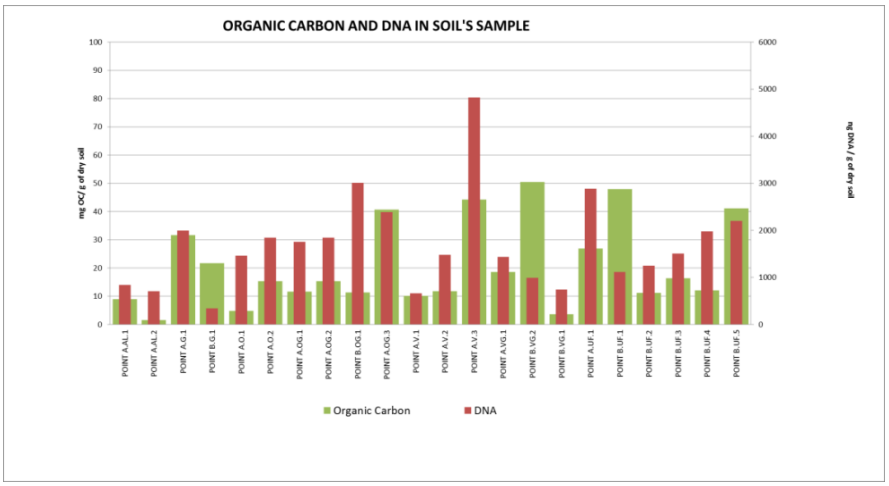

Figure no 5. *Concentration of Organic Carbon and DNA in soil's sample.*

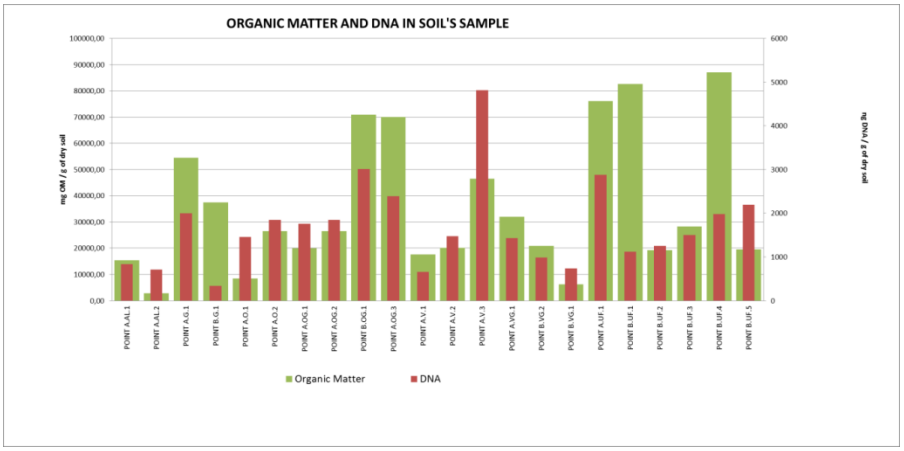

Figure no 6. *Concentration of Organic Matter and DNA in soil's sample*.

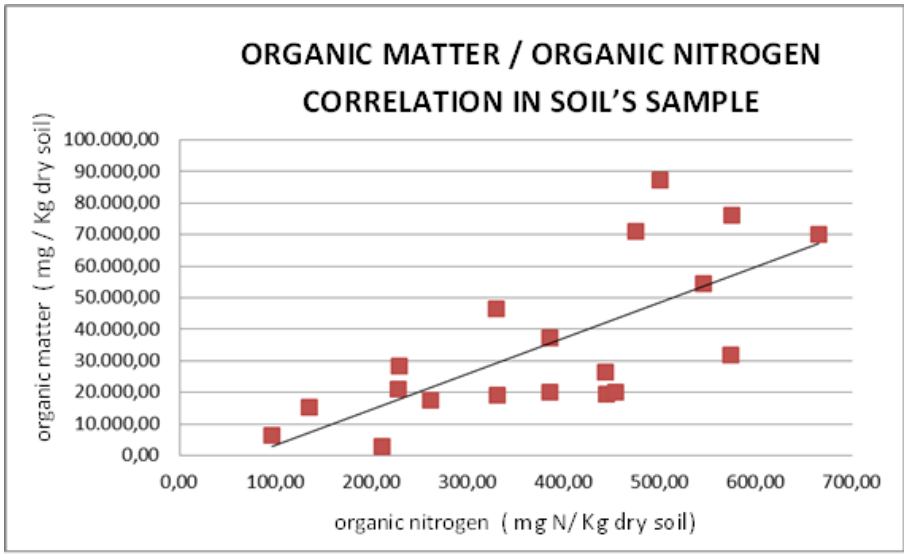

Figure no 7. *Correlation Organic Matter/organic nitrogen in soil sample*.

Biomolecular Analysis

DNA concentration in the soil samples resulted in the range (658.88 ng DNA/g - dry soil - 3012.67 ng DNA/g - dry soil), except POINT B.G.1 (338.59 ng DNA/g - dry soil) and POINT A.V.3 (4819.07 ng DNA/g - dry soil) (figure 6). The analysis of the DNA concentration had identified distinguishable range of DNA concentration in relation to the different use of soil: orchards present 1400 ng DNA/g - dry soil - 1900 ng DNA/g - dry soil; uncultivated field 1100 ng DNA/g - dry soil - 2900 ng DNA/g - dry soil; olive groves 1700 ng DNA/g - dry soil - 3000 ng DNA/g- dry soil; vegetable gardens 900 ng DNA/g - dry soil - 1500 ng DNA/g - dry soil; grasslands 300 ng DNA/g - dry soil - 600 ng DNA/g - dry soil ; arable lands 700 ng DNA/g - dry soil - 850 ng DNA/g - dry soil; vineyard 600 ng DNA/g - dry soil - 1500 ng DNA/g - dry soil. A high DNA concentration was present in the biodynamic vineyard (4800 ng DNA/g - dry soil) and in the field with a biological farming practice (2000 ng DNA/g - dry soil). Particularly relevant the results regarding the vegetable garden with a very low DNA concentration (740 ng DNA/g - dry soil) (figure 8).

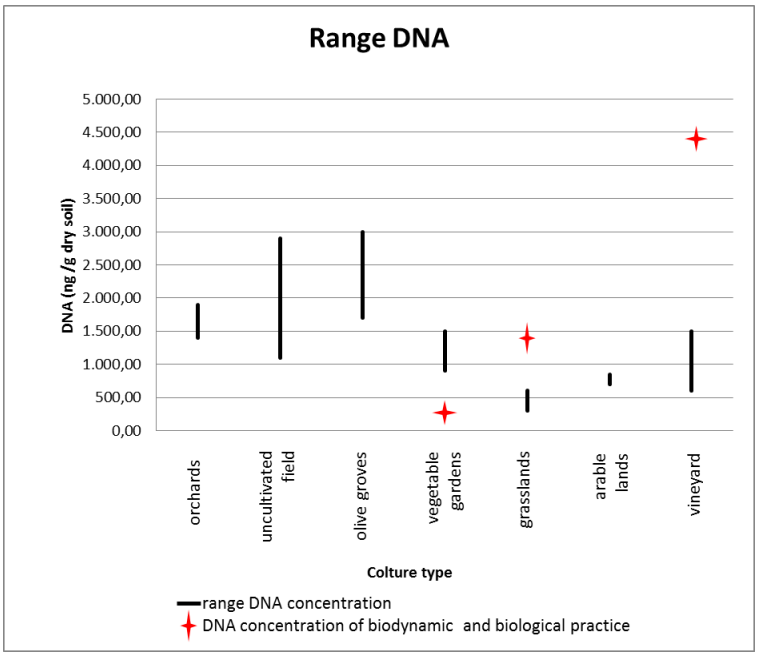

Figure no 8. *Range of DNA concentration in soil for different cultivation type and case of biodynamic and biological agriculture practice.*

The PCR analysis (figure 9) determined the presence of generic bacterial DNA in all samples and of Archaea DNA except in POINT B.UF.4 and POINT B.OG.1. In all samples the presence of Alfaproteobacteria, Betaprotobacteria and Gammaproteobacteria was detected, except samples POINT B.UF.4 and POINT B.OG.1.

The absence of Nitrospira and Bacteroidetes DNA has been shown in all samples.

The analyses of nitrifying species demonstrate the absence in all samples of Nitrobacter sp. DNA; the presence of Nitrosovibrio sp. and Nitrococcus spp. DNA, except for the sample POINT B.OG.1; the presence of Nitrosomonas spp. and Nitrococcus spp. DNA in all samples and absent in samples POINT B.UF.3, POINT B.VG.2, POINT B.UF.4, POINT B.OG.1, POINT B.UF.1, POINT B.G.1, POINT B.VG.1, POINT A.OG.3 and POINT A.G.1

The DNA of Nitrospiraceae sp. where always absent in all samples, thus confirming the observations Nitrospira class carried out and previously described.

The analyses of the denitrifying species demonstrate: the presence of Rhodopseudomonas sp. DNA in all samples except in POINT B.OG.1 and POINT A.OG.3; the presence of Hyphomicrobium sp. in all samples except for the samples POINT B.OG.1and POINT A.OG.3; the presence in all sample of the Thiobacillus sp. DNA and Pseudomonas spp. DNA, except for sample POINT B.OG.1; the absence in all sample of Kingella sp. DNA and Halomonas sp. DNA, the presence of Neisseria spp. DNA in POINT B.UF.2, POINT B.UF.4, POINT B.OG.1, POINT B.UF.1, POINT A.O.1, POINT A.V.1, POINT A.V.2 and POINT A.UF.1; the presence of Xanthomonas sp. DNA in all samples except for POINT B.OG.1, POINT A.OG.3 and POINT A.G.1; the presence of Hydrogenophilus sp. DNA is observed in all samples, except for POINT B.OG.1and POINT A.OG.3.

The analyses of the nitro-denitrifying species showed: the presence of Paracoccus spp. DNA only in POINT B.UF.4; completely absent is the Alcaligenes sp. DNA. A critical situation is represented by the POINT B.OG.1 only Neisseria spp. was found; not even one other bacteria of nitrogen cycle..

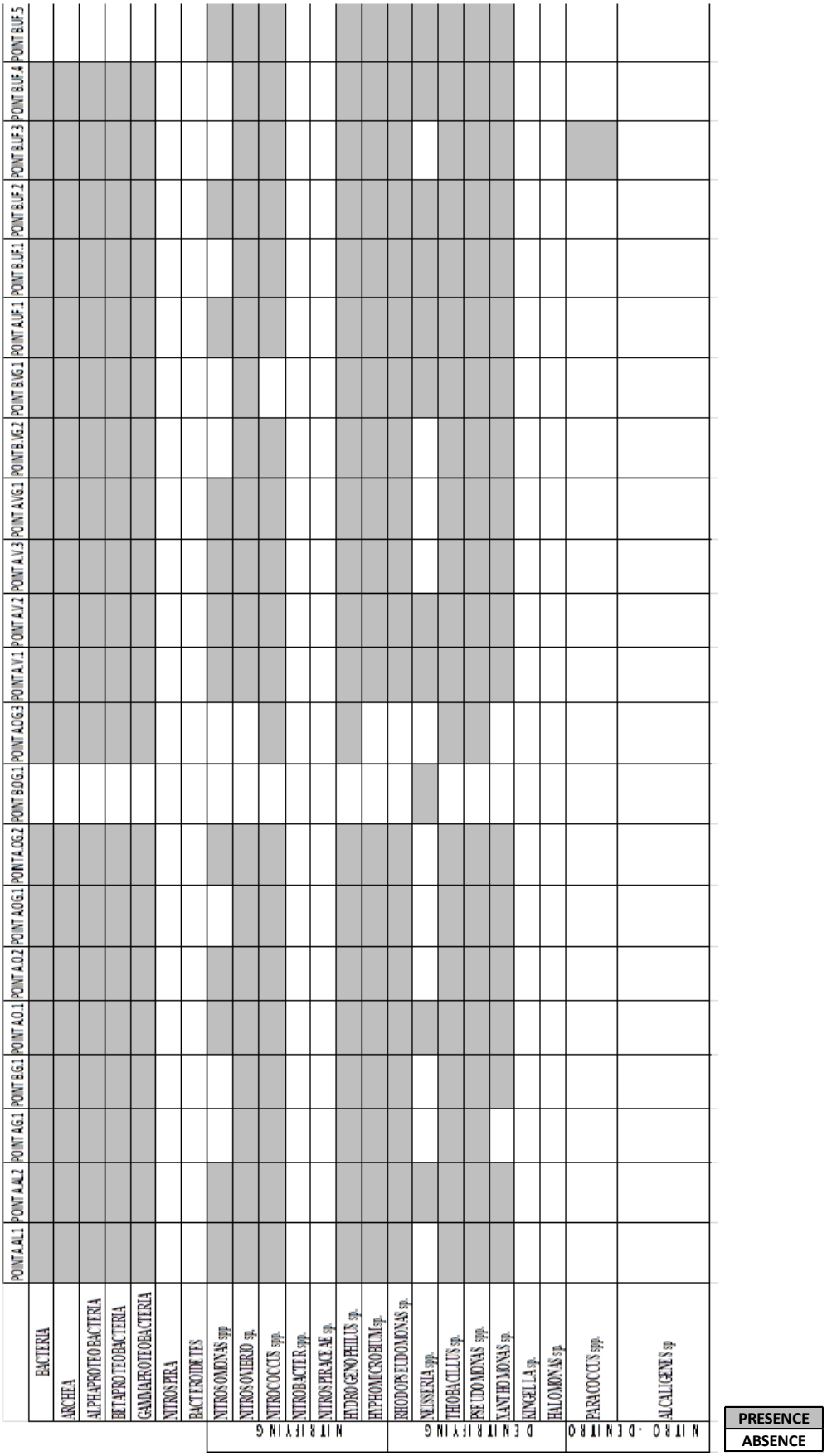

Figure no 9. *PCR schematic result of presence and absence of DNA amplification*.

IV. Discussion

The soil of the vineyard with long-lasting biodynamic practice (thirty years) shows a concentration of organic matter and organic carbon (46470.62 mg / kg of dry soil) 4 times higher than the soil of vineyards with traditional agricultural practices.

This situation, however, is not found in the olive grove cultivated with biodynamic practice for less time (about eight years); the concentration values of organic matter and organic carbon fall within the average range of the other olive groves analyzed.

The concentration of organic nitrogen is significantly higher in the soil of olive grove managed under biodynamic rules than in olive groves with traditional practices; instead, the soil of biodynamic vineyard falls within the average concentration value for this parameter.

Note the very low value of organic matter in the soil of a vegetable garden managed under biological rules (6288.03 mg / kg of dry soil), as well as in an arable land (2759.00 mg / kg of dry soil) and in an orchard (8375. 49 mg / kg of dry soil) conducted with traditional practices. The soil of the vegetable garden also presented an anomalous situation, with low concentration of organic nitrogen and high concentrations of nitrite and ammonia.

The concentrations of nitrite and ammonia are higher in the soil of biodynamic vineyard and in the soil of biodynamic olive grove than in sites with the same use of the soil but with traditional agricultural practices. The nitrate concentration, on the other hand, is higher in the biodynamic olive grove, while it falls within the average in the biodynamic vineyard.

In the soil of a lawn cultivated with advanced biological method has been found a higher concentration of organic matter and organic nitrogen, a lower concentration of nitrate, nitrite and ammonia compared to other sites with the same use of the soil.

The soil of the biodynamic vineyard has a high concentration of DNA, 4800 ng DNA / g of dry soil.

The soil of advanced biologically managed lawn shows a DNA concentration of 2000 ng DNA / g of dry soil, while the soil in the biodynamic olive grove the concentration of DNA falls within the average range of the other olive groves (from 1700 ng DNA / g of dry soil to 3000 ng DNA / g of dry soil).

An exceptional case is that of the soil in the vegetable garden managed under biological rules, where a very low DNA concentration (740 ng DNA / g of dry soil) has been found.

The distribution of the bacterial species of the nitrogen cycle, identified in the soil samples, is somewhat homogeneous. There are no particular differences of bacterial species between the soil of the vineyard conducted with biodynamic method and that of vineyards with traditional agronomic activity, except for the Neisseria species, absent in the biodynamic vineyard.

There are also no distinguishable characteristics relating to the microbiome of the biodynamic olive grove, the lawn and the organic garden conducted under biological rules

In the territory of the municipality of Gallipoli occurred a particular situation in the soil of an olive grove conducted with the traditional method: only one bacterial species of nitrogen cycle, Neisseria spp, was detected.

It may be interesting to remember that this territory is considered as the first outbreak of the “olive quick decline syndrome” in Puglia region.

V. Conclusion

Some first chemical and biome characterizations, related to the nitrogen cycle, were conducted in sites with different land use and with different agronomic practices.

There is a high variability in the concentration of organic substance, with values much lower and much higher than the maximum value found (gold standard).

The gold standard value has been found mainly in soils under application of biodynamic practices for a long time.

The low correlation of organic carbon concentration versus organic nitrogen concentration founded in the soil samples denotes differences in the quality of the organic matter present, in fact not always all "good" even if on average high concentrations.

The soil of the ancient biodynamic vineyard shows the highest concentration of DNA found, more than double the value of the second value detected, that was found in the soil of the lawn managed under advanced biological practice.

Unexpectedly, the vegetable garden managed under biological methods shows the lowest concentration of DNA found.

The composition of the biome for the bacterial species of nitrogen cycle shows a very complex picture.

Some species are always absent and some always present; in a worrying case only the Neisseria species was detected.

The work will continue by applying quantitative PCR techniques (Real Time), with which a complete photograph of the state of the microbiome of the analyzed soils will be possible.

The applied method seems particularly suitable for bio-geo-chemical insights on the nitrogen cycle in the soil, referred to different use of the soil and in relation to the agronomic practices adopted.

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